Section 12. Laboratory Considerations

12.1 Overview and General Guidance

This section contains information on to the laboratory procedures performed in HPTN 035.

Some laboratory procedures will be performed in study site clinics; others will be performed in study site local laboratories, at the HPTN Network Laboratory (NL) in Baltimore, MD, USA, and at the MTN NL in Pittsburgh, PA, USA. Figure 12-1 lists the testing location for each test. Figure 12-1 also lists specimen and kit requirements for each test. Protocol Sections 5.2.3, 5.3.3, 5.4.3, Appendix II, and Appendix III specify the timepoints 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 NL and/or MTN NL. In addition, package insert instructions must be followed for the following test kits:

- Abbott Determine HIV 1/2 Rapid Test
- Becton Dickinson ProbeTec ET *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Strand Displacement Assays
- Focus Technologies HerpesSelect-2 ELISA
- Genetic Systems HIV-1 Western Blot Test (manufactured by Bio-Rad Laboratories)
- OraSure OraQuick Rapid HIV Test
- Quidel QuickVue OneStep hCG Urine Pregnancy Test
- Quidel QuickVue UrinCheck 10+SG Urine Test Strips or Bayer Multistix 9 Reagent Strips or Bayer Uristix 4 Reagent Strips
- Trinity Biotech Uni-Gold Recombigen Rapid HIV Test

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 NL and MTN NL with any questions about these documents.

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 and MTN 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.

Figure 12-1
Overview of Laboratory Testing Locations, Specimens, and Methods for HPTN 035

Test	Testing Location	Specimen Type	Tube/Container	Kit	Kit Supplier
Pregnancy test	In clinic	Urine	Plastic screw top cup	Quidel QuickVue One Step hCG	US-based or local
Dipstick urinalysis	In clinic	Urine	Plastic screw top cup	QuickVue UrinChek 10+SG*	US-based or local
SDA for chlamydia and gonorrhea	Local lab	Urine	Plastic screw top cup	BD ProbeTec ET	Becton Dickenson
HIV antibody tests	In clinic	Anticoagulated blood	Lavender top tube	Abbott Determine	Local
	In clinic	Anticoagulated blood	Lavender top tube	OraSure OraQuick	US-based or local
	In clinic	Anticoagulated blood	Lavender top tube	Uni-Gold Recombigen	US-based or local
	Local lab	Anticoagulated blood	Lavender top tube	FDA-approved HIV EIA (US site only)	Local
	Local lab	Anticoagulated blood	Lavender 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	Plasma	Lavender top tube ⇒ cryovial	Focus HSV-2 ELISA	Local
Plasma archive	Local lab	Plasma	Lavender top tube ⇒ cryovial	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
Coagulation tests	Local lab	Anticoagulated blood	Blue top tube	NA	NA
Vaginal pH	In clinic	NA	NA	S/P pH Indicator Strips	HPTN NL
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	Slide	NA	NA
Gram stain for BV	MTN NL (Rabe)	Vaginal fluid swab	Slides	NA	NA
Multiplex PCR for chancroid, HSV-2, and syphilis	HPTN NL (Gaydos)	Genital ulcer swab	Plastic shaft Dacron swab ⇒ cryovial	NA	NA
HIV Status Verification	HPTN NL (Piwowar-Manning)	Plasma	Cryovial	FDA-approved HIV EIA Genetic Systems WB	NA

*As of 15 July 2005, Bayer Multistix 9 Reagent Strips and Bayer Uristix 4 Reagent Strips also may be used.

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 NL and MTN NL.

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

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

http://www.who.int/hiv/topics/precautions/universal/en/

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

http://www.hptn.org/research_studies/HPTN035Lab.htm

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

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. Microscope slides used for evaluation of vaginal fluids also will be labeled with SCHARP-provided PTID labels. PTIDs are pre-printed on these labels; however study staff must write the specimen collection date on each label. The visit code also may be written on the label. When specimens are tested at the local lab, any additional labeling required for on-site specimen management and chain of custody will be performed in accordance with site SOPs. Stored plasma specimens will be entered into the Laboratory Data Management System (LDMS) and labeled with LDMS-generated cryovial labels. Genital ulcer swabs collected for Multiplex PCR testing at the HPTN NL also will be entered into LDMS and labeled with LDMS-generated cryovial labels. Vaginal fluid slides prepared for Gram stain evaluation at the MTN NL will be entered into LDMS and labeled with LDMS-generated labels. See also Section 12.3.

12.3 Use of LDMS

LDMS must be used at all sites to track the collection, storage, and shipment of three types of specimens in HPTN 035: plasma, vaginal fluids (air-dried on microscope slides for Gram stain evaluation), and genital ulcer swabs. 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 section, the current version of LDMS is Version 5.5.4. All sites should upgrade to this version as soon as possible. All sites must use the "LDMS1" label format in order to ensure that both the Specimen ID and the Global ID assigned to each specimen are printed on LDMS-generated labels.

Questions related to use of LDMS in HPTN 035 should be directed to Estelle Piwowar-Manning (epiwowa@jhmi.edu, +410-614-6736). Technical support also is available from LDMS User Support. Usual business hours for LDMS User Support are 7:30 am to 6:00 pm ET on 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 if you are locked out of LDMS or experience errors that prevent you from completing LDMS lab work. To page LDMS User Support, email LDMS pager 1 (address shown in table below) and include the following information in the body of your email:

- LDMS lab number (this is a three-digit number that is different from your network assigned clinical site number)
- The full telephone number at which you can be reached, including the country code and city code if you are outside the United States
- A short description of the problem

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

The pagers also can be reached via telephone. When paging via telephone, after dialing you will hear a voice greeting followed by three quick beeps that indicate you are connected to the paging service. Please include the full telephone number at which you can be reached, including the country and city codes if you are outside the United States. Please call LDMS pager 1 first (telephone number shown in table below). If you do not receive a response within 15 minutes after calling LDMS 1, please try LDMS 2, then finally, LDMS 3.

LDMS User Support Paging Details		
Pager	Email Address	Telephone Number
LDMS 1	ldmspager1@fstrf.org	+716-556-0583
LDMS 2	ldmspager2@fstrf.org	+716-556-0584
LDMS 3	ldmspager3@fstrf.org	+716-556-0585

Each site must export its LDMS data to Frontier Science (FSTRF) on a weekly basis. Exported data are use 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 (e.g., plasma needed for confirmatory HIV testing) that appear to be missing, and works with the NL and site staff to undertake appropriate corrective action. All corrective action should be documented in paper-based clinic and/or laboratory records as appropriate, and entered in the details section of LDMS. The NL and SDMC will discuss and document any items that, although resolved, appear 'unresolvable' in LDMS.

12.4 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.

12.4.1 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^{o} C for subsequent chlamydia and gonorrhea testing.

12.4.2 Pregnancy Testing

At visits when pregnancy testing is required, aliquot approximately 5 mL of urine from the specimen collection cup and pipette from this aliquot for pregnancy testing.

Note: *Protocol-specified pregnancy testing is <u>not</u> 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 035 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 pregnancy tests for confirmatory purposes in the absence of consultation with the HPTN NL and MTN NL.

All pregnancy tests performed for HPTN 035 must be documented on testing logs that record the PTID of the participant tested, the lot number of the test kit used, QC/QA performed for the test, the time of the test, and the result of the test. As of 5 October 2005, at sites where pregnancy tests are performed in-clinic by clinic staff, test results may be recorded directly onto relevant case report forms, and then transcribed onto the testing logs (i.e., the case report form may serve as the source document for the test result). At sites where pregnancy tests are performed in the laboratory by laboratory staff, results must be recorded first on the testing logs and then transcribed onto the relevant case report forms.

Pregnancy status is a critical participant safety consideration in HPTN 035. 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). All sites will be required to report their kit inventories, including kit lot numbers, to the HPTN NL on a monthly basis. Notify the NL immediately if any kit inventory or quality control problems are identified, so that appropriate action can be taken.

12.4.3 Dipstick Urinalysis

At visits when dipstick urinalysis — for leukocytes and nitrites — 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 Quidel QuickVue UrinChek 10+ SG urine test strips, Bayer Multistix 9 reagent strips, and Bayer Uristix 4 reagent strips may be used at all sites. The Quidel test strips were approved for use at all sites from the outset of study implementation, however this strip is expected to be discontinued. The MTN NL evaluated the Bayer Multistix 9 and Uristix 4 in the presence of study gel and approved these strips for use at all sites as of 15 July 2005.

Perform this test according to site SOPs and the package insert. Assess and record results for leukocytes and nitrites only.

12.4.4 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^{o} C between the time of collection and the time of testing. Perform the assays according to site SOPs and the package insert. Tests should be performed within four days of specimen collection.

12.5 Blood Testing for HIV, Syphilis, Hematology, Liver and Renal Function, Coagulation, and Plasma 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, 5-10 mL of blood will be collected, however additional blood may be collected if clinically indicated.

12.5.1 Specimen Collection and Initial Processing

Figures 12-2 and 12-3 present the per-visit blood collection requirements for protocol-specified testing for Phase II/IIb and Phase IIb study participants, respectively. Sites needing to diverge from the specifications of these figures (e.g., to shift volumes across tube types) may do so in consultation with the HPTN NL. Additional blood may be collected at any visit if required to perform additional clinically indicated tests. 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. After collection:

- Allow red top tubes (no additive) to clot, then centrifuge per site SOPs to yield serum for syphilis, liver function, and renal function testing.

- Lavender top tubes (additive = EDTA) require no additional processing prior to testing, but should be gently inverted at least eight times after specimen collection to prevent clotting. At timepoints when rapid HIV testing is performed, pipette blood from the EDTA tube for the rapid test(s) and then deliver the remainder of the blood in the tube to the local laboratory for testing per protocol.

- Blue top tubes (additive = sodium citrate) require no additional processing prior to testing, but should be gently inverted at least eight times after specimen collection to prevent clotting. NCCLS recommends using 3.2% sodium citrate.

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.*

Figure 12-2
Blood Collection for Protocol-Specified Testing: Phase II/IIb Participants

Testing Timepoint	Total Blood Volume	Volume By Tube Type	Purpose
Screening Part 1	10 mL	4 mL red top	Syphilis, liver and renal function
		3 mL lavender top	HIV, hematology
		3 mL blue top	Coagulation
Enrollment	10 mL	10 mL lavender top	Plasma archive
Months 1 and 2	10 mL	4 mL red top	Liver and renal function
		3 mL lavender top	Hematology
		3 mL blue top	Coagulation
Month 3	10 mL	4 mL red top	Liver and renal function
		3 mL lavender top	HIV, hematology
		3 mL blue top	Coagulation
Months 6, 9, 15, 18, 21, and 27	5 mL	5 mL lavender top	HIV
Months 12 and 24	10 mL	2 mL red top	Syphilis
		5 mL lavender top	HIV, hematology
		3 mL blue top	Coagulation
"Sample 2" for Confirmatory HIV Testing	5 mL	5 mL lavender top	HIV, plasma archive
Study Exit	10 mL	2 mL red top	Syphilis
		5 mL lavender top	HIV, hematology, plasma archive
		3 mL blue top	Coagulation

Notes: Additional blood may be collected for additional clinically indicated testing. Red top tubes contain no additive. Lavender top tubes contain EDTA. Blue top tubes contain sodium citrate.

Figure 12-3
Blood Collection for Protocol-Specified Testing: Phase IIb Participants

Testing Timepoint	Total Blood Volume	Volume By Tube Type	Purpose
Screening Part 1	8 mL	2 mL red top	Syphilis
		3 mL lavender top	HIV, hematology
		3 mL blue top	Coagulation
Enrollment	10 mL	10 mL lavender top	Plasma archive
Month 3	6 mL	3 mL lavender top	HIV, hematology
		3 mL blue top	Coagulation
Months 6, 9, 15, 18, 21, and 27	5 mL	5 mL lavender top	HIV
Months 12 and 24	10 mL	2 mL red top	Syphilis
		5 mL lavender top	HIV, hematology
		3 mL blue top	Coagulation
"Sample 2" for Confirmatory HIV Testing	5 mL	5 mL lavender top	HIV, plasma archive
Study Exit	10 mL	2 mL red top	Syphilis
		5 mL lavender top	HIV, hematology, plasma archive
		3 mL blue top	Coagulation

Notes: Additional blood may be collected for additional clinically indicated testing. Red top tubes contain no additive. Lavender top tubes contain EDTA. Blue top tubes contain sodium citrate.

12.5.2 HIV Testing

Anticoagulated blood will be tested for evidence of HIV infection using tests that have been validated at the study site. These same tests will be used to test plasma specimens archived at enrollment for participants who test HIV-positive at their first HIV testing timepoint during follow-up. All tests, and associated QC procedures, must be documented on local laboratory log sheets or other laboratory source documents.

At all sites, the following rapid HIV tests may be used, provided the tests have been validated at the site:

- Abbott Determine
- OraSure OraQuick
- Uni-Gold Recombigen

When using the OraSure OraQuick and Uni-Gold Recombigen tests, test kits approved by the US Food and Drug Administration (FDA) must be used. See Sections 12.5.2.1 and 12.5.2.2 for specific kit requirements for HIV testing performed at screening and during follow-up, respectively.

Perform all 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 timeframes. Place appropriate timekeeping devices in all test settings to ensure that each test is read and verified at appropriate timepoints. 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 Screening Part 1 Laboratory Results case report form or the Follow-up Laboratory Results case report form, record rapid test kit codes as follows:

- Abbott Determine, test kit code = 01
- OraSure OraQuick, test kit code = 02
- Uni-Gold Recombigen, test kit code = 03

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. 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.

- Indeterminate: One or more bands are present, but the blot does not meet the criteria for a positive result as described above.

- Negative: No bands are present

HIV infection status is the primary endpoint for HPTN 035. 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 NL. All sites must maintain an adequate inventory of the HIV test kits they have selected and validated for use in HPTN 035. 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). All sites are required to report their kit inventories, including kit lot numbers, to the HPTN NL on a monthly basis. Notify the NL immediately if any kit inventory or quality control problems are identified, so that appropriate action can be taken.

Beginning in October 2006, all laboratory staff who read and interpret WB results are required to complete proficiency testing approximately every six months. The HPTN NL will post an image of an actual WB run on the HPTN 035 web page for this purpose. Relevant laboratory staff from each site will review these images and submit their interpretations of the images to the HPTN NL via the 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 WBs for study purposes.

12.5.2.1 HIV Testing at Screening

At non-US sites, HIV infection status at screening will be assessed using two different rapid HIV tests. If both rapid tests are negative, the participant will be considered HIV-uninfected; no further testing is required. If both rapid tests are positive, the participant will be considered HIV-infected; no further testing is required. If the two rapid tests are discordant, 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. If the WB is indeterminate, the participant will be asked to return to the study site in approximately one month for re-testing. At that time, the two rapid tests will be repeated and the above-described algorithm will be followed. A WB will only be performed if the two rapid tests are discordant.

At the US site, HIV infection status at screening will be assessed using an FDA-approved enzyme immunoassay (EIA); currently the Abbott HIV EIA is used at the US site. 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. 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 tests must be documented on local laboratory log sheets or other laboratory source documents. Also at all sites, a second independent clinic or laboratory staff member trained in proper HIV testing and result recording procedures must review, verify, and sign-off on screening HIV test results within the timeframe of the tests and prior to disclosure of results to participants. 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.

12.5.2.2 HIV Testing During Follow-up

At all sites, follow-up HIV testing will be performed according to the algorithm in protocol Appendix V, which is re-printed in Figure 12-4 below.

In Step One, an FDA-approved rapid HIV test (i.e., either the OraSure OraQuick test or the Uni-Gold Recombigen test) is performed. If the rapid test is negative, testing will stop after Step One. If the rapid test is positive, testing will proceed to Step Two, in which the same sample that tested positive in Step One will be tested with the FDA-approved Genetic Systems WB. The blood tested in Step One (referred to as "sample 1") is not archived.

At some sites, a second rapid may be performed in Step One. For example, HIV counseling and testing guidelines at some sites require that two rapid tests be performed whenever rapid testing is utilized. Sites required or otherwise wishing to perform a second test in Step One must specify their site-specific testing procedures in their local laboratory SOPs for HPTN 035, and must obtain HPTN NL approval of these SOPs prior to study activation. Once approved, these SOPs must be followed consistently for all HPTN 035 participants. For sites that perform two tests in Step One, testing will proceed to Step Two if either of the two tests is positive/reactive.

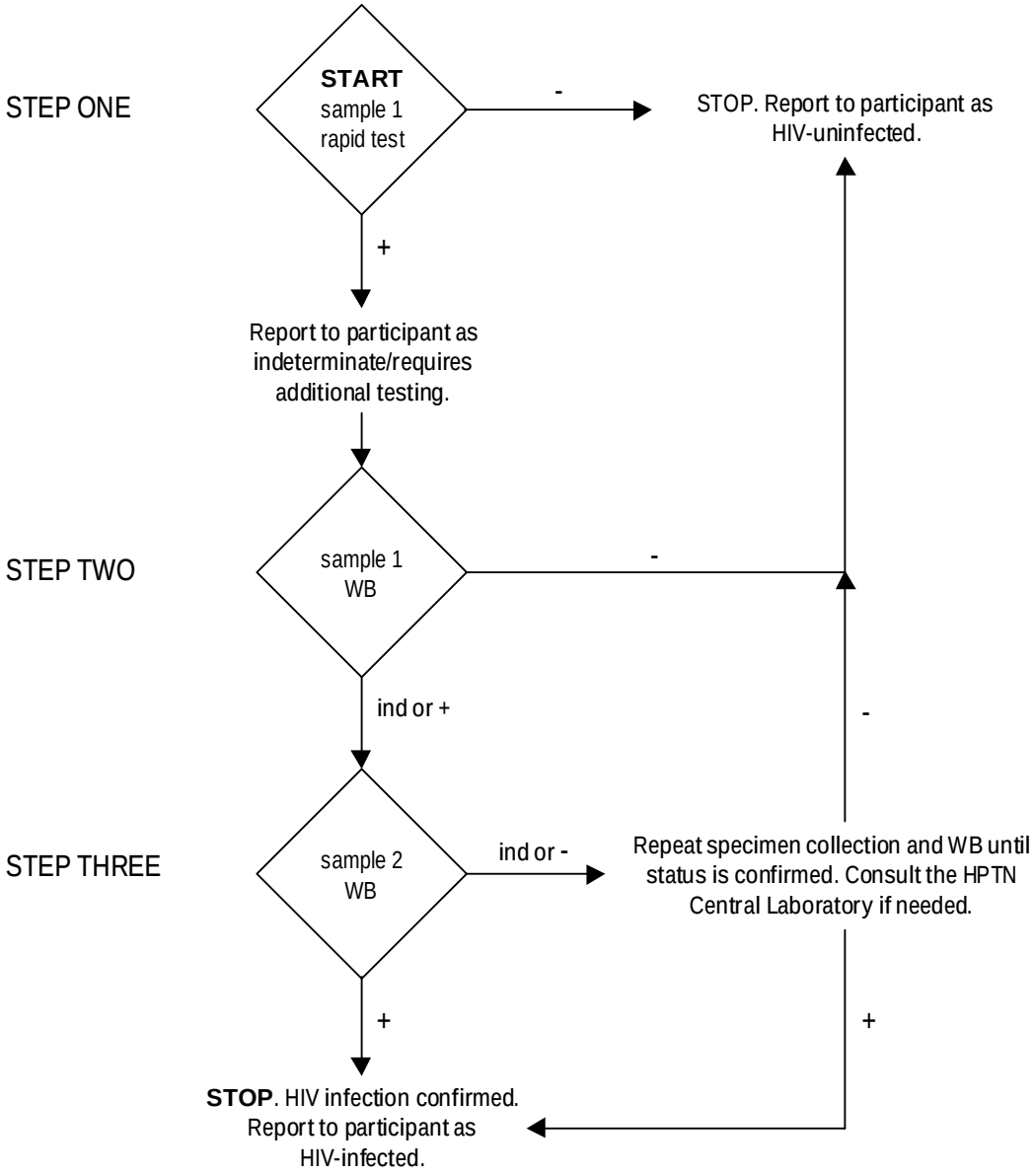
If the WB in Step Two is negative, testing will stop after Step Two. If the WB is positive or indeterminate, a second Genetic Systems WB must be performed on a second sample collected from the participant. This sample is referred to as "sample 2" in the algorithm and will be used for plasma archive if HIV infection is confirmed. For purposes of estimating the effectiveness of the gels tested in HPTN 035, only participants for whom infection is confirmed with two positive WB results on two different samples will be counted as having become HIV-infected. For participants with confirmed infection at their first HIV testing timepoint during follow-up, plasma archived at enrollment also will be tested for evidence of HIV infection, as described below.

If the sample 2 WB is negative or indeterminate, additional WB testing must be performed on additional samples. In this case, inform the HPTN NL via email of the sample 1 and sample 2 test results (copied to the HPTN CORE and SDMC) and request NL input on next steps and timeframes for additional specimen collection and testing. Plasma processed from sample 2 as well as any additional samples (e.g., sample 3, sample 4) must be archived as described in Section 12.5.8.

All tests must be documented on local laboratory log sheets or other laboratory source documents. A second independent clinic or laboratory staff member trained in proper HIV testing and result recording procedures must review, verify, and sign-off on follow-up HIV test results within the timeframe of the tests and prior to disclosure of results to participants. For positive/reactive results, review, verification, and sign-off must be performed by a nurse, clinician, or physician. 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.

Figure 12-4
Algorithm for HIV Antibody Testing During Follow-up in HPTN 035

NOTE: In order to correspond exactly with the HPTN 035 protocol, reference to the HPTN CL in this algorithm has not been modified; however, all HIV testing queries should be directed to the HPTN NL.



Notes:

WB=Western blot; + = positive; - = negative; ind = indeterminate.

If required by local HIV counseling and testing guidelines or regulations, and/or approved by the HPTN Central Laboratory, a second concurrent rapid test (at non-US sites) or enzyme immunoassay (at the US site) may be performed on sample 1 as part of Step One. In this case, testing will proceed to Step Two (sample 1 WB) if either of the two tests is positive/reactive.

12.5.2.3 HIV Testing of Plasma Archived at Enrollment

For participants who test HIV-positive at their first testing timepoint during follow-up, plasma archived at enrollment will be tested for evidence of HIV infection, as follows:

- Using LDMS, remove one aliquot of plasma archived for the participant at enrollment from the storage module.
- Obtain the designated aliquot from the freezer and confirm the PTID, global ID, and date on the cryovial label.
- Thaw the aliquot completely and then mix/vortex adequately.
- Perform and document testing per Step One and Step Two (if applicable) of the follow up testing algorithm according to site SOPs and the test kit package inserts.
- Return the aliquot to the storage module, documenting a thaw.

12.5.3 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 titres 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 performed, and appropriate clinical management action taken, prior to enrollment in the study. See Section 10.6 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 titre does not decrease four-fold or revert to seronegative within three months after treatment, treatment should be repeated.

Please consult the HPTN NL 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 035 Protocol Safety Review Team as described in Section 10.6.2 of this manual.

12.5.4 Hematology Testing

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

- Hemoglobin
- Hematocrit
- Mean corpuscular volume
- Platelets
- White blood cells
- Absolute neutrophil count
- Percent neutrophils
- Absolute lymphocyte count
- Absolute monocyte count
- Absolute eosinophil count
- Absolute basophil count

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.

12.5.5 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.

12.5.6 Coagulation Testing

The following coagulation tests will be performed on anticoagulated (sodium citrate) blood; NCCLS recommends using 3.2% sodium citrate:

- Prothrombin time (PT)
- INR
- Activated partial prothrombin time (aPTT)

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

PT tests must be performed within two to four hours of collection unless the specimen is frozen; otherwise, results will be erroneous.

Particular attention must be paid to the INR. There are regional differences in the strengths of the thromboplastin reagent used for the PT test which make it difficult to compare results from different areas. Recognizing this, the World Health Organization created the International Sensitivity Index (ISI), which is a correction factor based on the response of different thromboplastins. The formula for the INR is as follows:

INR = (participant PT/normal PT geometric mean)*ISI

Each site lab must establish its own normal PT geometric mean based on the local population.

Every lot of thromboplastin reagent will contain an ISI. It is extremely important to check the ISI of each new lot of thromboplastin reagent to determine whether the ISI has changed. If the ISI has changed, the new value must be used when calculating INR results. In addition, each new ISI requires verification of the PT normal range and re-calculation of the normal PT geometric mean. Before using a new lot of thromboplastin reagent, each site lab must enter the new ISI value and the new geometric mean into its coagulation instrument; otherwise, results will be erroneous. Because of the complexities associated with use of new lots of thromboplastin reagents, each site must inform the HPTN NL before a new lot is used, so the NL can assist site laboratory staff in correctly performing all required calculations. When normal ranges change, each site must inform the HPTN NL and SDMC using a format similar to the following:

	Upper Limit of Normal (ULN) for:		
Start Date	PT	PT INR	PTT
dd-MMM-yy	[value]	[value]	[value]
dd-MMM-yy	[value] or "no change"	[value] or "no change"	[value] or "no change"
dd-MMM-yy	[value] or "no change"	[value] or "no change"	[value] or "no change"

12.5.7 Herpes Simplex Virus 2 Testing

Herpes Simplex Virus 2 (HSV-2) testing will be performed in batches during the final year of study implementation, and thereafter, using the Focus Technologies HerpesSelect-2 ELISA. Each participant's study enrollment specimen will be tested. Study exit specimens will be tested only for those participants whose enrollment specimen tested HSV-2-negative.

Testing will be performed at either the MTN NL or at designated study site laboratories according to testing laboratory 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 MTN NL (Cherpes et al, J Clin Microbiol 2003; 41:2758).

At the designated study site laboratories, testing of that site's participants' plasma specimens will be performed per the instructions listed below. Additional instructions for shipping and testing specimens from other sites will be provided toward the end of the study. Documentation of each testing run must be reviewed by the NL prior to reporting of test results to the SDMC. Unless otherwise determined by the NL, optical densities greater than 1.1 will be considered positive for US participants and optical densities greater than 3.5 will be considered positive for non-US participants.

- Using LDMS, remove an appropriate (enrollment or exit) aliquot of plasma archived for the participant from the storage module.

- Obtain the designated aliquot from the freezer and confirm the PTID, global ID, and date on the cryovial label.

- Thaw the aliquot completely and then mix/vortex adequately.

- Perform and document the test according to site SOPs and the package insert.

- Return the aliquot to the storage module, documenting a thaw.

12.5.8 Plasma Archive

For all participants, plasma will be archived at enrollment and at study exit. For participants who become HIV-infected during follow-up, plasma also will be archived when blood is collected for confirmatory HIV testing (i.e., when sample 2, 3 or 4 in Figure 12-4 is collected), as follows:

- At enrollment, collect 10 mL of blood into a lavender top tube (EDTA) labeled with a SCHARP-provided PTID label. At study exit 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 the sample into LDMS (specimen type = BLD) and generate the appropriate number (see next bullet) of LDMS cryovial labels.

- 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 exit 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^{o} C.

All sites have established SOPs for weekly reconciliation and verification of plasma archive 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 timepoint, 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.

Archived plasma will be used for the following purposes:

- HSV-2 testing
- HIV testing, on site and at the HPTN NL
- Possible future research testing, if the participant provides written informed consent for such testing

All enrolled study participants consent to collection and storage of their plasma for the duration of their study participation and until all protocol-specified HSV-2 and 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 thereof.

Protocol Section 9.3 describes the HIV testing that the HPTN NL will perform on archived plasma for quality control and quality assurance purposes. Each site will ship plasma samples to the NL 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 NL at Johns Hopkins University (Estelle Piwowar-Manning: epiwowa@jhmi.edu, +410-614-6736) to coordinate the timing and logistics of the shipment. The US site may ship to the HPTN NL via Federal Express Monday through Thursday, with 24-hour fax notification. For non-US sites, the HPTN NL will work with each site to 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, 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.

- When shipping on carbon dioxide and/or liquid nitrogen (LN2), 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. World Courier will replenish dry ice as necessary. Please check with the manufacturer of the LN2 shipper for appropriate internal packaging. LN2 shippers are manufactured to maintain temperatures for 7-14 days, and World Courier should deliver the LN2 shipper within this time frame.

- IATA shipping regulations have recently been updated. Please follow the instructions provided on the HPTN NL web page:

  http://www.hptn.org/hptn_structure/NetworkLab/LabShippingLabels.htm

- Include a copy of the shipping manifest, box map, LDMS diskette, and CDC import permit in the shipment. For dry ice shipments and LN2 shipments, use diagnostics packing code 650, UN 3373. Use Non-Flammable Gas labels, Keep Upright stickers, and Do Not Drop – Handle With Care stickers, and address the shipment to:

  Estelle Piwowar-Manning/Dr Brooks Jackson
  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) 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.

12.6 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 of the required sequence of specimen collection and diagnostic procedures to be performed during study pelvic exams.

12.6.1 Vaginal pH

Vaginal pH will be assessed as part of on-site evaluations for bacterial vaginosis. S/P pH Indicator Strips 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. It is not necessary to record pH values onto laboratory log sheets or other source documents prior to recording values onto case report forms.

12.6.2 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 Figure 12-5.

Figure 12-5
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 (*Gardnerella vaginalis*) 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 in ink 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 coverslip.

- 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 quantify 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.

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 appropriate case report form.

Prior to study initiation, the HPTN Central Laboratory (now the MTN NL) 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 must ensure that all staff designated to perform wet mounts complete additional on-site proficiency testing approximately every six months. The MTN NL will post images of wet mount slides on the HPTN 035 web page for this purpose. Relevant laboratory staff from each site will review these images and submit their interpretations of the images to the MTN NL via the web page. After each proficiency testing cycle, the MTN NL will report results back to each site Laboratory Manager and specify any corrective action that may be needed. Contact the MTN NL for additional information and guidance on performing and documenting the proficiency testing. Also contact the MTN NL when new staff are hired, so that appropriate training can take place prior to such staff performing wet mounts for study purposes.

12.6.3 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 MTN NL. Two slides will be prepared at each required timepoint and both will be entered into LDMS. One will be shipped to the MTN NL 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.

12.6.3.1 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 in ink on each label.

- Immediately following specimen collection from the lateral vaginal wall via swab, roll the swab across each of the two slides. 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:

  http://www.hptn.org/research_studies/HPTN035Lab.htm

  Note: *The HPTN 035 protocol requires that dried smears be prepared for all potential study participants at Screening Part 2, however all slides will not be assessed for BV at the MTN NL. 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 035 Questions and Answers posted at the following website for further operational guidance on this topic:*

  http://www.hptn.org/research_studies/HPTN035QuestionsAndAnswers.htm

12.6.3.2 Slide Shipment

A schedule for shipping slides to the HPTN NL is posted at:

http://www.hptn.org/research_studies/HPTN035Lab.htm

All sites must follow the posted scheduled. First shipments for each site should contain slides from all pelvic exams conducted prior to the scheduled shipment date; it is not necessary for these shipments to contain full slide boxes. Thereafter shipments will take place approximately every two months and should contain only full boxes of 100 slides each.

- Prior to shipment, contact the MTN NL 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, box map, and LDMS shipping diskette for the slides to be shipped. The MTN NL is Lab 414.

- 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 copy of the shipping manifest, box map, and LDMS diskette.

- Address the shipment to:

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

- Notify the MTN NL 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 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.

12.6.4 Swab for Multiplex PCR

Genital ulcers observed during follow-up will be sampled for multiplex PCR testing at the HPTN NL 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. Otherwise use a different swab for each ulcer.

- 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.

- Place the cryovial(s) in a plastic ziplock biohazard bag and immediately place the bag in a refrigerator or a cooler with an ice pack. If necessary, the cryovials(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^{o} C.

- Use LDMS to generate a shipping manifest, box map, and LDMS shipping diskette for the swabs to be shipped. The appropriate HPTN NL (Gaydos) is Lab 416.

- Ship the cryovials(s), frozen, to the HPTN NL at Johns Hopkins University as part of the next routine shipment of plasma samples. These may be shipped to the HPTN NL attn Estelle Piwowar-Manning, and lab 416 will be asked to pick up the samples.

- Additional instructions are posted at:

  http://www.hptn.org/research_studies/HPTN035Lab.htm

12.6.5 Swab for Archive and Future Research Testing

At non-US sites, after all required IRB/EC approvals of Letter of Amendment #1 of protocol Version 3.0 are obtained, among consenting participants, a single swab of vaginal fluid will be collected during every pelvic examination performed for study purposes. After the study is completed, these specimens will be used for research testing at the MTN NL aiming to identify biomarkers of study product safety and effectiveness.

Instructions for specimen collection, preparation, and storage are as follows; instructions for specimen shipping will be provided after the study is completed:

- Swab the posterior fornix of the vagina with a plastic shaft Dacron swab; the vaginal tissue should be gently swabbed until the tip of the swab is saturated with fluid.

- Immediately place the swab in a 2 mL cryovial containing 0.4 mL phosphate buffered saline (calcium and magnesium free) and 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.

- Place the cryovial in a plastic ziplock biohazard bag and immediately place the bag in a refrigerator or a cooler with an ice pack. All cryovials should be frozen on the day of collection, such that specimens are ideally stored refrigerated for no more than eight hours prior to freezing. However, if necessary, cryovials may be stored refrigerated for up 24 hours. No cryovials should be stored refrigerated for more than 24 hours.

- Deliver the cryovial and an LDMS Specimen Tracking Sheet to the local LDMS laboratory.

- Using the LDMS Specimen Tracking Sheet, log the cryovial into LDMS (specimen type = VAG) and generate an LDMS cryovial label. Affix the LDMS label to the cryovial (over the SCHARP-provided PTID label).

- Within 24 hours of collection, store the cryovial in the freezer location assigned in LDMS at -70^{o} or -80^{o} C.

12.6.6 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 STDs (e.g., trichomoniasis). Because Pap smear methods are not adequately sensitive and specific for STDs, Pap smear findings associated with STDs should not be considered diagnostic of any infections. Rather, such findings should be handled as follows:

- Do not consider STD-related notations on Pap smear result reports when assessing participant eligibility for the study. Use only the results of protocol-specified STD tests for purposes of eligibility determination.

- If protocol-specified STD 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 STD-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 STD 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.

12.7 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.

12.8 Monthly Local Laboratory Reports

Each site must submit a detailed 'report card' to the HPTN NL by the third Wednesday of each month reporting on the site's status of LDMS, SOP revisions, IATA training, shipping concerns, and web-based proficiency testing for wet mounts and Western blots. Also reported are the results of the site's three previous proficiency panel results for protocol-specified tests. The report cards are reviewed by the HPTN NL and MTN NL prior to the HPTN 035 Laboratory Managers Group conference calls that take place on the fourth Wednesday of each month and any issues or concerns are addressed during the conference calls.

At the end of each month, each site also must submit to the HPTN NL quality control reports for all safety laboratory tests. 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.