Section 9. Laboratory Considerations

Table of Contents

9.1 Overview and General Guidance

This section contains information on the laboratory procedures performed in MTN-029/IPM 039.

As transmission of HIV and other infectious agents can occur through contact with contaminated needles, blood, blood products, rectal, and vaginal secretions, all study staff must take appropriate precautions when collecting and handling biological specimens. Sites must have appropriate written safety procedures in place before study initiation. Guidance on universal precautions available from the US Centers for Disease Control and Prevention can be found at the following website: **http://www.cdc.gov/hai/**

Laboratory procedures may be performed in the study site clinics or laboratories, approved commercial laboratories and in the MTN Laboratory Center (LC), including the MTN Pharmacology Core (Johns Hopkins University Clinical Pharmacology Analytical Lab or JHU CPAL). Table 9-1 lists for each test, the testing location, specimen type, specimen container and kit/method (if specified). Table 9-2 specifies specimen collection for storage and shipment.

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

Sites are responsible to ensure that specimen volumes do not exceed what is described in the informed consent process. The MTN LC may request details of collection containers and volumes for this purpose.

Note: Additional blood may be collected for any clinically indicated testing.

Ideally, one method, type of 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. The MTN LC must be notified before the change and can provide further guidance on validation requirements.

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 all regulatory authorities across study sites.

This section of the MTN-029/IPM 039 SSP Manual gives basic guidance to the sites, but is not an exhaustive procedure manual for all laboratory testing. This section must be supplemented with Standard Operating Procedures. The MTN LC is available to assist in the creation of any SOPs upon request. Essential SOPs include but are not limited to:

SOPs created by the site
Specimen Collection and transport*
Chain of Custody *

*Must be approved by the MTN LC for study activation

Table 9-1
Overview of Laboratory Testing Locations,
Specimens, And Methods for MTN-029/IPM 039

Sites are responsible to ensure that specimen volumes do not exceed what is described in the informed consent process. The MTN LC may request details of collection containers and volumes for this purpose.

Test	Testing Location	Specimen Type	Tube or Container and tube size (recommended)	Kit/Method
Urine hCG	Clinic/Local Lab	Urine	Plastic screw top cup	LC approved methodology
Dipstick Urinalysis/Culture	Clinic/Local Lab	Urine	Plastic screw top cup	LC approved methodology
Urine NAAT for GC/CT	Local Lab	Urine	Kit Specific Transport Tube	GenProbe Aptima or GeneXpert
HIV-1 Serology	Clinic/Local Lab	Plasma, serum or whole blood	EDTA or plain tube 4mL	FDA approved tests
Chemistries (ALT, AST)	Local Lab	Serum, plasma, or whole blood	Local lab requirements	Local Methodology
Syphilis Serology	Local Lab	Serum or Plasma	Local lab requirements	Local Methodology
Plasma archive	MTN LC	Plasma	EDTA tube 10mL	MTN LC Protocol
Plasma for PK	JHU CPAL	Plasma	EDTA tube 10mL	JHU CPAL Protocol
Trichomonas Test	Clinic/Local Lab	Vaginal Swab	Kit specific collection device	OSOM Kit
Wet prep	Clinic/Local Lab	Vaginal Swab	Sterile Tube	MTN LC Protocol
Vaginal pH	Clinic	Vaginal Swab	N/A	pH Indicator Strip
Vaginal gram stain	MTN LC	Vaginal Swab	Slide	MTN LC Protocol
Quantitative vaginal culture	MTN LC	Vaginal Swab	Culture Swab Max V+ or Starplex Starswab Anerobic System	MTN LC Protocol
Cervical NAAT for GC/CT	Local Lab	Cervical Swab	Kit specific collection device	GenProbe Aptima or GeneXpert
Pap smear	Local Lab	Cervical Cells	Slides	Local Methodology
Herpes Culture	Local Lab	Swab	Local lab requirements	Local Methodology
CVF PK	JHU CPAL	Cervicovaginal Swab	Cryovial	JHU CPAL Protocol
Vaginal Biomarkers	MTN LC	Vaginal Swab	Cryovial	MTN LC Protocol
Breastmilk for PD	MTN LC	Breast Milk	Cryovial	MTN LC Protocol
Breastmilk for PK	JHU CPAL	Breast Milk	Cryovial	JHU CPAL Protocol
Intravaginal Ring	MTN LC designation	Intravaginal Ring	Zippit Pouch	IPM Protocol

Volumes may vary depending on each site's testing platforms. Please confirm with the testing lab to determine minimum volume requirements. Sites are responsible for ensuring that specimen volumes do not exceed what is described in the informed consent process. The MTN LC may request details of collection containers and volumes for this purpose.

Notes: Additional blood may be collected for any clinically indicated testing.

Red top tubes contain no additive.
Purple top tubes contain EDTA.
Light Blue top tubes contain Na Citrate.

Table 9-2
Overview of Specimens for Storage and Shipment

Specimen and Subsequent Testing	Additive	Tube type or size recommendation	Processing and Storage	Ship to:
Plasma archive	EDTA	1x10mL	Spin 10 minutes at 1500xg (or double spin at 800xg). Aliquot and freeze.	Batch to MTN LC
Plasma for PK	EDTA	1x10mL	Spin 10 minutes at 1500xg. Aliquot and freeze within 8 hours of collection.	Batch to JHU CPAL
Vaginal Biomarkers	None	Swab in Cryovial	Place swab in PBS. Keep refrigerated. Freeze within 8 hours of collection.	Batch to MTN LC
Quantitative vaginal culture	None	Culture Swab Max V+ or Starplex Starswab Anerobic System	Store refrigerated within 4 hours of collection.	Ship to MTN LC overnight on cold pack
Vaginal gram stain	None	Slide	Allow slide to air dry and store at room temperature.	Ship to MTN LC overnight with culture
CVF PK	None	Swab in Cryovial	Keep refrigerated. Freeze within 2 hours of collection.	Batch to JHU CPAL
Breastmilk for PD	None	Cryovial	Aliquot approximately 1.5mL into each cryovial. Freeze immediately	Batch to MTN LC
Breastmilk for PK	None	Cryovial	Aliquot approximately 1.5mL into each cryovial. Freeze immediately	Batch to JHU CPAL
Intravaginal Ring	None	Zippit Pouch	Rinse and store dry at room temperature	Batch upon request by MTN LC

9.2 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. The date of specimen collection should also be included on the label. If the date is handwritten, it should be in indelible ink (such as a black 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. Specimens that are sent to the LC or are archived at the site will be entered into LDMS (Table 9-3) and labeled with LDMS-generated labels.

9.3 Procedures for Specimens that cannot be Evaluated

Specimen collection will be repeated (whenever possible) if samples cannot be evaluated per site SOPs. Site clinic and laboratory staff will monitor specimen collection, processing and management as part of ongoing quality assurance (QA) procedures and take action as needed to address any issues or problems. In cases where additional specimens need to be recollected either due to a laboratory error (lost, broken tube, clerical, etc.) or clinic error, a protocol deviation form may be required.

The site is responsible for notifying the MTN Laboratory Center in the following cases

- Any time a participant must return to the clinic for specimen collection
- When PK specimens are missed or not collected within the allowable time frames

- Insufficient blood volume is collected for the plasma archive
- Any time specimens have been mishandled, possibly compromised specimen integrity
- Any situation that may indicate a protocol deviation

If site staff has any question regarding time windows or collection processes, call MTN Laboratory Center staff (Pam Kunjara at +1-412-641-6393 or (*PKunjara@mwri.magee.edu*) as soon as possible for guidance.

9.4 Use of LDMS

The Laboratory Data and Management System (LDMS) is a program used for the storage and shipping of laboratory specimens. It is supported by the Frontier Science Foundation (FSTRF). LDMS must be used to track the collection, storage, and shipment of specimens in Table 9-3.
Detailed instructions for use of LDMS are provided at: *https://www.fstrf.org/ldms* (may require a password).

All sites will be required to maintain the current version of LDMS and monitor updates relating to use of the LDMS. It is crucial to be aware of proper label formats to ensure that specimens are correctly labeled. Sites will be responsible to back up their LDMS data (frequency determined by site) locally and to export their data to FSTRF (at least weekly).

Questions related to use of LDMS in MTN-029/IPM 039 may be directed to Pam Kunjara (or LDMS Technical (User) Support. Usual business hours for LDMS User Support are 7:00 am - 6:00 pm - (ET) from Monday through Friday. All other hours and weekends, an on-call user support -specialist will be available. Contact LDMS User Support at:

Email: *ldmshelp@fstrf.org*
Phone: +1-716-834-0900, ext. 7311
Fax: +1-716-898-7711

9.4.1 Off-Hours Contact Information

If you are locked out of your LDMS or are experiencing errors that prevent you from completing your LDMS lab work during off-hours, page LDMS User Support using the LDMS Web Pager utility. Alternatively, you may e-mail the paging system directly at *ldmspager1@fstrf.org*. Please allow at least 15 minutes to get a response before sending another e-mail to the paging system.

Each site must export its LDMS data to Frontier Science (FSTRF) on a weekly basis. Exported data are used by the MTN 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 the site. Sites are expected to resolve all discrepancies within two weeks of receipt of the report. The MTN LC 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 MTN SDMC reviews the discrepancy reports for critical samples (e.g., blood needed for confirmatory HIV testing) that appear to be missing, and works with the LC and site staff to undertake appropriate corrective action. All corrective action should be documented in paper-based clinic and/or laboratory records as appropriate, and entered in the details section of LDMS. The LC and SDMC will discuss and document any items that, although resolved, appear 'irresolvable' in LDMS.

Table 9-3
LDMS Specimen Management Guide to Logging in MTN-029/IPM 039 Specimens

The table below should be used as a guide when logging in MTN-029/IPM 039 specimens. Please use the LDMS codes listed below when logging in specimens for each test listed. Tests that are listed as local do not require that a sample be logged into the LDMS. The LDMS Tracking Sheet can be found on the MTN website (***www.mtnstopshiv.org***) under the MTN-029/IPM 039 study implementation materials.

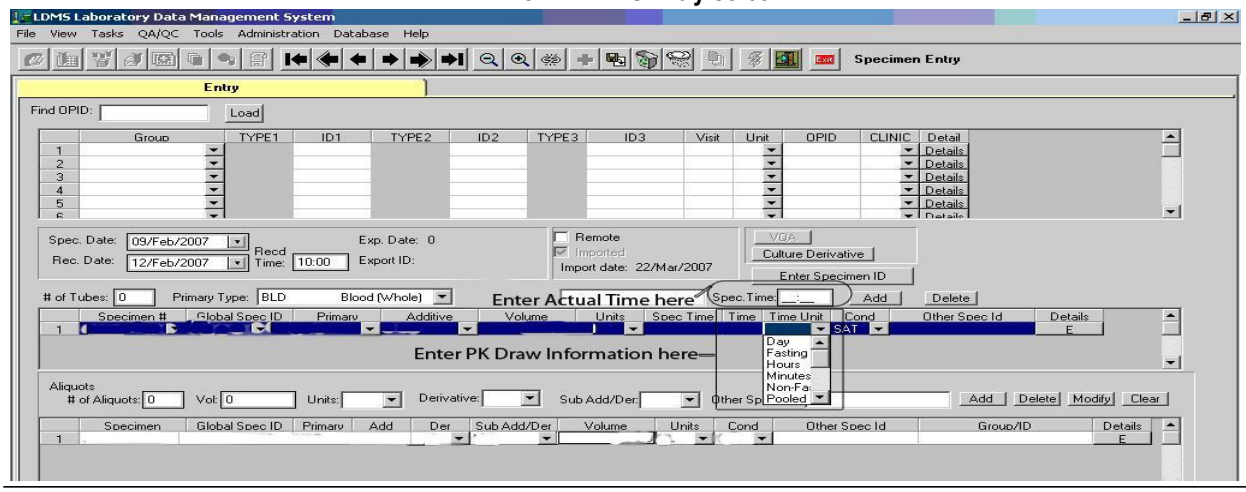
Test	Primary	Additive	Primary Volume	Time Unit	No. of Aliquots	Aliquot Volume	Units	Derv	Sub Add/ Derv	Other Spec ID
Plasma Archive/Storage	BLD	EDT	10.0 ML		4-5	1.0	ML	PL1/2	N/A	
Plasma for PK	BLD	EDT	10.0 ML	Pre-dose or hour	4-5	1.0	ML	PL1	N/A	PK
Vaginal Biomarkers	VAG	PBS	0.8 ML		2	0.4	ML	VAG	N/A	
Quantitative Vaginal Culture	VAG	CTK	2.0		1	2.0	EA	SWB	N/A	
Vaginal Gram Stain	VAG	NON	2.0		2	1	EA	SLD	GRS	
CVF for PK	CVF	NON	Net Weight	Pre-dose or hour	1	Net Weight	MG	SWB	N/A	
Breast milk for PD and PK	BMK	NON	8.0	Pre-dose or hour	4	2.0	ML	BMK	N/A	PD or PK
Breast milk Home Collection	BMK	NON	4.0	Day	2	2.0	ML	BMK	N/A	
Intravaginal Ring	IVR	NON	1		1	1	EA	IVR	N/A	

BLD: Whole Blood
BMK: Breastmilk
CTK: Culture Transport Kit
CVF: Cervicovaginal Fluid
EDT: EDTA
GRS: Gram Stain
IVR: Intravaginal Ring
NON: None
PBS: Phosphate Buffered Saline
PL1: Single spun Plasma
PL2: Double spun Plasma
SLD: Slide
SWB: Swab
VAG: Vaginal Swab
VSC: Vaginal Secretion

9.4.2 Logging in PK Samples

Enter the actual time using the 24 hour clock format in the Specimen Time area (Image 1) When applicable enter the PK time point information (0 pre-dose, 1 hour, 2 hour, etc.) in Time and Time Unit area (Image 1) otherwise leave blank.

IMAGE 1: LDMS Entry Screen



9.5 Urine Testing

The urine tests performed at the study visit will depend on the time point 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 aliquots will be made for each test when possible. When doing multiple tests from one specimen, the correct order is separation of urine for the Chlamydia and Gonorrhea first and then the urine dipstick last.

9.5.1 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 female participant not to clean the labia prior to specimen collection.
- Collect the first 15-60 mL of voided urine in a sterile collection cup. (Not mid-stream).
- Instruct the participant to screw the lid tightly onto the cup after collection.
- At visits when dipstick urinalyses and/or pregnancy testing is required, aliquot 5 to 10 mL for this test and store the remaining urine at 2-8°C or introduce the urine immediately into the UPT for subsequent Chlamydia and Gonorrhea testing.
- Note: only in situations where there is no NAAT testing and a clinician suspects a urinary tract infection, specimens may be collected per local specifications such as mid-stream clean catch.

9.5.2 Pregnancy Testing

At visits when pregnancy testing is required, aliquot approximately 5 to 10 mL of urine from the specimen collection cup and pipette from this aliquot for pregnancy testing. If the urine pregnancy test cannot adequately be interpreted because of interfering factors, for example excess blood or extreme cloudiness due to amorphous material, the sample can be spun down and the urine supernatant can be used. If the test continues to have interferences such as gross hemolysis making the test difficult to read, then another urine sample will need to be collected.

The Quidel QuickVue One-Step urine, Quidel Quick Vue Combo urine/serum, or Fisher HealthCare Sure-Vue urine hCG pregnancy test must be used at all sites. Perform the test according to site SOPs and the package insert.

The urine only kit and the combo kit are different kits and have different CAP method codes for EQA panels. If sites are running both kits, they must run CAP EQA panels on both kits. In most cases, the CAP results forms will only allow for entry of one kit. Sites can generally submit results to CAP for one kit and do a self-evaluation for the other kit. Consult SMILE, MTN LC or your PNL in case of questions regarding your EQA panels.

9.5.3 Urine Chlamydia and Gonorrhea Testing

This testing will be done using the Gen-Probe Aptima NAAT Method or the Cepheid GeneXpert NAAT method by the local or regional laboratory.

The laboratory that is performing the test must provide the clinic with the appropriate transport tube for the test being performed.

Instructions for transferring urine into the Gen-Probe UPT

1. Collect urine as noted above.
2. Open the UPT kit and remove the UPT and transfer pipette. Label the UPT with the participants PTID number and date.
3. 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.
4. 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.
5. Cap tightly and invert the tube 3-4 times to ensure that the specimen and reagent are mixed.
6. The specimen can now remain at 2-30°C for 30 days.

7. Place the transport tube in a biohazard zip-lock bag and transport to the local laboratory for testing.
8. The results are sent to the clinic and are reported on a STI Test Results CRF.

Instructions for transferring urine into the GeneXpert transport reagent tube

1. Collect urine as noted above.
2. Open the packaging of a disposable transfer pipette provided in the kit. Label the tube with the participants PTID number and date.
3. Remove the cap from the Xpert CT/NG Urine Transport reagent tube. Insert the transfer pipette into the urine cup so that the tip is near the bottom of the cup. Transfer approximately 7 mL of urine into the Xpert CT/NG Urine Transport reagent tube. The correct volume of urine has been added when the level reaches the black dashed line on the label.
4. Cap tightly and invert the tube 3-4 times to ensure that the specimen and reagent are mixed.
5. The specimen can remain at 2-30°C for 30 days.
6. Place the transport tube in a biohazard zip-lock bag and transport to the local laboratory for testing.
7. The results are sent to the clinic and are reported on a STI Test Results CRF.

9.5.4 Dipstick Urinalysis

Any of the Siemens urine reagent test strips may be used. Dip the urinalysis test strip into an aliquot of urine. Perform this test according to site SOPs and the package insert. Assess and record results for glucose, protein, leukocytes and nitrites. If leukocytes or nitrites are positive, perform a urine culture according to local SOP. To avoid overgrowth of bacteria, refrigerate specimen before and during transport to laboratory.

9.6 Blood Testing

The blood tests performed depend on the time point of the visit and potentially the clinical presentation of the participant. Perform all tests according to site SOPs and package inserts.

9.6.1 Specimen Collection and Initial Processing

Label all required primary tubes with a SCHARP-provided PTID label at the time of collection.

After collection:

- Allow plain tubes (no additive or serum separator) to clot, then centrifuge per site SOPs.
- Lavender top tubes (additive = EDTA) should be gently inverted at least eight times after specimen collection to prevent clotting. If whole blood for hematology testing and plasma are to be taken from the same tube, the hematology must be completed before the tube is centrifuged and aliquoted. If whole blood is to be used for multiple tests, ensure that the tube is well mixed before removing any specimen.

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 listed tube top colors.

9.6.2 HIV Testing

HIV testing must be validated at the study site per the CLIA standards, if applicable. All tests, and associated QC procedures, must be documented on local laboratory log sheets or other laboratory source documents.

HIV infection status at screening will be assessed using an FDA-approved HIV test per the HIV testing algorithm (see Appendix II in the current version of the MTN-029/IPM 039 protocol). If the test is negative, the participant will be considered HIV-seronegative. If the test is positive or indeterminate, an FDA-approved confirmatory test will be performed on the original sample. If there is insufficient sample to perform confirmatory testing, then additional blood must be collected. If the confirmatory test is negative or indeterminate, contact the LC for guidance.

If the participant enrolls and has a positive Sample 1 confirmation test, a second confirmation test will be conducted. If that second confirmatory test is negative or indeterminate, contact the LC for guidance. Please notify the MTN Virology Core (mtnvirology@mtnstopshiv.org) via e-mail of all possible seroconverters identified during a follow up visit by submitting a MTN Query Form which can be found on the MTN website. Once the MTN Virology Core has had an opportunity to review the form, a request for plasma storage to be shipped on dry ice to the MTN Virology Core may be issued. Be sure to provide the lab with the tracking number and details of each specimen prior to shipping.

Ship samples to MTN Virology Core (LDMS Lab 470)
Urvi Parikh
University of Pittsburgh
3550 Terrace Street
S804 Scaife Hall
Pittsburgh, PA 15261
Phone # 412-648-3103
Fax # 412-648-8521

Plasma storage (section 9.6.5) is required for further Laboratory Center HIV testing (HIV RNA and HIV drug resistance) of enrolled participants in the event of a positive HIV rapid or positive HIV EIA test result, and when additional samples are collected as part of algorithm testing at the site local lab to confirm a participant's HIV infection status. This sample will be logged into LDMS (Table 9-3) similar to plasma archive (Section 9.6.5).

All test results must be documented on local laboratory log sheets or other laboratory source documents. For non-CLIA sites, in addition to initialing or signing the testing logs to document review and verification of the results, the second lab staff member must also record the time at which the results were reviewed and verified.

9.6.3 Liver and Renal Function Testing

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

Liver Function

- Aspartate aminotransferase (AST)
- Alanine transaminase (ALT)

These chemistry tests will be collected and performed according to local laboratory SOPs.

9.6.4 Syphilis Testing

Syphilis testing can be performed using FDA approved tests in one of two ways:

1. Rapid Plasma Reagin (RPR) or Venereal Disease Research Laboratory (VDRL) screening test followed by a confirmatory test for Treponema pallidum. Any FDA approved Treponema pallidum confirmatory test can be used such as the Enzyme Immunoassay (EIA), microhemagglutinin assay for Treponema pallidum (MHA-TP), Treponema pallidum hemagglutination assay (TPHA), Treponema pallidum particle agglutination (TPPA), or fluorescent treponemal antibody (FTA-ABS). All positive RPR or VDRL results must have a titer reported. For reactive RPR or VDRL tests observed during screening, a confirmatory test is performed and appropriate clinical management action must be taken. MTN LC recommends for enrolled participants considered positive should include repeat non-treponemal assay tests at quarterly intervals following syphilis diagnosis to evaluate treatment effectiveness. If the RPR or VDRL titer does not decrease four-fold or revert to seronegative within three months after treatment, further investigation and/or treatment may be warranted.
2. Perform syphilis assessment using a specific FDA approved treponemal test (such as EIA, MHA-TP, TPHA, TPPA, or FTA-ABS) and confirming with a non-treponemal assay (RPR or VDRL). If the confirmatory non-treponemal assay is reactive at screening visit, appropriate clinical management action must be taken. These participants are not eligible

for enrollment in the study. If the RPR or VDRL is negative, this may indicate prior treatment, late latent disease, or a false positive test. MTN LC recommends additional testing using an alternative treponemal test other than the original treponemal test used for the original assessment so the participant can be correctly evaluated. (Of note, the FTA-ABS should not be used as the alternative confirmatory test due to performance issues). If the second confirmatory test is negative, the participant is not considered infected with syphilis. If the second confirmatory test is positive, the participant has had prior exposure to syphilis and depending on clinical scenario may or may not require treatment.

Please consult the MTN LC with any questions related to Syphilis testing to confirm treatment effectiveness and/or interpretation of unusual test results.

Questions related to result interpretation concerning eligibility and enrollment in the study should be directed to the MTN-029/IPM 039 Protocol Safety Physicians (***mtn029safetymd@mtnstopshiv.org***).

9.6.5 Plasma Archive/Storage

For plasma archive, use collection tubes with EDTA anticoagulant. Aliquot plasma into 2 ml cryovials, store at ≤-70°C, and batch onsite until the MTN LC study team requests shipping and/or testing.

1. If sample is collected and held at room temp, freeze plasma within 4 hours. If refrigerated or on ice after collection, freeze plasma within 24 hours.
2. If total whole blood volume is less than 10.0 mL, redraw as soon as possible.
3. Spin blood at room temperature in a centrifuge according to one of these techniques:
   - Single spun: Spin blood at 1500 x g for 10 minutes and remove plasma.
   - Double spun: Spin blood at 800 x g for 10 minutes, recover plasma and place in a tube to spin again at 800 x g for 10 minutes, remove plasma.
4. Prepare as many 1.0 mL aliquots as possible with a total volume of aliquots greater than or equal (≥) to 4ml
5. If less than 4 mL of plasma are available, store that plasma and inform the MTN LC for instruction.
6. If samples are hemolyzed, store the aliquots as per normal and enter comments in LDMS.
7. Log samples into LDMS (Table 9-3) and label aliquots with LDMS labels.
8. Store aliquots at ≤-70°C until requested.
9. The MTN LC will send instructions to the site when shipping and/or testing is required.

9.6.6 Blood for Dapivirine PK

Collect blood into a labeled 10 mL EDTA Vacutainer tube using either an indwelling venous catheter or direct venipuncture. Document collection time and product regimen on the LDMS tracking sheet.

1. Mix blood sample with the anticoagulant using gentle inversions (8 to 10 times).
2. Centrifuge the sample at approximately 1500 x g for 10 minutes at 4°C. The centrifugation must be completed and sample placed in the freezer within 8 hours of blood collection.
3. Transfer plasma to appropriately labeled 2.0 mL cryovials in as many 1.0 mL aliquots as possible.
4. Log samples into LDMS (Table 9-3) and label aliquots with LDMS labels
5. Store aliquots at ≤-70°C until batch shipped to JHU CPAL. The MTN LC will send instructions to the site when shipping is required.

Ship PK samples to JHU-CPAL (LDMS Lab 194)

James Johnson
Clinical Pharmacology Analytical Lab
Division of Clinical Pharmacology
The Johns Hopkins University School of Medicine
600 N. Wolfe Street, Osler 523
Baltimore, MD 21287

Lab Phone#: +1-410-955-9710 or +1-410-614-9978

9.7 Testing of Cervicovaginal Specimens

Cervicovaginal specimens will be collected in the order and manner stated in the clinical considerations section of this SSP Manual (section 7).

9.7.1 Test for *Trichomonas vaginalis* using OSOM Rapid Test

- Testing for *Trichomonas* is done using the OSOM Rapid *Trichomonas* test (manufactured by Sekisui Diagnostics).
- Use the rayon swab provided with the kit for collection.
- Affix a SCHARP-provided PTID label to a clean glass or plastic tube with a cap.
- Collect specimen using kit-provided swab from the lateral vaginal wall (fluids also may be collected from the posterior fornix; avoid collecting specimens from the cervix).
- Immediately place the swab in the labeled tube, break off the shaft of the swab, and cap the tube.
- Testing is expected to be performed during the participant visit. However, specimens may be stored at room temperature for 24 hours or refrigerated for 36 hours before testing.

9.7.2 Vaginal pH and Wet Preps, if indicated

BV will be diagnosed based on the presence of any three of the four Amsel's criteria:

- Homogenous vaginal discharge
- Vaginal pH greater than 4.5
- Positive whiff test
- At least 20% clue cells

Wet prep assessments used to diagnose BV and candidiasis are summarized in Table 9-4.

CLIA regulations require semi-annual wet mount proficiency testing. The MTN LC administers a web-based proficiency test approximately every six months. Wet mount slides on the MTN web pages are posted for this purpose every 6 months.

- Contact Lorna Rabe of the MTN LC (lrabe@mwri.magee.edu) to register names of clinicians who need to take the test.
- The registrants take the test and enter their answers directly on the website.
- The MTN LC sends a report of the results, including any necessary corrective action, to the Laboratory Manager.

Contact the MTN LC for additional information and guidance on performing and documenting the proficiency testing. Also contact the MTN LC when new laboratory staff is hired, so that appropriate training can take place prior to such staff performing wet mounts for study purposes.

Vaginal Fluid pH

Vaginal fluid pH will be assessed if clinically indicated for bacterial vaginosis. pH Indicator Strips (pH range 3.6 to 6.1) with brand names S/P Cardinal Health, Baker-pHIX, Whatman, or Machery-Nagel must be used at all sites.

Vaginal fluid pH swab (Dacron or cotton) may be collected in any of 2 ways depending on if a speculum is used at that particular visit:

- Obtained by the clinician during the pelvic examination
- Collected by the clinician in a non-speculum exam
- Note: a speculum is not required for pH sample collection.

Vaginal Fluid pH Procedure:

1. Swab onto the pH strip (Do not insert the pH strip into the vagina).
2. Match the resulting color of the indicator strip to the color scale provided with the strips to determine the pH value.

3. Record the pH value directly onto the appropriate case report form (CRF). It is not necessary to record pH values onto laboratory log sheets or other source documents prior to recording values onto CRFs.

Vaginal Fluid Wet Mount Testing, if indicated for BV and Yeast (KOH)

Wet mount procedures for this study are only performed if indicated, and consists of two different preparations: Potassium Hydroxide (KOH) and Saline. These procedures are for diagnosis of BV and candidiasis as summarized in Table 9-4.

Table 9-4
Summary of Wet Prep Assessments and Diagnostic Criteria

Assessment	Saline Prep	KOH Prep
Whiff Test	Not applicable	Positive if fishy amine odor detected
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.
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* and/or anaerobic GNR) to be counted as clue cells.	Not applicable (clue cells are lysed by KOH)

Preparation and Examination of Wet Prep Slides

Materials:

- Pencil
- 2 SCHARP labels, 3 if using optional tube
- 2 frosted end slides
- Glass or plastic tube, optional
- Sterile physiologic saline
- 10% KOH
- Dacron Swab
- 2 cover slips
- Microscope, 10× and 40× magnification

1. 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)
2. Immediately following collection from the lateral vaginal wall via swab (Dacron or cotton), 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.
3. Apply one drop of 10% KOH to one slide and immediately perform whiff test for a “fishy” amine odor. Then apply cover slip.
4. Apply one drop of sterile physiologic saline to the second slide, emulsify with the vaginal fluid specimen, and then apply cover-slip. Examine immediately at 10× magnification for epithelial cells, budding yeast, and pseudohyphae. Examine at 40× 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.
5. Examine the KOH slide at both 10× and 40× magnification for yeast and pseudohyphae.

RESULTS:

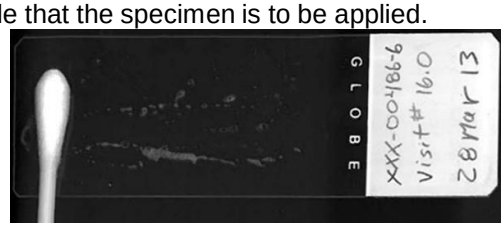
- If wet prep slides are read in-clinic by clinical staff, results may be recorded directly on to appropriate case report forms (CRFs).
- If slides are read by lab staff (either in the local laboratory or a designated in-clinic lab area), results must be recorded on laboratory log sheets or other laboratory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

9.7.3 Gram Stains of Vaginal Fluid

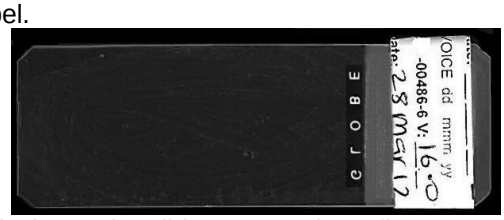
Dried vaginal fluid smears will be prepared for Gram staining and assessment for bacterial vaginosis at the MTN LC. Two slides (one designated as primary and the other as secondary) will be prepared at each required time point and both will be entered into LDMS. The primary slide will be shipped to the MTN LC and the secondary will be archived on site until written notification is received from the Statistical Center for HIV/AIDS Research & Prevention (SCHARP) that the slide may be discarded.

Instructions for slide preparation and shipping are provided below:

1. Use a pencil to write the PTID and specimen collection date on the frosted end of the slide. This is the side of the slide that the specimen is to be applied.



2. Immediately following specimen collection from the lateral vaginal wall via swab (Dacron or cotton), roll the swab across each of the slide. (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.
3. A SCHARP-provided PTID label is to be placed on the underside of the slides (on the frosted end, under the pencil markings); write the specimen collection date in indelible ink (e.g. Sharpie pen) on each label.



4. Allow the specimens to air-dry on the slides. Do not heat-fix.
5. Vaginal smears for gram stain are to be logged into LDMS (Table 9-3) and label the slides with LDMS labels. Place the LDMS label on the frosted end of the slide on top of the pencil markings (same side as sample).

6. The primary slides will be positioned in a plastic slide holder and sent to the MTN LC. If there is no culture on the visit for which a gram stain is collected, then hold the gram stain slides until other samples are to be sent to the Magee-Womens Research Institute. (See shipping instructions below).
7. Store the secondary slide in the slide box location assigned in LDMS at room temperature. (This is a backup slide in case the first is lost, broken, or unreadable).

9.7.4 Quantitative Vaginal Culture

In addition to the wet mounts and Gram stains, vaginal swabs will be collected for Quantitative cultures and sent to the MTN LC. Shipping instructions follow.

- Collect the specimen for culture by rotating 2 Dacron swabs several times over the lateral wall of the vagina. Do not collect culture swabs in the exact same area that another sample was collected (i.e. If the PK swab was collected first, then collect in a different location in the vagina preferably closer to the introitus). Insert the two swabs attached to the cap into the tube.
- The specimen may be kept at controlled room temperature for up to 4 hours. After four hours, the specimen must be refrigerated.
- Deliver the specimen and the LDMS specimen tracking sheet to the local LDMS laboratory.
- Log the specimen into LDMS (Table 9-3) and label the tube with LDMS labels.
- Use LDMS to generate a shipping manifest for the cultures to be shipped to lab 414.
- Ship the specimen and the vaginal smear for Gram stain the same day of collection by overnight courier.
- Place the specimen in a biohazard bag and secure in the leak-proof container with absorbent material. Place the container, ice packs, slides, and a copy of the manifest in a cardboard box lined with Styrofoam.
- Use diagnostics packing code 650, UN3373.
- Confirm the address is correct (see below). The Research Institute is not open for weekend deliveries. Therefore, specimens collected on Friday must be sent to the hospital address for delivery on Saturday.

  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 of UPMC
  300 Halket St.
  Pittsburgh, Pa. 15213
  Phone # 412-641-4191 (contact number for Safety and Security)
  ** Be sure to check Saturday delivery on the Fed Ex label

Notify the MTN LC via email (***lrabe@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.

9.7.5 Cervicovaginal Testing for GC/CT (Neisseria gonorrhea and Chlamydia trachomatis) by NAAT

Testing for chlamydia and gonorrhea is performed at screening and when clinically indicated. Sites can choose to use the Gen-Probe Aptima or Cepheid GeneXpert.

- Swab the cervix.
- Immediately place the swab in the transport tube, break off the shaft of the swab, and cap the tube.
- Transport the specimen at ambient temperature to the local laboratory.

9.7.6 Papanicolaou (Pap) Test

At visits when Pap smears are indicated, 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. There is no required external review of these procedures by the MTN.

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 (including HPV), Pap smear findings associated with STIs should not be used to diagnose any STIs.

9.7.7 Herpes Culture

Testing will be performed per local lab SOP.

9.7.8 Cervicovaginal Fluid for PK

- Each day of collection of cervicovaginal fluid for PK, perform QC that would be required for the analytical scale to accurately weigh samples to a weight of at least 0.1 milligrams. Do not turn off balance until weighing for the day is completed.
- PK swab must be collected within one hour of PK blood draw.
- Ensure that new or sterilized supplies are used for each sample as Dapivirine is very sensitive to cross-contamination.
- There are two methods to collecting and weighing the swab for PK. Collection may be obtained with a pre-cut swab or swab may be cut after collection. Please see instructions below. Sites may choose either method based on site preference.

<u>Pre-cut Swab Collection Method</u>
Materials for each collection:

- SCHARP label with PTID, visit number, and visit date
- 2-mL Nalgene cryovial containing pre-cut Polyester-Tipped (Dacron) Swab
- Hemostat or Ring Forceps (recommend 8 inches or longer)
- Analytical scale (accurate to 0.1 milligrams)

1. Affix SCHARP label to the cryovial containing the pre-cut swab.
2. Perform pre-weight measurement by weighing the labeled capped cryovial with pre-cut swab and record on the LDMS Tracking Sheet.
3. Uncap the pre-weighed cryovial. Use a clean hemostat or forceps to clamp on to the shaft of the swab.
4. Insert the hemostat/forceps holding the swab into the upper vagina near the cervix to the location nearest to where the ring resides without touching the ring. For 10-20 seconds, rotate in a circular motion touching all walls to absorb as much fluid as possible.
5. Immediately place swab into the cryovial after sampling and recap.
6. Perform post-weight measurement by weighing the capped cryovial containing the absorbed swab tip and record on the LDMS Tracking Sheet.
7. Calculate and record the NET weight on the LDMS Tracking Sheet.
8. Within 2 hours, place the sample tubes in the freezer at ≤-70°C.
9. Log into LDMS (Table 9-3) and label specimen with LDMS label.
10. Batch ship to JHU CPAL (LDMS Lab 194) upon request.

<u>Post-cut Swab Collection Method</u>

Materials for each collection:

- 2 SCHARP labels with PTID, visit number, and visit date
- 2-mL Nalgene cryovial
- Polyester-Tipped (Dacron) Swab
- Zip-lock biohazard sample bag
- Plastic cup (without lid) or similar lightweight container, placed on middle of scale, to contain items to be weighed. (Some balances have an optional basket.)
- Scissors to cut swab shaft

1. Place identically-labeled SCHARP labels on the cryovial and the biohazard sample bag.
2. Perform pre-weight. Handle items to be weighted with gloves.

a. Zero the cup or similar container on the scale.
b. Place the labeled 2-mL cryovial and the packaged sterile Dacron swab upright in the cup. (Make sure it is not leaning on a part of the scale.)
c. Record this pre-weight on the LDMS Tracking Sheet.
d. Place the cryovial and the packaged Dacron swab in a labeled biohazard sample bag.

3. Sample collection

NOTE: **All** of the items in the bag should return to the bag. Nothing will be thrown into the garbage.

a. Remove swab from packaging. Do **NOT** discard the packaging. Place all of the packaging back into the bag.
b. Collect vaginal fluid holding the swab into the upper vagina near the cervix to the location nearest to where the ring resides without touching the ring. For 10-20 seconds, rotate in a circular motion touching all walls to absorb as much fluid as possible.
c. Place the swab in the cryovial and cut the swab shaft using scissors at the pivot point. Be sure to hold onto the shaft to avoid losing it. Do **NOT** discard the shaft!
d. Place the cut shaft in the specimen bag.
e. Screw the lid back on the cryovial and place sample in the bag with the swab packaging and the swab shaft.
f. Document the collection time on to the LDMS tracking sheet.

6. Perform Post Weight:

a. Zero the cup or similar lightweight container on the scale.
b. Weigh the capped cryovial containing the absorbed swab tip, the swab packaging and the remainder of the swab shaft (Suggestion: Place the swab shaft into the packaging and have it upright during weighing.)
c. Record post-weight on the LDMS Tracking sheet then calculate and record the NET weight.

7. Within 2 hours, place the sample tubes in the freezer at ≤-70°C.
8. Log into LDMS (Table 9-3) and batch ship to JHU CPAL (LDMS Lab 194) upon request.

9.7.9 Vaginal Biomarkers

At each pelvic exam, vaginal fluids are collected from the posterior fornix using two separate Dacron swabs with a plastic shaft for biomarker analysis at the MTN LC.

1. Collect vaginal fluid using a Dacron swab from the posterior fornix.
2. Place the swab in a SCHARP labeled cryovial containing 400 µL PBS (1× Concentration).
3. Break shaft of swab at a minimum of 1cm beyond the swab and cap the vial.
4. Repeat with the second Dacron swab as described above.
5. Store refrigerated or on ice for up to 8 hours prior to delivery to the lab.
6. Deliver the tubes and an LDMS Specimen Tracking Sheet to the local LDMS laboratory within 8 hours.
7. Log the cryovial into LDMS (Table 9-3) and label each vial with a LDMS label. Avoid covering the entire PID on the original SCHARP label.
8. Freeze at ≤ -70ºC until the LC requests shipment

9.8 Testing of Breast Milk Samples

Breast milk samples will be collected at various time points specified by the protocol for PK and PD evaluation. Samples may be collected at home or in the clinic.

Breast milk collection may be obtained from one or both breasts. The participant should empty all the milk from at least one side. If milk is collected from both breasts, the milk can be combined in a single container.

1. Gently swirl to mix.
2. Label cryovials with the participant's PID, collection date and time.
   - During clinic visits, 4 cryovials of breast milk will be required per collection. Two cryovials

will be collected for PK analysis while another two cryovials will be used for PD analysis.
    - When collecting breast milk at home, only two cryovials are required per collection.
3. Using a disposable pipette, transfer approximately 2.0 ml breast milk into each cryovial.
4. Freeze aliquots immediately at approximately -20°C or colder.
    - When using a household freezer to store sample temporarily, place the vials toward the back of the freezer.
    - When bringing the samples collected at home to the clinic please be sure to transport them on ice in a cooler to minimize thawing of breast milk.
5. Complete the LDMS Tracking Sheet.
6. Log aliquots into LDMS (Table 9-3).
    - For specimens collected during study visits, be sure to identify two PK aliquots with “PK” in the LDMS Other Spec ID fields and two PD aliquots with “PD” in the corresponding Other Spec ID fields.
    - For specimens collected at home enter the study day into the PK time and time unit (See MTN 029 Breast Milk Home LDMS Tracking Sheet).
7. Label the aliquots with LDMS labels.
8. Breastmilk for PK analysis will be batched shipped to JHU CPAL (LDMS Lab 194). Breast milk for PD analysis and home collected samples will be batched and shipped to the MTN LC (LDMS Lab 414) upon request.

9.9 Testing of Intravaginal Ring (IVR)

Used rings will be analyzed for residual levels of Dapivirine, and will be collected at visit 5. The used rings may contain vaginal secretions and therefore must be treated as a biohazard. The rings will remain in the amber pouch and stored at room temperature until further notice from the MTN LC. Rings that are defective or inserted briefly and removed for various reasons may be destroyed at the site via biohazard procedures.

Important notes:
If the ring is removed by the participant prior to the clinic visit and will not be reinserted, then the used ring is still prepared for residual drug analysis (see Counseling Considerations Section 10). After the used ring is taken out of the participant’s bag (bag or pouch they returned the ring in), follow directions starting with step 1.

1. Wear lab coat, gloves, and protective face guards when performing this step. The clinician will remove the used ring and place in a sterile container (disposable cup) with tap water. Move the ring around in the water or swirl the container to remove vaginal material. Take the ring out of the water and blot dry with paper towels or gauze. The ring should be dry before storing in pouch. Dispose of blotting materials and contaminated water according to your institution biohazard policy.
2. Site staff will place the ring into a new 3 5” amber Zippit pouch. Label the pouch with the participant ID number and visit number. Add a biohazard sticker if one is not already attached to the pouch, making sure not to cover the identifier information.
3. Store the used ring within the biohazard labeled amber pouch at room temperature.
4. Log the ring into LDMS (Table 9-3) and batch ship upon request by the MTN LC.