

Section 11 Laboratory Procedures

11.1 Overview and General Guidance

This section contains information on the laboratory procedures performed in HPTN 061. Some laboratory procedures will be performed in study site clinics; others will be performed in study site local laboratories, or at the HPTN Network Laboratory (NL) in Baltimore, MD, USA. Table 11-1 lists the time points, testing location and specimen requirements for each test. In all settings, laboratory procedures will be performed according to study site standard operating procedures (SOPs). Laboratory procedures performed in-clinic will be performed according to study site SOPs that have been approved by the HPTN NL. In addition, package insert instructions must be followed.

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

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

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

11.1.1 Biohazard Containment

As transmission of HIV and other infectious agents can occur through contact with contaminated needles, blood, blood products, and rectal swabs, all study staff must take appropriate precautions when collecting and handling biological specimens. Guidance on standard precautions available from the US Centers for Disease Control and Prevention (CDC) and can be found at:

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

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

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

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

11.2 Specimen Labeling

All containers into which specimens are initially collected (e.g., blood collection tubes) will be labeled with SCHARP-provided Participant ID (PTID) labels. PTIDs are pre-printed on these labels; however study staff must write the specimen collection date on each label. The visit code may also be written on the label. When specimens are tested at the local lab, any additional labeling required for on-site specimen management and chain of custody will be performed in accordance with site SOPs. Stored plasma specimens will be entered into the Laboratory Data Management System (LDMS) and labeled with LDMS-generated cryovial labels. See also Section 11.5.3.

11.3 Use of LDMS

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

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

As of the date of this version of Section 11, the current version of LDMS is Version 5.7. All sites should upgrade to this version as soon as possible. All sites must use the HPTN Barcode label format for bar-coded labels in order to ensure that both the Specimen ID and the Global Specimen ID assigned to each specimen are printed on LDMS-generated labels. All sites must use the bar-coded labels designed for a LDMS suitable printer, utilizing the appropriate label size. Contact LDMS user support for further information.

Questions related to use of LDMS in HPTN 061 should be directed to Estelle Piwowar-Manning (epiwowa@jhmi.edu, + 410-614-6736/Vanessa Cummings, 410-502-5296). Technical support also is available from LDMS User Support. Usual business hours for LDMS User Support are 12 am to 6:00 pm ET Monday through Friday. During business hours, please contact LDMS User Support as follows:

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

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

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

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

LDMS User Support

LDMS 1: ldmspager1@fstrf.org

LDMS 2: ldmspager2@fstrf.org

LDMS 3: ldmspager3@fstrf.org

Each site must export its LDMS data to Frontier Science (FSTRF) on a weekly basis. Exported data are used by the HPTN SDMC to generate a monthly specimen repository report and to reconcile data entered in LDMS with data entered on study case report forms. Any discrepancies identified during the reconciliation are included in a monthly discrepancy report for each site. Sites are expected to resolve all discrepancies within two weeks of receipt of the report. The HPTN NL is responsible for reminding sites to adhere to the two-week timeframe and for following up with sites that do not resolve discrepancies within two weeks. The HPTN SDMC reviews the discrepancy reports for critical samples that appear to be missing, and works with the NL and site staff to undertake appropriate corrective action. All corrective action should be documented in paper-based clinic and/or laboratory records as appropriate, and entered in the details section of LDMS. The NL and SDMC will discuss and document any items that, although resolved, appear ‘unresolvable’ in LDMS.

11.3.1 LDMS Specimen Processing

When the specimens are received at the local LDMS Laboratory, each specimen will be checked to ensure that the information on the label for each specimen matches the information on the accompanying LDMS Specimen Tracking Sheet, and that the type and number of specimens marked on the Specimen Tracking Sheet are correct. If discrepancies are noticed, the clinic staff should be contacted and the lab should follow their specific procedures for discrepancies.

If there are any problems associated with sample aliquots (e.g. inadequate number of aliquots due to a short draw, lost sample, lab error), describe the problem in the LDMS (details section for the specimen) and cascade the information to the aliquot level. Any

consistent problems (e.g. with short draws, lost samples, etc.) need to be recorded on a Note to File, which is sent to the HPTN NL, FHI and SCHARP.

Detailed below is information on logging plasma and rectal swab samples into the LDMS.

Test	Primary	Additive	Derivative	Sub Add/Der
Plasma Archive	BLD	EDT	PL2	NA
Rectal Swab	REC	NON	SWB	NA

11.4 Protocol related testing

- HIV rapid test.
- HIV Western Blot (WB) for participants with a reactive HIV rapid test.
- CD4 cell count testing for participants with a reactive HIV rapid test and for HIV positive subjects at 52 weeks.
- Syphilis serology (including titer).
- CT/NG NAAT from urine.
- HIV viral load for subjects with a positive WB result.
- Prepare rectal swabs for shipment to NL (for CT/NG NAAT)
- Plasma sample archive, for subsequent shipment to NL.

The tests performed at each study visit vary depending on the time point of the visit and the clinical presentation of the participant. At most visits in which blood testing is required, up to 40 mLs of blood will be collected; however additional blood may be collected if clinically indicated.

For CD4, WB testing (plasma required per protocol), HIV viral load and syphilis serology testing, please consult the local laboratory for sample tube sizes and volumes noting that the total volume to include plasma archive should not exceed 40 mLs.

11.4.1 Specimen Collection and Initial Processing

Table 11.1 indicates the per-visit blood collection requirements for protocol-specified testing for study participants. Sites need to consult with and obtain approval from the HPTN NL on the specific tube types and volumes for their specific setting. Additional blood may be collected at any visit if required to perform additional clinically indicated tests. It is recommended that pediatric size tubes should be used when collecting 2 mL and 3 mL blood volumes.

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

11.4.1.2 Blood Collection

After collection:

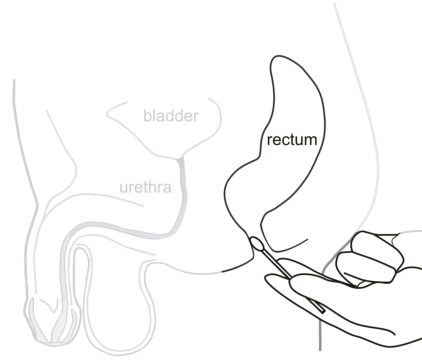
EDTA tubes require no additional processing prior to testing, but should be gently inverted at least eight times (or as specified by manufacturer) after specimen collection to prevent clotting. At time points when rapid HIV testing is performed, pipette blood from the EDTA tube for the rapid test and then discard the remainder of the blood. Serum separator tubes (SST) and plain red top tubes require no additional processing prior to testing.

11.4.1.3 Rectal Swab Collection

The rectal swabs are to be collected by the clinician or the participant as determined by the site. See collection instructions below. The swab to be used for collection is the BD CultureSwab EZ single swab BD 220144. These can be obtained through Fisher Scientific using catalog number B220144.

11.4.1.3.1 Self-Collection of Rectal Swab

1. Before opening the swab, go to a place where you feel comfortable squatting down or putting one leg up on something such as a toilet, ledge, or chair.
2. Wash your hands.
3. Pull underwear down or off before collecting.
4. Peel open the wrapper containing the swab.
5. Remove the swab from its wrapper; **DO NOT TOUCH THE TIP OF SWAB.**
6. Place your fingers 1½ inches away from the tip of the swab.
7. Position your bare buttock and lift one cheek for easy access to the rectum.
8. **DO NOT USE ANYTHING** (such as soap, saliva, or any kind of lubricant) on the swab or rectum before insertion.
9. Insert the swab 1½ inches into your rectum until you feel your fingers touch your anus.
10. Once swab is inserted, release your hold on the shaft of the swab by moving your fingers halfway down the shaft for stability.
11. Gently rub the swab touching the walls of your rectum in a circle to collect the specimen.
12. When removing the swab from your rectum, slowly turn it in a circle while pulling it out.
13. Screw the cap off a labeled 2 mL cryovial and place the swab in the cryovial .
14. Bend the shaft of the swab until the part sticking out breaks off.
15. Place the cap tightly on the tube to prevent leakage during transporting.
16. Place tube into plastic zip-lock bag and seal the bag. Specimen should be kept in refrigerator or wet ice until stored in -70°C freezer (rectal swabs should be frozen at -70°C within 24 hours of collection). If they have to be transported to the lab to be frozen there, then ice packs can be placed in the container they are being transported in. Follow steps 6 and 7 below.



11.4.1.3.2 Clinician Collected

1. Participant will be asked to remove or pull down pants and either lie on left side on exam table or bend over table with chest on table and buttock exposed.
2. Provider will at same time wash hands and put on disposable latex free gloved.
3. Provider will peel open the wrapper containing the swab taking care to not touch the tip of the swab (do not place any fluid or lubricant on swab).
4. Insert swab into rectum 1 ½ inches from the tip of the swab and rotate 180 degrees making sure to touch the walls of the rectum. Remove swab in a circular motion.
5. Place the swab in a labeled 2 mL cryovial (hold cryovial with clean hand), bend shaft of the swab until end breaks off. Place the cap tightly back on the tube with the swab inside and keep in refrigerator or on wet ice until it can be stored in -70⁰C freezer (rectal swabs should be frozen at -70⁰C within 24 hours of collection). If they have to be transported to the lab to be frozen there, then ice packs can be placed in the container they are being transported in.
6. Log the swab into the LDMS system, generate LDMS label and place label on cryovial.
7. Store the swab in the -70C freezer until shipment to Network lab.

11.4.1.3.3 Urine Collection

Urine will be collected for Chlamydia and gonorrhea (CT/NG NAAT). The same assay must be used throughout the study; Gen-Probe Aptima for CT/NG. Prior to urine collection, the urine collection cup (one per participant per visit) should be labeled (participant ID label and hand-written date). Urine should be collected as follows:

- Participant should not have urinated within one hour prior to collection.
- Provide participant with a labeled sterile screw-top urine collection cup without preservatives; obtain Aptima Urine Collection Kit (confirm with testing lab choice of urine collection container).
- Instruct participant not to clean the penis prior to collecting the urine specimen.
- Instruct participant to collect the first portion (confirm with testing lab, volume to be collected) of urine stream (*i.e.*, first void / dirty catch urine).

- Instruct participant to screw lid on tightly, determine from testing lab if urine needs to be transferred to transport tube, urine specimens can be transported to the lab at 2⁰C to 30⁰C (confirm with testing lab specimen transport requirements).

Table 11.1 Specimen Collection and Storage by Visit in HPTN 061

Table abbreviations: mL = milliliter; RT = real-time; spec = specimen

Study Visit	Collection Tube Type or Container	Test		Specimen Type collected	Specimen Amount		Test Processing Location
		RT test	Stored Spec				
Enrollment	EDTA	Rapid HIV test ¹		Whole Blood	Site Dependent	Total blood volume drawn not to exceed 40 ml	Clinic Site
		CD4+ cell count ²		Whole Blood K2EDTA recommended	Site Dependent		Local Lab
		HIV Western Blot ³		Whole Blood	Site Dependent		Local Lab
		HIV Viral Load ²		Whole Blood K2 or K3 EDTA	Site Dependent		Local Lab
				Plasma Archive ⁴	Whole Blood		After drawing off whatever blood is required for clinical tests, spin down for plasma archive. Aliquot and store as many 1.8 mL samples as possible. (minimum 4 aliquots)
	Plain/SST	Syphilis serology plus titer	Whole Blood	Site Dependent	Local Lab		
	Rectal Swab	CT/NG NAAT	Rectal Swab	1 swab	Local lab storage to be shipped to NL		
	Urine	CT/NG NAAT		Urine	Site Dependent		Local Lab
Week 26	EDTA	Rapid HIV test		Whole Blood	Site Dependent	Total blood volume drawn not to exceed 40 ml	Clinic Site
		CD4+ cell count		Whole Blood K2 EDTA recommended	Site Dependent		Local Lab
		HIV Western Blot		Whole Blood	Site Dependent		Local Lab
		HIV Viral Load		Whole Blood K2 or K3 EDTA	Site Dependent		Local Lab
				Plasma Archive	Whole Blood		After drawing off whatever blood is required for clinical tests, spin down for plasma archive. Aliquot and store as many 1.8 mL samples as possible. (minimum 4 aliquots)
	Plain/SST	Syphilis serology plus titer	Whole Blood	Site Dependent	Local Lab		
	Urine	CT/NG NAAT		Urine	Site Dependent		Local Lab
Week 52	EDTA	Rapid HIV test ¹		Whole Blood	Site Dependent	Total blood volume drawn not to exceed 40 ml	Clinic Site
		CD4+ cell count ²		Whole Blood K2 EDTA recommended	Site Dependent		Local Lab
		HIV Western Blot ³		Whole Blood	Site Dependent		Local Lab
		HIV Viral Load ²		Whole Blood K2 or K3 EDTA	Site Dependent		Local Lab
					Whole Blood		After drawing

Study Visit	Collection Tube Type or Container	Test		Specimen Type collected	Specimen Amount		Test Processing Location
		RT test	Stored Spec				
			Plasma Archive ⁴		off whatever blood is required for clinical tests, spin down for plasma archive. Aliquot and store as many 1.8 mL samples as possible. (minimum 4 aliquots)		
	Plain/SST	Syphilis serology plus titer		Whole Blood	Site Dependent		Local Lab
	Rectal Swab	CT/NG NAAT		Rectal Swab	1 swab		Local lab storage to be shipped to NL
	Urine	CT/NG NAAT		Urine	Site Dependent		Local Lab
Post Test Visit for WB Confirmation or Ad Hoc visit	EDTA	Rapid HIV test ¹		Whole Blood	Site Dependent	Total blood volume drawn not to exceed 40 ml	Clinic Site
		HIV Western Blot ³		Whole Blood	Site Dependent		Local Lab
			Plasma Archive ⁴	Whole Blood	After drawing off whatever blood is required for clinical tests, spin down for plasma archive. Aliquot and store as many 1.8 mL samples as possible. (minimum 4 aliquots)		

1. HIV rapid test is performed for all study subjects at enrollment, except for participants who refuse testing. HIV rapid testing is not performed at 26 or 52 weeks if HIV infection was confirmed at prior visit.
2. CD4 cell count is performed at any visit where a reactive HIV rapid test is obtained. CD4 cell count is not performed at the 26 week visit if HIV infection was confirmed at enrollment. A CD4 cell count is obtained at 52 weeks for all subjects with confirmed HIV infection. HIV viral load is performed at enrollment and week 52 visits for any subjects with a positive WB or previously confirmed HIV infection
3. A WB is performed at any visit where a reactive HIV rapid test is obtained. A WB is not performed at the 26 or 52 week visits if HIV infection was confirmed at a prior visit. Subjects who have an indeterminate or positive WB should have a repeat (confirmatory) WB performed within one or two weeks. If a subject provides prior documentation of HIV-infection, rapid HIV testing and Western Blot may be waived but CD4 and viral load should still be performed and samples should be stored as indicated for HIV-infected individuals. For participants who were not diagnosed with HIV infection prior to their study visit, two positive WBs are required to document HIV infection; additional testing using samples collected at a third visit may be required in some cases.
4. Plasma is stored for all participants at all study visits.
5. Rectal swabs are shipped to the NL for testing and are obtained at enrollment and week 52.

11.4.2 HIV Testing

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

(NOTE: The HIV rapid test will be performed at time of enrollment and also at subsequent visits, if the participant has not previously been confirmed HIV-infected. Rapid test kits must meet specifications described at <http://www.fda.gov/cber/products/testkits.html>. Testing at clinic sites must be performed under the oversight of the Study Coordinator for the CRS.

Perform all rapid tests according to site SOPs and package inserts. All staff involved in HIV testing and verification of HIV test results should be aware of the different testing timeframes for each rapid test, so that all tests are performed and verified within the specified timeframe. Place appropriate timekeeping devices in all test settings to ensure that each test is read and verified at appropriate time points. Document the testing start and stop times, as well as result verification times, on testing log sheets. When transcribing rapid test results from log sheets to the HPTN 061 Laboratory Results case report forms, sites must record the correct test code to indicate the type of test used. These codes are provided on the instruction sheet for the CRF.

All participants who receive a non-reactive or negative HIV rapid test result are considered to be HIV seronegative. No further testing at the local laboratory is necessary. Blood specimens already drawn should be sent to the local laboratory for plasma archive.

All participants who receive a reactive or positive HIV rapid test result are to be considered to be potentially HIV seropositive and must have the appropriate blood specimen(s) sent to the local laboratory for a CD4 cell count, confirmatory HIV testing by WB, and if the WB confirms positivity, a viral load must be performed.

All participants who are known to be HIV positive at enrollment will have the HIV rapid test and WB performed at enrollment to confirm serostatus. This is the only situation in which the HPTN NL will allow a single positive WB to serve as confirmation of HIV infection. Rapid HIV testing and WB confirmation may be waived at enrollment for participants who were diagnosed with HIV infection prior to enrollment who provide documentation of HIV infection status per SSP Section 4.

NOTE: “HIV indeterminate by rapid testing” is not an appropriate term. If the rapid test does not have a clear reactive or non-reactive result, the test is considered invalid and should be repeated. If there are still problems with the rapid test results, contact the HPTN NL for guidance.

The remainder of the specimens already drawn should be sent to the local LDMS laboratory for plasma archive.

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

- **Positive:** At least two of the major bands — gp160/gp120, gp41, p24 — must be present and must be at least as intense as the low positive control gp120 band. The gp41 band must be broad and diffuse. (NOTE if any sites use the Cambridge HIV Western blot kit please inform the HPTN NL. The Cambridge WB kit uses a different control band for comparison).
- **Indeterminate:** One or more bands is/are present, but the blot does not meet the criteria for a positive result as described above.
- **Negative:** No bands are present.

All laboratory staff that read and interpret WB results for study participants are encouraged to complete proficiency testing approximately every six months. The HPTN NL will post an image of an actual WB run on the HPTN 061 web page for this purpose. Relevant laboratory staff from each site can review these images and submit their interpretations of the images to the HPTN NL via the HPTN 061 web page. After each proficiency testing cycle, the HPTN NL will report results back to each site Laboratory Manager and specify any corrective action that may be needed. Contact the HPTN NL for additional information and guidance on performing and documenting the proficiency testing. Also contact the HPTN NL when new laboratory staff are hired, so that proficiency testing can take place prior to such staff interpreting Western blots for study purposes.

Sites should follow the procedures below based upon the WB results obtained after a positive rapid HIV test:

- **Negative 1st WB:** If a participant receives a negative result by WB on the first test, site should consult Network Laboratory.
- **Indeterminate or Positive 1st WB:** Participants who receive an indeterminate or positive result by WB should have a repeat blood draw 1 – 2 weeks later (unless the participant was known to be HIV positive at enrollment.) This repeat blood draw should be sent to the local laboratory for WB analysis. For sample volumes, please refer to the local laboratory requirements. Blood for plasma archive should also be obtained.
- **Positive 2nd WB:** All participants who receive a positive result by WB on the 1st and 2nd samples are to be considered to be confirmed HIV seropositive. If the 1st sample was indeterminate and the second sample is positive, a third sample should be drawn to confirm HIV seropositivity. Blood for plasma archive should also be obtained.
- **Negative or Indeterminate 2nd WB:** If the laboratory reports a negative or indeterminate WB on the 2nd WB following a positive WB result, or a negative WB following an indeterminate WB, the site should consult the HPTN NL for guidance. Additional sample collection and testing may be needed.

- **Indeterminate 1st and 2nd WB** All participants who receive an indeterminate result by WB on the 1st and 2nd samples should be reported as HIV indeterminate. Contact the Network Laboratory for further instructions.

11.4.3 CD4 cell count

CD4 cell count testing must be performed at a laboratory that is CLIA-certified. Laboratories that are certified through the DAIDS IQA program are preferred. CD4 cell counts are performed at any visit where a reactive HIV rapid test result is obtained and for all HIV-positive participants at the 52-week visit. A CD4 cell count is not performed at the 26-week visit if HIV infection was confirmed at an earlier visit.

11.4.4 HIV viral load

HIV viral load must be performed at a laboratory that is CLIA-certified. An HIV viral load is performed after a participant receives a confirmatory positive HIV result by WB at any visit. Viral load specimens can be sent out at the same time as the Western Blot or may be spun down and plasma kept frozen at -70⁰C until the positive Western Blot result is obtained and then sent to lab for testing. Viral load will be repeated at the 52-week visit for all HIV positive participants.

11.4.5 Urine CT/NG NAAT

Urine will be collected for CT/NG NAAT testing at the site's local CLIA-certified laboratory. The local lab should provide collection instruction and volumes needed for CT/NG NAAT.

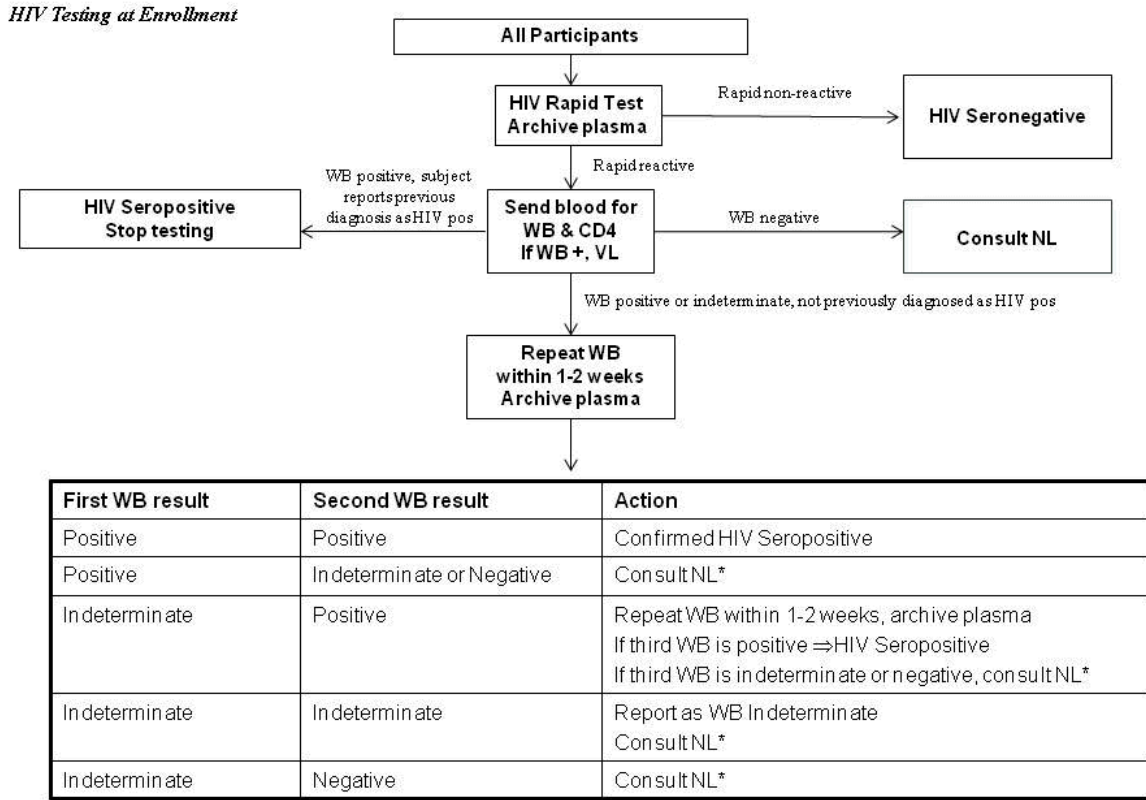
11.4.6 Rectal Swab CT/NG NAAT

Rectal swabs will be collected and sent to the local LDMS laboratory for storage. Samples will be stored in an Ultra low freezer (-65 to -95°C). Do not store the swabs at -20 to -30°C. The local lab will ship the swabs to the NL for CT/NG NAAT testing.

11.4.7 Syphilis serology

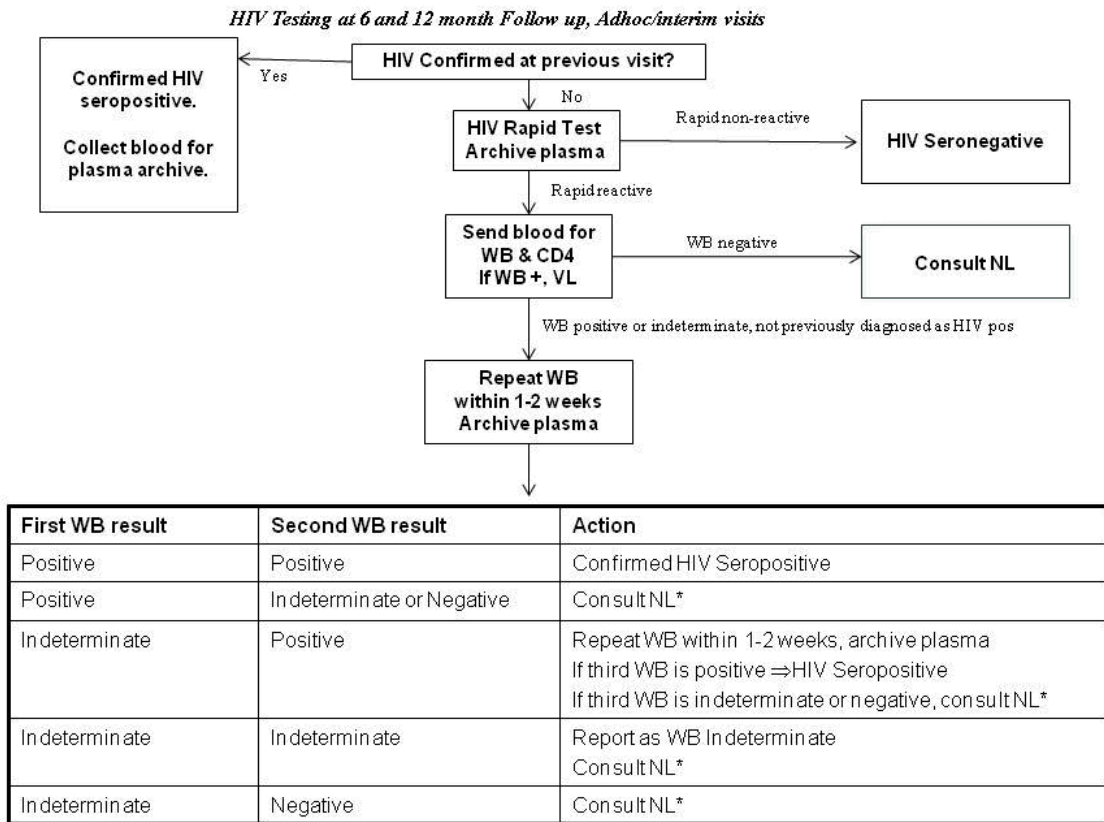
Blood will be collected for syphilis serology including titers at the site's local CLIA-certified laboratory.

Figure 11.2 HIV Testing at Enrollment



*Additional sample collection and testing may be needed.

Figure 11.3 HIV Testing at 26 and 52 weeks follow up



*Additional sample collection and testing may be needed.

11.5 HPTN NL samples: archived plasma and rectal swabs.

HIV Incidence testing

The HPTN NL will perform testing to optimize algorithms for identification of recent HIV infection. If an algorithm is successfully validated, the NL will use the algorithm to identify participants who tested HIV positive at study enrollment and were likely to have been recently infected. The algorithm is likely to include BED testing, CD4 cell count results, and other assays, to be determined by the NL. Some specialized assays may be performed at a commercial laboratory or at the CDC. Testing for HIV incidence will be batched to reduce cost and maximize consistency of test procedures. Assays used by the NL determining HIV incidence (including some HIV-1 RNA assays) are not currently FDA-cleared for diagnosis of HIV infection. All testing will be performed retrospectively. Results from these tests will not be provided to study sites, clinicians, or study subjects and will not be used for clinical management. However, as noted, if a participant has a positive HIV NAAT result at 52 weeks the laboratory will seek to have a stored sample from the participant retested a CLIA-certified lab using an FDA-cleared HIV RNA assay. If such testing also suggests acute infection at the time of sample collection, attempts will be made to contact the participant and direct him to local resources for a diagnostic HIV test and appropriate counseling.

All subjects with a reactive HIV rapid test or previous positive WB

Plasma for HIV incidence testing:

An initial aliquot will be tested with screening assays, such as the BED and avidity assays, to identify samples that are potentially incident. Additional aliquots are needed for further testing, to increase the precision of the HIV incidence estimate. This testing may include viral load, antiretroviral drug screens, further serological characterization, analysis of HIV diversity, etc. Other tests will be performed to characterize the viruses in the samples (e.g. HIV subtype).

All subjects with a negative HIV rapid test

Plasma for pooled HIV RNA testing and/or HIV antigen testing (52 week visit only, pooling performed at NL):

From selected study visits (e.g. last study visit sample, or sample preceding a positive HIV test), an aliquot will be tested with assays to screen for acute HIV infection (i.e. assays for detecting HIV RNA or HIV antigen). Additional aliquots from selected study subjects (e.g. those found to have possible acute HIV infection) are needed for further testing. This testing may include further serological characterization (e.g. testing with more sensitive screening assays) and other assays for optimization of incidence measures (e.g. evaluation of different pooling strategies), as well as other tests to characterize the viruses in the pre-seroconversion samples (e.g. HIV subtype, HIV sequence).

11.5.1 Plasma Archive

For all enrolled participants, plasma will be archived at enrollment and at the 26 week and 52 week visits for these who receive follow-up. Plasma will be archived for any participant who comes for a post test visit for HIV confirmation. For participants who suspect they may have become HIV-infected during the course of the study and attend an unscheduled (ad hoc) visit, plasma will also be archived.

Study staff will store plasma collected in this study until all protocol-related testing is complete. Note that some testing will be performed retrospectively after the last participant completes the final study visit. Protocol related testing at the NL using plasma collected at the study sites will include:

- Quality Assurance (QA) HIV testing at the HPTN NL
- Determination of HIV incidence including comparison of BED results to those obtained by other assays.
- Characterization of HIV viruses (e.g. HIV genotyping, HIV subtyping, HIV sequencing, and phylogenetics, HIV tropism) and other immunologic studies.
- Possible future research testing, if the participant provides written informed consent for such testing

Plasma of study participants consenting to HIV testing will be stored for the duration of their study participation and until all protocol-specified HIV 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. Therefore, after all protocol-specified testing has been completed, the stored plasma of participants who do not consent to indefinite storage and possible future research testing must be destroyed. After the study is completed, the SDMC will provide each site with a list of participants who did not consent to indefinite storage and possible future research testing, and the HPTN NL will provide detailed instructions for specimen destruction and documentation for those participants.

Protocol Section 11.5 described the HIV testing that the HPTN NL will perform on archived plasma for QC and QA purposes. Each site will ship plasma samples to the NL on a routine basis throughout the study; the SDMC will provide a list of samples (by PTID and specimen collection date) to be included in each shipment.

11.5.3 Plasma Processing

- A minimum of 20 mL of EDTA whole blood should be reserved for plasma archive at each visit.

- 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; The HPTN NL recommends the Sarstedt cryovial (Cat # 72.694.006) but other 2.0 mL cryovials can be used. The HPTN NL recommends that the plasma be double spun (PL2).
- Blood processing and plasma archive should be performed within 6 hours of sample collection. Note any time related processing issues in the LDMS (specimen management, Details section).
- Document the volume of whole blood received by the lab in the LDMS.
- Centrifuge tube at 800 x g for 10 minutes to separate cells and plasma.
- Carefully remove plasma and avoid disturbing the cell layer. Transfer the plasma to another sterile centrifuge tube.
- Centrifuge plasma again at 800 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 as much of the plasma as possible in 1.8 mL aliquots and store the aliquots in the freezer locations assigned in LDMS in an Ultra- low freezer (-65C to -95°C).
- Store plasma in aliquot number order. For example, if there is insufficient plasma for archive, store 1.8 mL in aliquots 1, 2 and 3. Store remainder in aliquot 4 and adjust the aliquot volume in LDMS to indicate volume stored. Aliquot 5 can be discarded and should be deleted from LDMS.
- All aliquots will be stored on site. Selected samples will be shipped to the HPTN NL on request.
- This plasma archive is for protocol-related testing only. Plasma should be stored for every participant at every visit, regardless of whether they have agreed to indefinite storage.

11.5.4 Plasma shipment

Upon receipt of each listing from the SDMC:

- Contact the HPTN NL at Johns Hopkins University (Estelle Piwowar-Manning: epiwowa@jhmi.edu, and Vanessa Cummings: vcummin1@jhmi.edu to coordinate the timing and logistics of the shipment. Sites may ship to the HPTN NL via Federal Express Monday through Thursday, with 24-hour fax notification.
- Working from the SDMC list of specimens to be shipped, use LDMS to generate a shipping manifest, box map, and LDMS shipping diskette for the selected samples.
- Obtain the selected specimens (one or more aliquots for each PTID and date as instructed) from the freezer and confirm the PTID, global ID, and date on the cryovial labels.
- Place the aliquots in a 5x5 or 9x9 cryovial box in the order of the shipping manifest.

- Personnel involved in the shipping process must be trained and certified for the shipping of Category B Biological specimens UN 3373 (Diagnostic) Packing Instructions 650.
- When shipping on carbon dioxide (dry ice), wrap the cryovial box in absorbent material and place it inside a shipping bag. Seal the bag, place it into a shipping envelope (STP 710) and then place it in a shipping box. Fill the box with sufficient dry ice to last at least 48 hours.
- Include a copy of the shipping manifest, box map, and LDMS diskette in the shipment. Ensure boxes are correctly labeled, for dry ice shipments with the samples, use diagnostics packing code 650, affix labels UN 3373 Biologic substance category B and for dry ice, UN 1845 to the outside of the shipping box. Address the shipment to:

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

- Notify the HPTN NL via email (epiwowa@jhmi.edu, and vcummin1@jhmi.edu) when the shipment has been picked up from the site by the courier/shipping company. Attach an electronic copy of the shipping manifest and LDMS batch to the email notification, and include the following information in the notification: name of courier/shipping company, shipment tracking number, number of boxes shipped, date of shipment, and expected date of arrival.

11.5.5 Rectal Swabs

Rectal swabs will be collected and shipped for testing at the HPTN NL for CT/NG NAAT from all study participants consenting to STI testing at enrollment and 52 weeks. The HPTN NL has a validated and approved methodology for the rectal swab testing. The rectal swabs are frozen at Ultra low temperature (-65C to -95°C) prior to shipment to the NL. Results will be returned to the sites within 2 weeks of receipt.

11.5.6 Rectal Swab shipping.

Approximately every 2 weeks, the local LDMS lab will ship the rectal swabs to the NL following the shipping instructions detailed in the plasma shipment Section 11.5.4. The local LDMS lab will prepare a shipping manifest and batch to lab 416, HPTN STD NL. The 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

11.6 LDMS reconciliation

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

11.7 Laboratory Monitoring

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

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

Appendix A. Specimen Collection and Storage by Visit in HPTN 061

Typical Specimen Collection for Person Consenting to HIV and STI Testing. Blood Draw Volumes Will Be Determined by Site Requirements, Visit Number and Participant History, But Should Not Exceed 40mL, and Should Allow for a Minimum of 8-10mL Stored Plasma for Shipping to Network Lab.

Table abbreviations: mL = milliliter

Enrollment Visit:

Sample Type Collected	Volume	Used For			Processing/ Storage
		All pts	If Rapid HIV positive	If Rapid HIV negative	
EDTA	5mL	Rapid HIV Test			Clinic Site
SST/Red Top	5mL	Syphilis Test			Local Lab
EDTA	5mL		CD4+ Cell Count	Plasma	Local Lab
EDTA	5mL		HIV WB	Plasma	Local Lab
EDTA	4 x 5mL		Plasma Archive and HIV VL	Plasma	Local Lab
Total Plasma Volume (stored in 2mL aliquots. 10mL plasma minimum needed)			10mL	15mL	Plasma stored at local site until shipped to Network Lab
Total Blood Volume Drawn			40mL	40mL	
Rectal Swab	1 swab	CT/NG NAAT			Stored at local site until shipped to Network Lab
Urine		CT/NG NAAT			Local Lab

26 Week Visit

Sample Type Collected	Volume	Used For			If HIV positive at enrollment	Processing/Storage
		All pts	If Rapid HIV positive	If Rapid HIV negative		
EDTA	5mL	Rapid HIV Test			X	Clinic Site
SST/Red Top	5mL	Syphilis Test			5mL	Local Lab
EDTA	5mL		CD4+ Cell Count	Plasma	X	Local Lab
EDTA	5mL		HIV WB	Plasma	X	Local Lab
EDTA	4 x 5mL		Plasma Archive and HIV VL	Plasma	X	Local Lab
Total Plasma Volume (stored in 2mL aliquots. 10mL plasma minimum needed)			10mL	15mL	10mL	Plasma stored at local site until shipped to Network Lab
Total Blood Volume Drawn			40mL	40mL	25mL	
Urine		CT/NG NAAT				Local Lab

52 Week Visit

Sample Type Collected	Volume	Used For			If HIV positive at a prior visit	Processing/Storage
		All pts	If Rapid HIV positive	If Rapid HIV negative		
EDTA	5mL	Rapid HIV Test			X	Clinic Site
SST/Red Top	5mL	Syphilis Test			5mL	Local Lab
EDTA	5mL		CD4+ Cell Count	Plasma	5mL	Local Lab
EDTA	5mL		HIV WB	Plasma	X	Local Lab
EDTA	4 x 5mL		Plasma Archive and HIV VL	Plasma	Plasma Archive and HIV VL	Local Lab
Total Plasma Volume (stored in 2mL aliquots. 10mL plasma minimum needed)			10mL	15mL	10mL	Plasma stored at local site until shipped to Network Lab
Total Blood Volume Drawn			40mL	40mL	30mL	
Rectal Swab	1 swab	CT/NG NAAT				Stored at local site until shipped to Network Lab
Urine		CT/NG NAAT				Local Lab

APPENDIX B FREQUENTLY ASKED QUESTIONS (FAQ)

HIV Algorithm

What do we do if a participant has two indeterminate Western blot results?

Contact the NL. The NL will request an aliquot from the visit at which the second indeterminate WB results was obtained, and will perform the GenAptima HIV RNA test to help establish the HIV infection status of the study subject.

What happens if the participant refuses the second blood draw for confirmatory testing?

Contact the NL. The NL will convene the end-point committee. The NL may request a sample from the first draw for additional confirmatory testing.

What if a participant has a reactive HIV rapid test result, with a negative WB?

Consult the NL. NL will recommend a repeat blood draw in two weeks. If the new sample from the participant has a non-reactive HIV rapid test result, the participant will be considered to be HIV-uninfected. If the new sample from the participant has a reactive HIV rapid test result, the NL will recommend that the site perform a WB. If the WB is positive, another sample will be needed to confirm HIV serostatus. If the WB is negative or indeterminate, the NL may request an aliquot for GenAptima HIV RNA testing.

Can we send an aliquot for the viral load assay at the same time that we send the sample for WB testing (rather than waiting for the WB result)?

The NL requires two separate blood draws (two separate days) before considering any participant to be HIV-infected. One reason for this requirement is to rule-out the possibility of a sample mix-up. If the site wishes to send the HIV RNA test at the time that the first WB is performed, that is acceptable, but a second sample will still be required to confirm HIV infection (i.e. to rule out a sample mix-up).

If a participant is previously known HIV positive, do we need to have 2 Western blots done?

If participant is known to be HIV positive, after obtaining a reactive HIV rapid result and the first positive WB result, you can stop testing (this is the only time NL will accept one positive WB to confirm HIV positive serostatus).

PROCESSING/LDMS

What do we do with a clotted sample?

Please check with your clinic. The clinic should consult their testing laboratory to see if the CD4 sample is also clotted. If all tubes are clotted, continue to process and store the samples according to the SSP. Indicate in the Condition drop down box of the LDMS that the specimen was clotted (CLT). Let the clinic know about the issue, so that they can provide retraining to the phlebotomist, if deemed necessary. If the participant is still in the clinic and a redraw is possible, draw a new sample, and indicate that the new sample is a redraw in the comment field. If only one tube is clotted, we suggest that you process the samples separately and do not pool the plasma. Keep the plasma from each tube separate and aliquot them separately. Indicate in the condition drop down box which sample was clotted so that this follows into the aliquots.

We pre-label our cryovials before aliquoting. We did not have enough plasma for the 5th aliquot tube and we only had 1 ml for the 4th aliquot tube. What do we do?

Delete the 5th aliquot from the LDMS, ensuring that you have checked the correct global ID. Adjust the volume for the 4th aliquot tube to 1 ml. If it is your practice, and in your SOP to relabel the tubes, relabel the sample. Otherwise, just comment in the LDMS that you have adjusted the volume. This comment will travel with the aliquot via the shipping manifest.

How do we enter interim visits into the LDMS? Will there ever be more than 9 interim visits.

Interim visits use the number after the decimal point to keep track of the visit. For example if someone comes in after their enrollment visit as an interim visit, you would use 1.1 as the visit code. The next time they came in for an interim visit, it would be 1.2. If someone is coming in for multiple interim visits, FHI, SCHARP, NL and the protocol chairs should be contacted.

How do we store the rectal swabs?

The rectal swabs should be collected in the clinic and placed in a cryovial. The clinician or nurse should break or cut the shaft so that it fits properly in the cryovial. They should seal the cryovial with the cap. The cryovials should be labeled with the PTID label, specimen date, and visit code. The samples should be sent to the laboratory and frozen within 24 hours. Do not store at -20°C. Store at -65°C to -95°C. Ship on dry ice to the NL for testing. Place the LDMS label over the PTID label, ensuring that the white part of the LDMS label covers the PTID label.

Do we need to accurately measure the volume of plasma in the cryovials?

The NL suggests that the plasma be pipetted into the cryovial to give an accurate measure. The NL realizes that this may not always be feasible. A disposable transfer pipette may be used to add the plasma to the cryovial. Deliver approximately 1.8 ml of plasma into the cryovial, but do not go above the 1.8 ml mark on the cryovial. You should have a procedure in place to estimate your volumes that the NL will review.

We received a sample that is greater than 6 hrs old. What do we do?

Please look into the cause of the delay. If this is a persistent problem, please notify the NL (Paul's direct number is 443-631-4671), so that we can work with you to find an appropriate solution. Process the sample and enter the collection time, received time, and processing time in the LDMS so that the NL will be able to see this information. If the sample is greater than 24 hours old, process the sample and indicate that the sample is >24 hours old in the Comment field of the Specimen Management Screen of the LDMS. Contact the clinic and request a redraw.

APPENDIX C Requisition for Rectal Swabs:

Chlamydia Laboratory
 Johns Hopkins University
 Division of Infectious Diseases
 855 N. Wolfe Street
 520-530 Rangos Bldg.
 Baltimore, MD 21205-2196
 410-614-0931

Clinic Site

Clinic Name Address Phone/FAX

Collection Date

M	M	D	D	Y	Y

Client Name:	Participant Study ID:
Address:	
	Laboratory ID:
Phone:	

Demographics

Date of Birth M M D D Y Y <input type="text"/>	Age Years <input type="text"/>	Gender M F <input type="text"/>	Pregnant Yes No <input type="text"/>	Race B W O <input type="text"/>	Hispanic Yes No <input type="text"/>
Patient Zip Code <input type="text"/>	Study Consent Yes No <input type="text"/>	Visit Code <input type="text"/>			

Laboratory

Date Received: M M D D Y Y <input type="text"/>	Date Tested: M M D D Y Y <input type="text"/>
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Condition if unable to process

<input type="checkbox"/>	Broken/leaked in transit
<input type="checkbox"/>	Inadequate ID/Ship Detail
<input type="checkbox"/>	Inappropriate Specimen
<input type="checkbox"/>	Inadequate Specimen
<input type="checkbox"/>	Other

NAAT Assay Test Results

Chlamydia:	<input type="checkbox"/> Positive	<input type="checkbox"/> Negative
Gonorrhea:	<input type="checkbox"/> Positive	<input type="checkbox"/> Negative

Comments _____

Technician _____ Date _____

Reviewer: _____ Date _____