

Section 12. Laboratory Considerations

12.1 Overview and General Guidance

This section contains information on the laboratory procedures performed in HPTN 059.

Some laboratory procedures will be performed in study site clinics, others will be performed in study site local laboratories, and others in HPTN Central Laboratories (CL). Table 12-1 lists the testing location for each test. Table 12-1 also lists specimen and kit requirements for each test. Protocol Sections 5.2-5.6, Appendix II, and Appendix III specify the time points at which each test is to be performed.

In all settings, laboratory procedures will be performed according to study site standard operating procedures (SOPs) that have been approved by the HPTN CL. In addition, package insert instructions must be followed for the following test kits:

- Becton Dickinson ProbeTec ET *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Strand Displacement Assays
- Focus Technologies HerpesSelect-2 ELISA
- Quidel Quick Vue OneStep hCG Pregnancy Test
- Bayer Multistix 9 Urine Dipsticks
- Genetic Systems HIV-1 Western Blot Test (manufactured by Bio-Rad Laboratories).

A package insert is not available for the S/P pH Indicator Strips, however a material safety data sheet is available. Copies of all applicable package inserts and material safety data sheets should be accessible for reference in on site testing locations. Please contact the HPTN CL with any questions about these documents.

Regardless of whether tests are performed in clinic or laboratory settings, study staff that performs 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 quality control (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 CL.

See Table 12.1 for a summary of tests, testing locations and methods.

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 CL for further guidance on validation requirements.

**Table 12-1
Overview of Laboratory Testing Locations, Specimens, and Methods for HPTN 059**

Test	Testing Location	Specimen Type	Tube/Container	Kit	Kit Supplier
Pregnancy Test	In Clinic	Urine	Plastic screw top cup	Quidel Quick Vue	US based or Local
Dipstick Urinalysis	In Clinic	Urine	Plastic screw top cup	Bayer Multistix 9	US based or Local
Urine Microscopy	In Clinic	Urine	Slide	NA	NA
Urine Culture	Local Lab	Urine	Plastic screw top cup	NA	NA
SDA for Chlamydia and Gonorrhea	Local Lab/Central Lab	Urine	Plastic screw top cup	BD Probe Tec ET	Becton Dickenson
HIV Antibody Tests	Local Lab	Anticoagulated Blood / Serum	Lavender Top Tube / Red Top Tube	FDA approved HIV EIA kit	US based or Local
	Local Lab	Anticoagulated Blood / Serum	Lavender Top Tube / Red Top Tube	Genetic Systems WB	US based or Local
Syphilis Serology	Local Lab	Serum	Red Top Tube	RPR (any)	Local
	Local Lab	Serum	Red Top Tube	MHA-TP or TPHA (any)	Local
HSV-2 Serology	Local Lab/Central Lab	Plasma	Lavender Top Tube ⇒ cryovial	Focus HSV-2 ELISA	Local
Plasma Archive	Local Lab	Plasma	Lavender Top Tube ⇒ cryovial	NA	NA
Serum Archive	Local Lab	Serum	Red Top Tube	NA	NA
Hematology Tests	Local Lab	Anticoagulated Blood	Lavender Top Tube	NA	NA
Liver Function Tests	Local Lab	Serum	Red Top Tube	NA	NA
Renal Function Tests	Local Lab	Serum	Red Top Tube	NA	NA
Hepatitis B Viral Loads	Central Lab	Plasma	Lavender Top Tube ⇒ cryovial	NA	NA
Hepatitis B Surface Antigen (HBsAg)	Local Lab	Serum	Red Top Tube	Not specified	US based or Local
Vaginal pH	In Clinic	NA	NA	S/P pH Indicator Strips	HPTN CL
Wet mount for BV, Candidiasis, and Trichomoniasis	In Clinic / or Local Lab	Vaginal fluid swab	Slides	NA	Local
Pap Smear	Local Lab	Ecto- and Endocervical cells	Slides	NA	NA

Table 12-1 continued
Overview of Laboratory Testing Locations, Specimens, and Methods for HPTN 059

Cytokine / Chemokine Testing	HPTN CL (Rabe)	Cervical swab /	2 Swabs ⇒ cryovials w/ PBS	NA	NA
Cervical Gram Stain	HPTN CL (Rabe)	Cervical fluid swab	Slides	NA	NA
Vaginal Culture – Quantitative**	HPTN CL (Rabe)	Vaginal swab	Port-a-Cul transport tubes by BD	NA	HPTN CL
Vaginal Gram Stain	HPTN CL (Rabe)	Vaginal fluid swab	Slides	NA	NA
Multiplex PCR for Chancroid, HSV-2, and Syphilis	HPTN CL (Gaydos)	Genital ulcer swab	Plastic shaft Dacron swab ⇒ cryovial	NA	NA
PK Testing	HPTN CL (JHU)	Plasma	Lavender Top Tube *spray dried EDTA	NA	NA
HIV Status Verification	HPTN CL (JHU)	Plasma	Cryovial	FDA-approved HIV EIA Genetic Systems WB	NA

** *US sites only*

As transmission of HIV and other infectious agents can occur through contact with contaminated needles, blood, blood products, and vaginal secretions, all study staff must take appropriate precautions when collecting and handling biological specimens. Guidance on universal precautions available from the US Centers for Disease Control and Prevention and the World Health Organization can be found in Appendices 12-1 and 12-2 of this SSP Section and at the following websites:

- http://www.cdc.gov/ncidod/dhqp/bp_universal_precautions.html
- <http://www.who.int/hiv/topics/precautions/universal/en/>

Additional laboratory reference information can be found in Section 13 of the HPTN Manual of Operations and the HPTN Laboratory Standard Operating Procedures Manual.

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

12.2 Specimen Labeling

All containers into which specimens are initially collected (e.g., urine collection cups, blood collection tubes) will be labeled with SCHARP-provided Participant ID (PTID) labels. PTIDs are pre-printed on these labels; however, study staff must handwrite the visit code and the date the specimens are collected on the label in the spaces provided. Study staff should use indelible ink (such as a Sharpie pen).

Microscope slides used for evaluation of vaginal/cervical fluids also will be labeled with SCHARP provided PTID labels. Only the smaller size SCHARP labels should be used for slides. PTIDs are pre-printed on these labels; however study staff must handwrite the specimen collection date and visit code on

each label in indelible ink (such as a Sharpie pen). 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.

The following specimens will be entered into LDMS and labeled with LDMS-generated labels: stored plasma specimens, stored serum specimens, genital ulcer swabs collected for multiplex PCR testing, cervical swabs for cytokines/chemokines, vaginal/cervical fluid slides prepared for Gram stain evaluation, and vaginal cultures (US sites only). These specimens will be shipped to the HPTN CL for testing.

12.3 Use of LDMS

LDMS must be used at all sites to track the collection, storage, and shipment of seven types of specimens in HPTN 059: plasma, serum, cervical swabs for cytokines/chemokines, vaginal cultures (US sites only), vaginal and cervical fluids (air-dried on microscope slides for Gram stain evaluation), and genital ulcer swabs. A Sample Laboratory Specimen Processing form can be referenced in Appendix 12-3 of this SSP. Detailed instructions for use of LDMS are provided at:

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

As of the date of this section, the current version of LDMS is Version 5.4.1. All sites should upgrade to this version as soon as possible. Version 5.4.1 includes two types of label formats. Upon upgrading to Version 5.4.1, all sites must use the “LDMS1” label format in order to ensure that both the Specimen ID and the Global ID assigned to each specimen is printed on LDMS-generated labels.

Questions related to use of LDMS in HPTN 059 should be directed to Estelle Piwowar-Manning. LDMS Technical support also is available from LDMS User Support. Usual business hours for LDMS User Support are 7:30 am - 6:00 pm (ET) on Monday and Fridays and 7:30 am - 8:00 pm (ET) on Tuesdays, Wednesdays, and Thursdays. 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 also can be paged during off business hours for emergency LDMS problems. To leave a page, dial the number listed below as “LDMS 1.” Then, at the prompt, enter a phone number at which you can be reached. If you do not receive a response within 15 minutes after paging LDMS 1, please try LDMS 2, and then finally, LDMS 3.

LDMS 1: +716-556-0583
LDMS 2: +716-556-0584
LDMS 3: +716-556-0585

Sites should export their LDMS data weekly to FSTRF.

12.4 Procedures for Specimens that cannot be Evaluated

Specimens will be redrawn or recollected if it is found that they cannot be evaluated. Specimens that are redrawn or recollected due to reasons beyond the sites’ control (e.g. clotting) will not be considered protocol events. Specimens that are redrawn or recollected due to site error (lost or broken) will be considered protocol events.

12.5 Urine Testing for Pregnancy, Urinary Tract Infection, Chlamydia, and Gonorrhea

The urine tests performed at each study visit will depend on the timepoint of the visit and the clinical presentation of the participant. In general, at study visits when urine testing is required, a single specimen will be collected and then aliquotted for each test.

12.5.1 Specimen Collection

- The participant should not have urinated within one hour prior to urine collection.
- Provide the participant with a sterile, plastic, preservative-free screw-top urine collection cup labeled with a SCHARP-provided PTID label.
- Instruct the participant **not** to clean the labia prior to specimen collection.
- Show the participant the 20 mL mark on the specimen collection cup and instruct her to collect approximately 20 mL only from the first portion of her urine stream.
- Instruct the participant to screw the lid tightly onto the cup after collection.
- At visits when pregnancy testing and/or dipstick urinalysis is required, aliquot 5 mL for these tests and store the remaining urine at 2-8° C for subsequent chlamydia and gonorrhea testing.
- The New York site is using the Urine Transport Tube for the transport of the urine for GC/CT testing and should refer to section 12.5.5 for collection and shipping instructions.

12.5.2 Pregnancy Testing

Pregnancy testing is required, per protocol, at all study visits through Week 24/Early Termination; this includes both regularly scheduled visits and interim visits. Aliquot approximately 5 mL of urine from the specimen collection cup and pipette from this aliquot for pregnancy testing. If the collected urine is grossly bloody, centrifuge the urine aliquot and use the supernatant for testing. If the supernatant is too dark to read the pregnancy test, another urine sample will need to be collected.

Note: Protocol-specified pregnancy testing is not discontinued during pregnancy.

The Quidel QuickVue One-Step hCG urine pregnancy test must be used at all sites. This test was selected for use in HPTN 059 because of its ease of use and the validity of test results in the presence of the study gels. Perform the test according to site SOPs and the package insert. Do not perform any other urine pregnancy tests for confirmatory purposes.

Pregnancy status is a critical participant safety consideration in HPTN 059. All sites must maintain an adequate inventory of the QuickVue One-Step test kits at all times. Inventory should be monitored closely and re-supply orders placed at least 8-12 weeks in advance of actual need (or longer if needed per site procurement policies and procedures). The India site will be required to report their kit inventories, including kit lot numbers, to the HPTN CL on a monthly basis. Notify the CL immediately if any kit inventory or quality control problems are identified, so that appropriate action can be taken.

12.5.3 Dipstick Urinalysis

At visits when dipstick urinalysis is required to test for possible urinary tract infections, dip the urinalysis test strip into a 5 mL aliquot of urine. At visits when both pregnancy testing and dipstick urinalysis are

required, the same aliquot should be used for both tests, but the urinalysis should be performed after urine has been pipetted from the aliquot for the pregnancy test.

The Bayer Multistix 9 urine test strips must be used at all sites. Perform this test according to site SOPs and the package insert. For the required urinalysis at Screening, perform the test within the 56-day window for enrollment. Assess and record results for blood, glucose, protein, leukocytes and nitrites. If leukocytes or nitrites are positive, perform both a urine microscopy and a urine culture according to local SOP. To avoid overgrowth of bacteria, refrigerate specimen before and during transport to laboratory.

Inventory should be monitored closely and re-supply orders placed at least 8-12 weeks in advance of actual need (or longer if needed per site procurement policies and procedures). The India site will be required to report their kit inventories, including kit lot numbers, to the HPTN CL on a monthly basis. Notify the CL immediately if any kit inventory or quality control problems are identified, so that appropriate action can be taken.

12.5.4 Chlamydia and Gonorrhea Testing

At visits when chlamydia and gonorrhea testing are required, the 15 mL of urine remaining after pregnancy testing and/or dipstick urinalysis is used for this testing. The Becton Dickinson ProbeTec ET Strand Displacement Assay must be used at all sites. Store the urine at 2-8° C between the time of collection and the time of testing, unless the site is using the Urine Transport Tube (New York site) to ship the samples to Magee in Pittsburgh. For the New York site see instructions below. Perform the assays according to site SOPs and the package insert. For the required chlamydia and gonorrhea testing at Screening, perform the tests within the 56-day window for enrollment. Tests should be performed within four days of specimen collection if using the Urine Preservation Pouch (UPP) and within 7 days if using neat samples or 30 days if urine is preserved in the Urine Transport Tube. The UPP is being discontinued and all the sites are switching over to either the neat sample or Urine Transport Tube (UPT) preserved samples. Until the sites have validated the new method, the UPP will be used.

The Pune site will use the Gen-Probe Aptima as the backup instrument for testing CT/GC. The Gen-Probe will only be used for screening and enrollment samples. All samples tested with the Gen-Probe must be saved and re-tested with the ProbeTec. **Specimens from follow-up visits can only be tested on the ProbeTec.** If the ProbeTec is not working, the follow-up urine specimen will be processed through the lysing step and then frozen at -20°C until the ProbeTec instrument is working. For specimens that will be tested on both instruments urine samples will be processed in the following way:

- Before adding the UPP, transfer 2ml of urine into the Gen-Probe urine transport tube. The urine must be transferred within 24 hours of collection. Once in the transport tube the specimen can be stored at 2°C to 30°C for up to 30 days after collection.
- To the remaining urine add a ProbeTec UPP pouch. Follow the SOP for the ProbeTec through the lysing step. Freeze the sample at -20°C for up to 2 months. Complete the testing when the ProbeTec is working properly.
- Process the urine in the Gen-Probe transport tube according to the SOP for the Gen-Probe test.

12.5.5 Shipping instructions for urine samples to Magee-Womens Research Institute (US site only) Urine collection and preservation for GC/CT testing

- The participant should not have urinated for at least 1 hour prior to specimen collection.
- Collect the first 15-60 mL of voided urine in a sterile collection cup. (Not mid-stream)

Transfer urine to the Urine Preservation Tube (UPT)

- Open the UPT kit and remove the UPT and transfer pipette. Label the UPT with the participants PTID number and date.
- Hold the UPT upright and firmly tap the bottom of the tube on a flat surface to dislodge any large drops from inside the cap.
- Uncap the UPT and use the transfer pipette to transfer enough urine to fill the tube to the level indicated on the tube between the black lines. Do not under fill or overfill the tube.
- Cap tightly and invert the tube 3-4 times to ensure that the specimen and reagent are mixed.
- The specimen is stable for 30 days at 2-30°C.

Shipping instructions for urine samples

- Urine specimens can be batched and sent 1 or 2 times a week depending on your expected turn-around time
- Urine specimens must be received and tested within 30 days of collection.
- Fill out a shipping manifest with the following information (Do not use LDMS for urine specimens. See Appendix 12-5 for example of shipping manifest to be used).
 - Study site, contact name, phone and fax number, and e-mail address
 - List of specimens in shipment (include the HPTN PTID#, collection date, visit code, and volume of urine)
- Package the specimens according to the IATA packing instructions 650 for non-refrigerated specimens.
- Place a copy of the shipping manifest in the outer box.
- Confirm the address is correct (see below). Because the Research Institute is not open for delivery on the weekend the specimens taken on Friday must be sent to the hospital address in order for us to get the package on Saturday.
- The day of shipment, send Lorna Rabe an e-mail at rsilkr@mwri.magee.edu with the FedEx tracking number.

If sending Monday through Thursday, send to:

Lorna Rabe
Magee-Womens Research Institute
204 Craft Ave, Room 530
Pittsburgh, PA 15213
Phone # 412-641-6042

If sending on Friday for Saturday delivery, send to:

Lorna Rabe, % Safety and Security
Magee-Womens Hospital
300 Halket St.
Pittsburgh, PA 15213
Phone # 412-614-4191 (this is the Safety and Security #)
****Be sure to check Saturday delivery on the FedEx label****

12.6 Blood Testing for HIV, Syphilis, Hematology, Liver and Renal Function, and Plasma/Serum Archive

The blood tests performed at each study visit vary depending on the timepoint of the visit and the clinical presentation of the participant. At most visits in which blood testing is required, the amount of blood will be collected according to Site Specific SOPs, however additional blood may be collected if clinically indicated.

12.6.1 Specimen Collection and Initial Processing

After specimen collection, label all required tubes with a SCHARP-provided PTID label.

After collection:

- Allow red top tubes (no additive) to clot, then centrifuge per site SOPs to yield serum for syphilis, serum archive, liver function and renal function testing, Hepatitis B Surface Antigen testing, (and HIV testing, if needed).
- Lavender top tubes (additive = EDTA) for CBC's require no additional processing prior to testing, but should be gently inverted at least eight times after specimen collection to prevent clotting.

Note: If locally available tube top colors do not correspond with the tube additives specified above, use appropriate tubes based on the additives, not the tube top colors.

12.6.2 HIV Testing

Plasma or serum 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 worksheets or other laboratory source documents.

Perform all tests according to site SOPs and package inserts. Screening HIV testing must be conducted within the 56-day window for enrollment.

At all sites, when Western blot (WB) testing is required, the FDA-approved Genetic Systems WB, manufactured by Bio-Rad Laboratories, must be used. Perform this test according to site SOPs and the package insert.

Use of the HIV test kits listed above has been negotiated with the FDA, and no other tests may be used without approval from the HPTN CL. The India site must maintain an adequate inventory of the HIV test kits they have selected and validated for use in HPTN 059. Kit inventories should be monitored closely and re-supply orders placed at least 8-12 weeks in advance of actual need (or longer if needed per site procurement policies and procedures). The India site will be required to report their kit inventories, including kit lot numbers, to the HPTN CL on a monthly basis. Notify the CL immediately if any kit inventory or quality control problems are identified, so that appropriate action can be taken.

12.6.2.1 HIV Testing at Screening

At all sites, HIV infection status at screening will be assessed using an FDA-approved enzyme immunoassay (EIA). If the EIA is non-reactive, the participant will be considered HIV-uninfected. If the EIA is reactive, the FDA-approved Genetic Systems WB will be performed. If the WB is negative, the participant will be considered HIV-uninfected. If the WB is positive, the participant will be considered HIV-infected. A second specimen will be drawn for confirmatory testing. If the WB is indeterminate, the participant will be asked to present to the study site in approximately one month for re-testing. At that time, the EIA will be repeated and the above-described algorithm will be followed. A WB will only be performed if the EIA is reactive.

At all sites, all test results must be documented on local laboratory worksheets or other laboratory source documents

12.6.2.2 HIV Testing During Enrollment, Week 24 Visit/Early Termination, and When Clinically Indicated

At all sites, HIV testing will be performed according to the algorithm in protocol and in this SSP section (see algorithm at the end of section 12).

If the WB in Step Two is negative, testing will stop after Step Two. If the WB is positive or indeterminate, a second Genetic Systems WB must be performed on a second sample collected from the participant. This sample is referred to as “sample 2” in the algorithm and will be used for plasma archive if HIV infection is confirmed. For purposes of estimating the effectiveness of the gels tested in HPTN 059, only participants for whom infection is confirmed with two positive WB results on two different samples will be counted as having become HIV-infected.

If the sample 2 WB is negative or indeterminate, additional WB testing must be performed on additional samples. In this case, inform the HPTN CL via email of the sample 1 and sample 2 test results (copied to the HPTN CORE and SDMC) and request CL input on next steps and timeframes for additional specimen collection and testing.

All test results must be documented on local laboratory worksheets or other laboratory source documents. In addition to initialing or signing the testing logs to document review and verification of the results, the second staff member must also record the time at which the results were reviewed and verified.

12.6.3 Syphilis Testing

Syphilis testing will be performed using a rapid plasma reagin (RPR) screening test followed by a confirmatory microhemagglutinin assay for treponema pallidum (MHA-TP) or treponema pallidum haemagglutination assay (TPHA). Any RPR, MHA-TP, and/TPHA test may be used at each study site; however titers must be obtained and reported for all positive RPR tests. RPR tests may be performed on either serum or plasma. MHA-TP and TPHA tests must be performed on serum. All testing and QC procedures must be performed and documented in accordance with study site SOPs.

For reactive RPR tests observed during screening, a confirmatory test result must be received and appropriate clinical management action taken, prior to enrollment in the study. See Section 10.7 of this manual for more information on screening and enrollment considerations and clinical management of syphilis infections. Clinical management should include repeat RPR tests at quarterly intervals following syphilis diagnosis to confirm treatment effectiveness. If the RPR titer does not decrease fourfold or revert to sero-negative within three months after treatment, treatment should be repeated.

Please consult the HPTN Central Laboratory with any questions related to quarterly testing to confirm treatment effectiveness and/or interpretation of unusual test results.

Questions related to result interpretation vis-à-vis eligibility and enrollment in the study should be directed to the HPTN 059 Protocol Safety Review Team as described in Section 10.6.2 of this manual.

12.6.4 Hematology Testing

Complete blood counts with five-part differentials will be performed at all sites. Each of the following must be analyzed and reported:

- Hemoglobin
- Mean corpuscular volume
- Platelets

- White blood cells
- Absolute neutrophil count or percent neutrophils
- Absolute lymphocyte count or percent lymphocytes
- Absolute eosinophil count or percent eosinophils
- Absolute band count or percent bands (UAB site only)

Hematology testing must be performed on anticoagulated (EDTA) blood. All testing and QC procedures must be performed and documented in accordance with study site SOPs.

12.6.5 Liver and Renal Function Testing

The following tests will be performed to evaluate liver and renal function:

Liver Function

- Alkaline phosphatase (Alk Phos)
- Aspartate aminotransferase (AST)
- Alanine transaminase (ALT)
- Gammaglutamyl transaminase (GGT)
- Total bilirubin

Renal Function

- Creatinine
- Blood urea nitrogen (BUN)

These tests must be performed on serum. All testing and QC procedures must be performed and documented in accordance with study site SOPs.

12.6.6 Herpes Simplex Virus 2 Testing

Herpes Simplex Virus 2 (HSV-2) testing will be performed at the conclusion of the protocol at the Local Lab (LL) or at the HPTN CL Magee-Pittsburgh. Samples are collected at screening, enrollment, Week 24/Early Termination and when clinically indicated, during the study implementation, and thereafter, using the Focus Technologies HerpesSelect-2 ELISA. Testing will be performed and documented according to site SOPs and the package insert, with the exception that testing will be performed on archived plasma specimens, rather than sera. Use of plasma for this purpose has been validated by the HPTN CL (Cherpes et al, J Clin Microbiol 2003; 41:2758). Optical density ratios less than 1.1 will be considered negative; ratios of 1.1 and higher will be considered positive.

12.6.7 Plasma Archive

For all participants, plasma will be archived at screening, enrollment and at Week 24/Early Termination. For participants who become HIV-infected during follow-up (testing done only as clinically indicated), plasma also will be archived when blood is collected for confirmatory HIV testing (i.e., when “sample 2” in Figure 12-4 is collected), as follows:

- At screening, enrollment and Week 24/Early Termination, collect blood into a lavender top tube (EDTA) labeled with a SCHARP-provided PTID label. The visit code and date the specimens are collected should also be included on the label. If the date is handwritten, it should be in indelible ink (such as a Sharpie pen). At Week 24/Early Termination and time of seroconversion (if applicable), retain all anticoagulated blood from the lavender top (EDTA) tube after protocol-specified testing has been performed.

- Deliver the anticoagulated blood and LDMS Specimen Tracking Sheet to the local LDMS laboratory.
- Using the LDMS Specimen Tracking Sheet, log samples into LDMS and generate LDMS labels. (BLD/EDT/PL1)
- Within 24 hours of collection, process the blood for plasma according to site SOPs. At enrollment, prepare at least four 1 mL plasma aliquots in cryovials labeled with LDMS-generated labels. At study Week 24/Early Termination and time of seroconversion (if applicable), prepare at least four 0.5 mL plasma aliquots in cryovials labeled with LDMS generated labels.
- Store the aliquots in the freezer locations assigned in LDMS at -70° C.

Archived plasma will be used for the following purposes:

- HIV testing at the HPTN CL
- HSV-2 testing at HPTN CL (Magee) US sites only
- Other Safety Reasons

All enrolled study participants with chronic hepatitis B virus (CHBV) must provide consent for collection and storage of their plasma 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 CL will provide detailed instructions for specimen destruction and documentation thereof.

Protocol Section 9.3 describes the HIV testing that the HPTN CL will perform on archived plasma for quality control and quality assurance purposes. Each site will ship plasma samples to the CL on a routine basis throughout the study, and the SDMC will provide a listing of samples (by PTID and specimen collection date) to be included in each shipment.

Upon receipt of each listing from the SDMC:

- Contact the HPTN CL at Johns Hopkins University (Estelle Piwowar-Manning: epiwowa@jhmi.edu +410-614-6736) to coordinate the timing and logistics of the shipment. The U.S. sites may ship to the HPTN CL via Federal Express Monday through Thursday, with 24-hour fax notification. For non-US sites, the HPTN CL will arrange for shipping with World Courier.
- Working from the SDMC list of specimens to be shipped, use LDMS to generate a shipping manifest, box map, and LDMS shipping diskette for the selected samples.
- Obtain the selected specimens (one aliquot for each PTID and date) from the freezer and confirm the PTID and date on the cryovial labels.
- Place the aliquots in a 5x5 or 9x9 cryovial box in the order of the shipping manifest.
- Wrap the cryovial box in absorbent material and place it inside a shipping bag. Seal the bag and then place it in a shipping box. Fill the box with sufficient carbon dioxide (dry ice) to last at least 48 hours.
- Include a copy of the shipping manifest, box map, and LDMS diskette in the shipment. Use diagnostics packing code 650, UN 3373, and address the shipment to:

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

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

12.6.8 Serum Archive

For all participants, serum will be archived at enrollment and week 24/Early Termination. Sites should prepare at least two 1.0 mL serum aliquots in cryovials labeled with LDMS generated labels. Store the aliquots in the freezer locations assigned in LDMS at -70 degrees C. For CHBV participants who provide optional informed consent for storage and possible HBV future resistance testing, serum also will be collected for the HBV serum archive at enrollment, weeks 12, weeks 24 or early termination, and weeks 28, 32, and 36 (See 12.7.2 for additional information on HBV serum archive).

Archived serum will be used for the following purposes:

- Other Safety Reasons

12.7 Hepatitis Testing

12.7.1 HbsAg Testing

All participants will be tested for HbsAg at screening, enrollment, and week 24 (or early termination). HbsAg testing will only be drawn at follow-up visits if clinically indicated. Those who test positive will be considered Hepatitis B infected (CHBV).

12.7.2 HBV Viral Loads and HBV Serum Archive

- Once a participant is considered Hep B infected, samples for HBV viral load testing and the HBV serum archive (if participant provides consent) will be drawn according to the following schedule: enrollment, weeks 12, 24/Early Termination, 28, 32, and 36. Plasma specimens must be processed within 4 hours of collection. Store the aliquots in the freezer locations assigned in LDMS at -70 degrees C. The minimum plasma volume of aliquot is 0.5 mLs. A minimum of two aliquots should be prepared, including one back up aliquot. Sites are recommended to prepare three aliquots. Plasma must be frozen at -70°C and transported on Dry Ice. **HBV viral load specimens will not be entered into the LDMS.** Specimens for HBV viral loads will be shipped in real time. Test results will be sent from the HPTN CL back to the sites who will record the results on the HBV Lab Results form and fax it to the SDMC. Since the HBV viral load specimens will not be entered into LDMS (and thus will not be labeled with an LDMS-generated cryovial label), it is especially important that these aliquots are appropriately labeled with the PTID, collection time and date, visit code, and the type of specimen (plasma).

- For the HBV serum archive, sites should prepare at least two 1.0 mL serum aliquots in cryovials labeled with LDMS generated labels. Specimens must be labeled with the PTID, collection time and date, and a special JHH billing account number must be included on the paperwork accompanying the specimens.
- Prepare the specimens for shipping according to IATA regulations. Fill the box with sufficient carbon dioxide (dry ice) to last at least 48 hours.
- Include a copy of the shipping manifest in the shipment. Use diagnostics packing code 650, UN 3373, and address the shipment to:

**Johns Hopkins Hospital
Molecular Micro Lab
Meyer Bldg B1-110
600 North Wolfe Street
Baltimore, Maryland 21287
USA
Phone number: 410-955-2642**

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

12.8 PK Testing

Pharmacokinetic testing samples will be drawn at weeks 4, 12, and 20 and will be shipped to the Central Lab for analysis at the end of the study.

- At weeks 4, 12, and 20 collect blood into a lavender top tube (EDTA) labeled with a SCHARP-provided PTID label.
- PK specimens must be drawn in tubes with sprayed on EDTA. These tubes should be spun down and plasma separated and frozen ideally within 1 hour. (2500 rpm for 10 min at 4°C). If not within 1 hour, keep track of the time between drawing and separating and freezing. Plasma samples should be frozen at -70°C.
- Record the time the blood was collected.
- Samples must be collected into tubes which have sprayed dried EDTA, liquid EDTA tubes are unacceptable. Dried heparin anticoagulant is also acceptable.
- Deliver the anticoagulated blood and LDMS Specimen Tracking Sheet to the local LDMS laboratory.
- Using the LDMS Specimen Tracking Sheet, log samples into LDMS and generate LDMS labels. BLD/EDT/PL1
- Prepare at least two 0.75 mL plasma aliquots in cryovials labeled with LDMS-generated labels. If there is sample remaining, a back up aliquot should be prepared.
- Store the aliquots in the freezer locations assigned in LDMS at -70° C.

12.9 Testing of Vaginal and Cervical Specimens

Refer to the Screening and Follow-up Pelvic Exam checklists in Section 7 of this manual for further information on the required sequence of specimen collection and diagnostic procedures to be performed during study pelvic exams.

12.9.1 Vaginal pH

Vaginal pH will be assessed as part of on-site evaluations for bacterial vaginosis. S/P pH Indicator Strips provided by HPTN CL must be used at all sites, as follows:

- During pelvic examination, touch a pH indicator strip to the vaginal wall just until the paper is moistened. Avoid contact with cervical mucus, which has a high pH. Alternatively, vaginal fluids may be collected via swab and then swabbed onto the pH strip (instead of inserting the pH strip into the vagina).
- Match the resulting color of the indicator strip to the color scale provided with the strips to determine the pH value.
- Record the pH value directly onto the appropriate case report form. If the case report form is listed as source documentation for vaginal pH in the site's local source documentation SOP, it is not necessary to record pH values onto laboratory log sheets or other source documents

12.9.2 Vaginal Fluid Wet Mount Testing

Wet mount procedures for this study consist of two different preparations — saline prep and potassium hydroxide (KOH) prep — for diagnosis of bacterial vaginosis, trichomoniasis, and candidiasis, as summarized in Table 12-2.

If wet prep slides are read in-clinic by clinical staff, results may be recorded directly onto appropriate case report forms. If slides are read by lab staff (either in the local laboratory or a designated in-clinic lab area), results must be recorded onto laboratory log sheets or other laboratory source documents and then transcribed onto the appropriate case report form.

Prior to study initiation, the HPTN 059 CL conducted on-site training and proficiency testing for clinic and laboratory staff designated to perform wet mounts. CLIA regulations require semi-annual proficiency testing; therefore site Laboratory Managers should administer additional on-site proficiency testing approximately every six months. The HPTN CL will post wet mount slides on the HPTN 059 web page for this purpose, and result reports should be submitted to the CL via fax (Lorna Rabe: rsilkr@mwri.magee.edu, fax +412-641-5290) after each proficiency testing cycle. The HPTN CL will report results back to the Laboratory Manager and also specify any corrective action that may be needed based on the results. Contact the HPTN CL for additional information and guidance on performing and documenting the proficiency testing. Also contact the HPTN CL when new laboratory staff is hired, so that appropriate training can take place prior to such staff performing wet mounts for study purposes.

Table 12-2
Summary of Wet Prep Assessments and Diagnostic Criteria

Assessment	Saline Prep	KOH Prep
Whiff test	Not applicable.	Positive if fishy amine odor detected.
Clue cells	Individual cells rather than clusters of cells should be examined. Positive if at least 20% clue cells observed. Cells must be completely covered with bacteria (<i>Gardnerella vaginalis</i> and anaerobic GNR) to be counted as clue cells.	Not applicable (clue cells are lysed by KOH).
Trichomonads	Positive if at least one motile trichomonad is observed. Actively motile organisms are easily seen upon low power (10X). High power (40X) may be needed to detect less vigorously motile organisms when only the flagella may be moving.	Not applicable (organisms are lysed by KOH).
Yeast	Positive if pseudohyphae and/or budding yeast are observed. Pseudohyphae and budding yeast may be obscured by epithelial cells. These cells will be lysed by KOH, thus pseudohyphae and budding yeast not observed in saline prep may be observed in KOH prep.	Positive if pseudohyphae or budding yeast are observed.

Note: Bacterial vaginosis will be diagnosed based on the presence of any three of the following Amsel's criteria: homogenous vaginal discharge, vaginal pH greater than 4.5, positive whiff test, at least 20% clue cells.

Prepare and examine wet prep slides according to study site SOPs as follows:

- Use a pencil to write the PTID and specimen collection date on one side of the frosted end of two microscope slides. Affix a SCHARP-provided PTID label to the other side of the slides (on the frosted end, under the pencil markings) and write the specimen collection date and visit code in indelible ink (e.g. Sharpie pen) on each label.
- Immediately following collection from the lateral vaginal wall via swab, smear vaginal fluid specimens onto each slide. Alternatively, the swab may be placed in a glass or plastic tube with approximately six drops (100 µL) sterile physiologic saline to allow for non-immediate slide preparation. In this case, vaginal fluid specimens should be smeared onto the two slides upon receipt from the collecting clinician.
- Apply one drop of 10% KOH to one slide and immediately perform whiff test for a “fishy” amine odor. Then apply cover slip.

- Apply one drop of sterile physiologic saline to the second slide, emulsify with the vaginal fluid specimen, and then apply coverslip. Examine immediately at 10X magnification for epithelial cells, motile trichomonads, budding yeast, and pseudohyphae. Examine at 40X magnification to determine whether observed epithelial cells are clue cells and quantitate the cells. Clue cells are irregularly bordered squamous epithelial cells that are completely covered with bacteria (*Gardnerella vaginalis*). Clue cells must comprise at least 20 percent of the observed epithelial cells in order for the saline prep to be considered positive for clue cells.
- Examine the KOH slide at both 10X and 40X magnification for yeast and pseudohyphae.

12.9.3 Vaginal Fluid Dried Smears for Gram Staining

In addition to the wet mounts described above, dried vaginal fluid smears will be prepared for Gram staining and assessment for bacterial vaginosis at the HPTN CL. Two slides will be prepared at each required timepoint and both will be entered into LDMS. One will be shipped to the CL and the other will be archived on site until written notification is received from the SDMC that the slide may be discarded. Instructions for slide preparation and shipping are provided below.

12.9.3.1 Slide Preparation and Storage

- Use a pencil to write the PTID and specimen collection date on one side of the frosted end of two microscope slides. Affix a SCHARP-provided PTID label to the other side of the slides (on the frosted end, under the pencil markings) and write the specimen collection date and visit code in indelible ink (e.g. Sharpie pen) on each label. Also write “V” for vaginal on each label.
- Immediately following specimen collection from the lateral vaginal wall via swab, roll the swab across each of the two slides. (Be sure to collect the specimen from opposite the vaginal wall used for the wet mount specimen collection.) Do not place the swab in saline, transport medium, or any transport container prior to slide preparation.
- Allow the specimens to air-dry on the slides. **Do not heat-fix.**
- Deliver both slides and an LDMS Specimen Tracking Sheet to the local LDMS laboratory.
- Using the LDMS Tracking Sheet, log the slides into LDMS (specimen type = VAG) and label the slides with LDMS labels. Place the LDMS label on the frosted end of the slide, on the opposite side of the slide from the SCHARP-provided label, on top of the pencil markings.
- Store the slides in the slide box locations assigned in LDMS at room temperature. A guide for configuring slide boxes in LDMS is available at the following web site:
http://www.hptn.org/research_studies/HPTN059Lab.htm
- Vaginal and cervical Gram Stain Slides may be combined into the same LDMS shipping manifest. Gram Stain Slides (both cervical and vaginal) CANNOT be combined into the same shipping manifest as the vaginal swab for quantitative culture.

For the US sites only: One slide will be sent with the vaginal culture on the day of collection. The screening slide will be sent with the enrollment slide and the culture. Place the slides in plastic slide holders and send to the Magee lab. See sections 12.9.3.2 and 12.9.3.3 for shipping instructions. The second slide will be kept at the site.

Note: The HPTN 059 protocol requires that dried smears be prepared for all potential study participants at Screening, however all slides will not be assessed for BV at the HPTN CL. Slides will only be assessed for participants who enroll in the study and, for enrolled participants who undergo more than one screening pelvic exam, only slides from the exam that confirmed eligibility will be assessed. Please refer

to the HPTN 059 Questions and Answers posted at the following website for further operational guidance on this topic:

http://www.hptn.org/research_studies/HPTN059QuestionsAndAnswers.htm

12.9.3.2 Vaginal Swab for Quantitative Culture (US site only)

In addition to the wet mounts and Gram stains, vaginal swabs will be collected for Quantitative cultures and sent to the CL in Pittsburgh. See shipping instructions below. Cultures will be collected at enrollment, weeks 4, 12, and Week 24/early termination.

- Collect the specimen for culture by rotating one Dacron swab several times over the lateral wall of the vagina. Insert the swab into a Port-A-Cul transport tube (labeled with a SCHARP label), submerging the swab into the gel and breaking off the shaft of the swab, and capping. (The Port-A-Cul transport tubes will be provided by CL, Pittsburgh.)
- Deliver the Port-A-Cul and the LDMS specimen tracking sheet to the local LDMS laboratory.
- Using the LDMS Tracking Sheet, log the slides into LDMS (specimen type = VAG) and label the Port-A-Cul tube with LDMS labels.
- Use LDMS to generate a shipping manifest for the cultures to be shipped. Vaginal swabs and Gram Stain slides require SEPARATE shipping manifests (in order to distinguish between the two specimen types); they CANNOT be combined into the same shipping manifest.
- Following the guidance posted on the above-listed web page, generate the manifest as a .csv file. Then email the file to Lorna Rabe and print a copy for inclusion in the shipment.
- Ship the Port-A-Cul tube and the vaginal Gram stain the same day of collection by overnight courier.
- Place the Port-A-Cul in a biohazard bag, place in the leak-proof container with absorbent material. Place the container with **gel ice packs** in a cardboard box lined with Styrofoam.
- Include a copy of the shipping manifest. Use diagnostics packing code 650, UN3373.

Confirm the address is correct (see below). Because the Research Institute is not open for delivery on the weekend the specimens taken on Friday must be sent to the hospital address in order for delivery on Saturday.

The day of shipment send Lorna Rabe an e-mail at rsilkr@mwri.magee.edu with the FedEx tracking number and attach an electronic copy of the manifest as a .csv file.

If sending Monday through Thursday Send to:

Lorna Rabe
Magee-Womens Research Institute
204 Craft Ave, Room 530
Pittsburgh, Pa. 15213
Phone# 412-641-6042

If sending on Friday for Saturday delivery, send to:

Lorna Rabe, C/O Safety and Security
Magee-Womens Hospital
300 Halket St.
Pittsburgh, Pa. 15213
Phone # 412 641-4191 (this is the Safety and Security #)
**** Be sure to check Saturday delivery on the Fed Ex label**

12.9.3.3 Slide Shipment

For the US sites: Ship the slide with the culture the same day as collected. The screening slide should be shipped with the enrollment slide and culture.

For the Pune site: The first shipment should include the first 50 slides collected to assess the quality of the slides. The second shipment should be the next 200 slides and the last shipment will be at the end of the study.

- Prior to shipment, contact the HPTN CL at the University of Pittsburgh (Lorna Rabe: rsilkr@mwri.magee.edu, +412-641-6042) to coordinate the timing and logistics of the shipment.
- Use LDMS to generate a shipping manifest for the slides to be shipped. Vaginal swabs and Gram Stain slides require SEPARATE shipping manifests (in order to distinguish between the two specimen types); they CANNOT be combined into the same shipping manifest.
- Following the guidance posted on the above-listed web page, generate the manifest as a .csv file. Then email the file to Lorna Rabe and print a copy for inclusion in the shipment.
- Obtain the slides to be shipped and place them in slide boxes suitable for shipping. Add paper towels inside the box to prevent rattling/breaking. Wrap the boxes in several layers of bubble wrap or wadded paper. Place the wrapped boxes in a shipping box, together with a printed copy of the shipping manifest.
- Address the shipment to:

Lorna Rabe
Magee-Women's Research Institute
204 Craft Avenue, Room 530
Pittsburgh, PA 15213
USA

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

12.9.4 Cervical Sample Collection, Slide Preparation and Storage

12.9.4.1 Cervical swab for Cytokines / Chemokines

- Collect two swabs. Gently insert the Dacron swabs 1 cm into the cervical os and rotate 360 degrees to absorb the fluid.
- Place the swabs into separate cryovials each with 400ul of PBS. Break off the end of the swab to allow closure of the cryovial and securely attach the cap. Attach a SCHARP-provided label to the vial.
- Samples must be refrigerated immediately and frozen at -70°C within 6 hours after collection.
- Deliver both cryovials and an LDMS Specimen Tracking Sheet to the local LDMS laboratory.
- Using the LDMS Tracking Sheet, log the cryovials into LDMS (specimen type=CXS) and generate an LDMS cryovial label for each tube. Affix the LDMS label to the cryovial (over the SCHARP-provided PTID label).

- Store the cryovial(s) in the freezer locations assigned in LDMS at -70°C.
- Specimens will be batched and shipped to the CL on dry ice **at the end of the study**.

12.9.4.2 Cervical swab for Gram Stain

- Use a pencil to write the PTID and specimen collection date on one side of the frosted end of two microscope slides. Affix a SCHARP-provided PTID label to the other side of the slides (on the frosted end, under the pencil markings) and write the specimen collection date in indelible ink (e.g. Sharpie pen) on each label. Also write “C” for cervical on each label.
- Gently insert a Dacron swab 1 cm into the cervical os and rotate 2 times (don’t just hold the swab against the cervix otherwise there won’t be enough cells).
- Then roll the swab across each of the two slides. It’s important that they roll the swab as opposed to dragging it across the slide. Dragging the swab distorts the cells and makes the smear more difficult to read.
- **The slide is air dried and not fixed.** Label the same way they label the vaginal smear but indicate cervical on the smear to distinguish from the vaginal.
- Deliver both slides and an LDMS Specimen Tracking Sheet to the local LDMS laboratory.
- Using the LDMS Tracking Sheet, log the slides into LDMS (specimen type = CVL) and label the slides with LDMS labels. Place the LDMS label on the frosted end of the slide, on the opposite side of the slide from the SCHARP-provided label, on top of the pencil markings.
- Store the slides in the slide box locations assigned in LDMS at room temperature.
- Vaginal and cervical Gram Stain Slides may be combined into the same LDMS shipping manifest. Gram Stain Slides (both cervical and vaginal) CANNOT be combined into the same shipping manifest as the vaginal swab for quantitative culture.
- Shipment schedule:

For the New York and UAB sites only: place the slide in a plastic slide holder and ship to the Magee lab with the vaginal culture and Gram stain.

For the Pune site: The first shipment should include the first 50 slides collected to assess the quality of the slides. The second shipment should be the next 200 slides and the last shipment will be at the end of the study. See section 12.6.3.3 for shipping instructions.

12.9.5 Genital Ulcer Swab for Multiplex PCR

Genital ulcers observed during follow-up will be sampled for multiplex PCR testing at the HPTN CL for chancroid, HSV-2, and syphilis. Instructions for specimen collection, preparation, storage, and shipment are as follows:

- Swab the base of each observed ulcer using a plastic shaft Dacron swab. If a cluster of ulcers is observed, sample each ulcer in the cluster with the same swab.
- Immediately place each swab in a 2 mL cryovial labeled with a SCHARP provided PTID label. Break off the end of the swab to allow closure of the cryovial and securely attach the cap. Be sure to write “GUD” swab on the label (so the specimen is not confused with the cytokine/chemokine cryovials).
- Place the cryovial(s) in a plastic zip lock biohazard bag and immediately place the bag in a refrigerator or a cooler with an ice pack. If necessary, the cryovial(s) may be stored refrigerated for up to 24 hours prior to freezing.
- Deliver the cryovial(s) and an LDMS Specimen Tracking Sheet to the local LDMS laboratory.

- Using the LDMS Specimen Tracking Sheet, log the cryovial(s) into LDMS (specimen type = GLU) and generate an LDMS cryovial label for each tube. Affix the LDMS label to the cryovial (over the SCHARP-provided PTID label).
- Store the cryovial(s) in the freezer locations assigned in LDMS at -70°C .
- Ship the cryovial(s) to the HPTN CL at Johns Hopkins University as part of the next routine shipment of plasma samples (follow instructions in Section 12.6.7).

Detailed instructions for preparing the shipment in LDMS are available at the following web site:
http://www.hptn.org/research_studies/HPTN059Lab.htm

12.9.6 Papanicolaou (Pap) Test

Pap smears will be performed at sites with the capacity and expertise to prepare and interpret the smears and provide referrals to appropriate follow-up care to participants with abnormal results. At visits when Pap smears are required, ecto- and endocervical cells will be collected after all tissues have been visually inspected and all other required specimens have been collected. Specimen collection, slide preparation, slide interpretation, and QC procedures must be performed and documented in accordance with study site SOPs.

At some study sites, Pap smear results may include notations of findings associated with certain STIs (e.g., trichomoniasis). Because Pap smear methods are not adequately sensitive and specific for STIs, Pap smear findings associated with STIs should not be considered diagnostic of any infections. Rather, such findings should be handled as follows:

- Do not consider STI-related notations on Pap smear result reports when assessing participant eligibility for the study. Use only the results of protocol specified STI tests for purposes of eligibility determination.
- If protocol-specified STI testing was performed on other specimens (i.e., blood, urine, vaginal fluids) collected on the same day as specimen collection for Pap smear, the results of the protocol-specified testing overrule STI-related findings noted on the Pap smear result report. Provide treatment as needed based on the results of the protocol-specified tests.
- If protocol-specified testing was not performed on other specimens (i.e., blood, urine, vaginal fluids) collected on the same day as specimen collection for the Pap smear, collect specimens for indicated protocol-specified STI testing at the participant's next study visit that takes place after receipt of the Pap test result report. Provide treatment as needed based on the results of the protocol-specified tests.

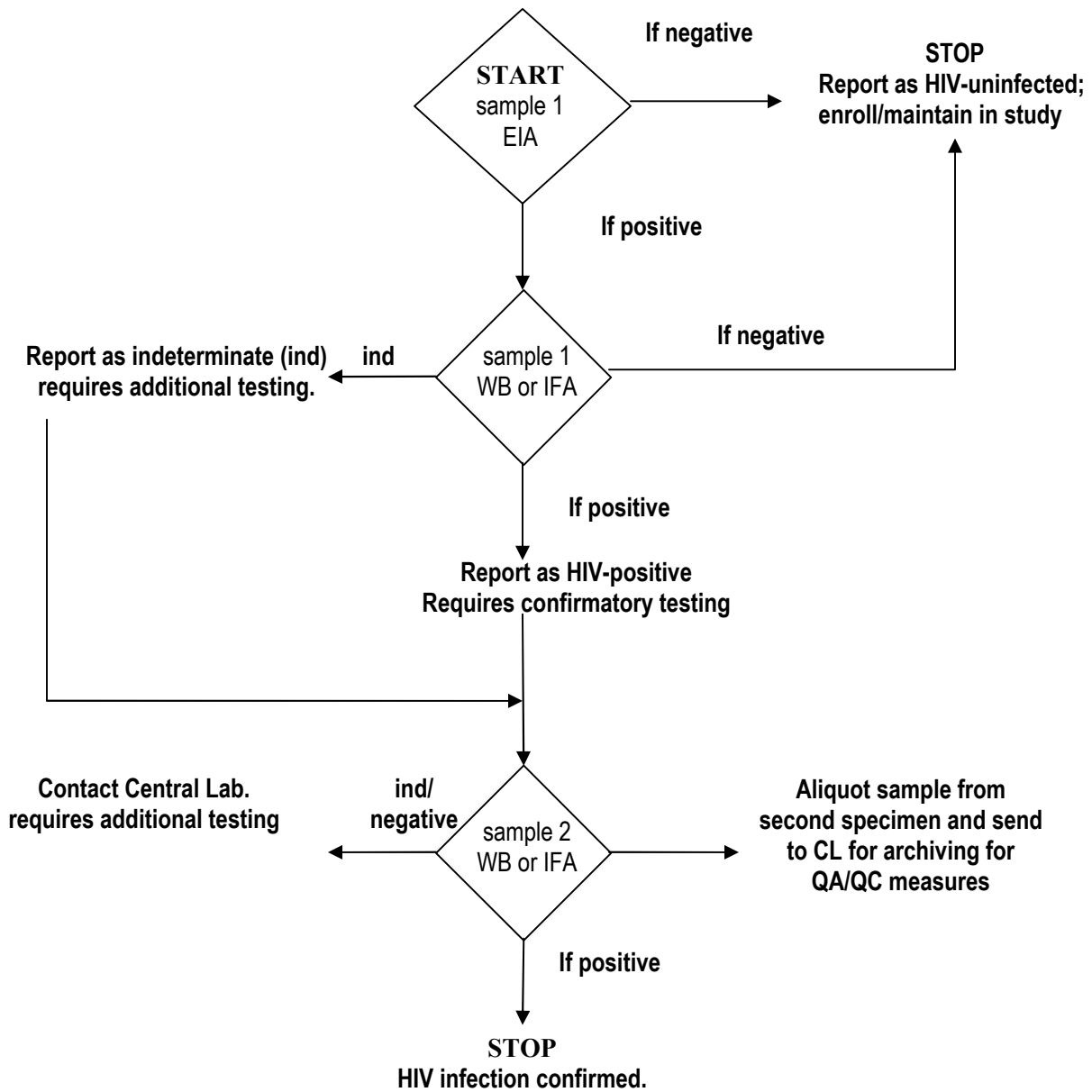
12.10 Local Laboratory Monitoring

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

12.11 Local Laboratory Quality Control Reports

The India site must submit monthly laboratory quality control (QC) reports to the HPTN CL for all safety laboratory tests (Hematology and Chemistry). These reports should contain monthly Levy-Jennings plots and corrective actions, if applicable. Please contact Paul Richardson (pricha18@jhmi.edu, +410-502-0435) to determine the required content and format of your site's report.

**HPTN HIV ANTIBODY ALGORITHM
NON-RAPID TESTING**



Appendix 12-1
CDC Universal Precautions for Prevention of Transmission of HIV and Other Bloodborne Infections

Appendix 12-2
World Health Organization Universal Precautions, Including Injection Safety

Appendix 12-3
Sample LDMS Laboratory Specimen Processing Form

HPTN 059 LDMS LABORATORY SPECIMEN PROCESSING

Test	Primary	Additive	Derivative	Sub Add/Derv	Primary Volume	Aliquot Volume
Cervical Swab for Gram Stain to CL	CXS	NON	SLD	NON	100 µL	100 µL
Vaginal Swab for Culture to CL	VAG	PAC	SWB	N/A	100 µL	100 µL
Cervical Swab for Cytokines / Chemokines to CL	CXS	PBS	CXS	N/A	100 µL	100 µL
Vag Swab for Gram Stain to CL	VAG	NON	SLD	NON	100 µL	100 µL
Genital Ulcer swab for multiplex PCR	GLU	NON	SWB	N/A	100 µL	100 µL
Pharmacokinetics to CL	BLD	DPE for potassium edta or DSE for sodium edta	PL1	N/A	3-5 mL	0.75 mL
Plasma for storage- LL	BLD	EDT	PL1	N/A	8-10 mL	1.0 mL
Serum for storage-LL	BLD	NON	SER	N/A	8-10 mL	1.0 mL

**Appendix 12-4
Sample LDMS Laboratory Specimen Tracking Sheet**

DO NOT FAX THIS FORM TO DATAFAX

**HPTN 059
LDMS Specimen Tracking Sheet**

Group: HPTN

Visit Code .

Participant ID:

Specimen Collection Date

- -
Site Number Participant Number Chk

dd MMM yy

Protocol #: 059.0

# of TUBES (or Specimens)	PRIMARY SPECIMEN TYPE	ADDITIVE
<input type="checkbox"/>	Vaginal Gram Stain Slide (VAG)	<input type="checkbox"/> No Additive <input type="checkbox"/> Other, specify: _____
<input type="checkbox"/>	Cervical Gram Stain Slide (CXS)	<input type="checkbox"/> No Additive <input type="checkbox"/> Other, specify: _____
<input type="checkbox"/>	Cervical Swab (CXS)	<input type="checkbox"/> PBS <input type="checkbox"/> No Additive <input type="checkbox"/> Other, specify: _____
<input type="checkbox"/>	U.S. Only: Vaginal Swab (VAG)	<input type="checkbox"/> PAC <input type="checkbox"/> No Additive <input type="checkbox"/> Other, specify: _____
<input type="checkbox"/>	GUD Swab (GLU)	<input type="checkbox"/> Viral Transport Medium (VTM) <input type="checkbox"/> No Additive <input type="checkbox"/> Other, specify: _____
<input type="checkbox"/>	Blood (BLD)	<i>If more than one additive is used, write in each box the number of tubes collected for each additive.</i> <input type="checkbox"/> SST <input type="checkbox"/> EDT <input type="checkbox"/> No Additive <input type="checkbox"/> Other, specify: _____ <input type="checkbox"/> Screening—lab to make at least two (2) 0.5 mL plasma aliquots <input type="checkbox"/> Enrollment—lab to make at least four (4) 1.0 mL plasma aliquots and at least two (2) 1.0 mL serum aliquots <input type="checkbox"/> Weeks 4, 12, and 20—lab to make at least two (2) 0.75 mL plasma aliquots <input type="checkbox"/> Week 24/Early Termination—lab to make at least four (4) 0.5 mL plasma aliquots and at least two (2) 1.0 mL serum aliquots
<input type="checkbox"/>	Other, specify: _____	<input type="checkbox"/> No Additive <input type="checkbox"/> Other, specify: _____

Comments: _____

Clinic Staff Initials: _____
Sending Staff

LDMS Data Entry Date: /
dd MMM yy Receiving Staff

Version 2.0, 01-AUG-2006

N:\hivnet\forms\IPTN_059\forms\p059_nonDF_spec_track_ldms.fm

Appendix 12-5

Shipping Manifest for CT/NG Urine Specimens

HPTN 059

Site (fill in)

Contact person: (fill in)

(Fill in address)

Phone number:

Fax number:

E-mail address:

Shipment Date _____

PTID	Collection Date	Visit Code	Specimen type
			Urine

Comments _____

For Monday- Thursday shipment	For Friday shipments
Lorna Rabe Magee-Womens Research Institute 204Craft Ave. Room 530 Pittsburgh, Pa. 15213 412-641-6041	Lorna Rabe, C/O Safety and Security Magee-Womens Hospital 300 Halket St. Pittsburgh, Pa. 15213 412-641-6041 Check Saturday delivery

On the day of shipment E-mail Lorna at rsilkr@mwri.magee.edu the FedEx tracking # .