Section 9. Laboratory Considerations

9.1 Overview and General Guidance

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. The site 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 Johns Hopkins University Clinical Pharmacology Analytical Lab (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, the study staff that performs the tests must be trained in proper testing methods and associated quality control (QC) procedures prior to performing the tests for study purposes; training documentation should be available for inspection at any time.

All site laboratories will be monitored by the MTN LC which will utilize information from DAIDS monitoring groups (PNL, IQA, VQA, etc.) to monitor and certify laboratories for testing. US laboratories that are certified by CLIA (Clinical Laboratory Improvement Amendment) will be able to substitute this for some of the documentation requirements required of other labs. Valid CLIA certificates must be provided in these cases. Please refer all questions related to laboratory testing to the MTN LC using the following e-mail address: MTNNetworkLab@MTNStopsHIV.org

The site is responsible for ensuring specimen volumes do not exceed what is described in the informed consent and/or during 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 clinically indicated testing.

Ideally, one method, one type of test kit, and/or a 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 test 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. Similarly, the MTN LC must be notified of changes to normal lab ranges.

Provided in the remainder of this section is information intended to standardized laboratory procedures at the site. Adherence to the specifications of this section is essential to ensure that data derived from laboratory testing will be considered acceptable to all regulatory authorities.

This section of the MTN-014 SSP manual gives basic guidance to the site, but is not an exhaustive procedures manual for all laboratory testing. This section must be supplemented with site standard operating procedures (SOPs). 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-014

The site is 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
Qualitative hCG	In Clinic	Urine	Plastic Screw Top Cup	Quidel QuickVue One-Step, Quidel QuickVue Combo or Fisher Healthcare Sure Vue
Dipstick Urinalysis[1]	In Clinic	Urine	Plastic Screw Top Cup	Siemens Multistix® 10 SG or Uristix 4 or other LC approved method
Urine Culture[1]	Local Lab	Urine	Plastic Screw Top Cup	Local Methodology
Urine NAAT for Gonorrhea and Chlamydia[1]	Local Lab	Urine	Kit Specific Transport Tube	GenProbe Aptima, BD ProbeTec, or GeneXpert
CBC with platelets	Local Lab	Whole Blood	EDTA Tube 4mL	Local Methodology
Chemistries (Creatinine, ALT, AST)	Local Lab	Serum, Plasma, or Whole Blood	Consult Local Lab Requirements	Local Methodology
HIV-1 Serology	Local Lab or MTN Virology Core (WB only)	Plasma, Serum or Whole Blood	EDTA or Plain Tube 4mL	FDA approved tests
Syphilis Serology	Local Lab	Serum or Plasma	EDTA, Plain or Serum Separator Tube 4mL	Local Methodology
Plasma archive	On-Site until notified by MTN LC	Plasma	EDTA tube 10mL	MTN LC Protocol
Plasma for PK	JHU CPAL	Plasma	EDTA tube 10mL	JHU Protocol
PBMCs for PK	JHU CPAL	Whole Blood	Na Citrate-CPT tube 3x8mL	JHU Protocol
PT/INR	Local Lab	Whole Blood	Light Blue (Na Citrate) 4mL	Local Methodology
Vaginal NAAT for Gonorrhea and Chlamydia	Local, Regional or MTN LC	Vaginal Swab	Kit Specific Transport tube	GenProbe Aptima, BD Probe Tec, or GeneXpert
Vaginal Gram Stain	MTN LC	Vaginal Swab	Slides x2	MTN LC Protocol
Rapid Trichomonas	Local Lab or In Clinic	Vaginal Swab (supplied with kit)	Sterile Tube with No Additives	OSOM Kit
Vaginal saline wet preparation on females (for BV and/or KOH wet mount)[1]	In clinic	Vaginal Swab	Tube with 6 drops of Saline	MTN LC Protocol
Vaginal pH	In clinic	Vaginal swab	N/A	S/P pH Indicator Strips
PAP Smear[2]	Local Lab	Ecto & Endo-Cervical Cells	Local Lab Requirements	Local methodology

Test	Testing Location	Specimen Type	Tube or Container and tube size (recommended)	Kit/Method
Vaginal biomarkers	MTN LC	Vaginal Swab	Cryovial with 400ul PBS	MTN LC Protocol
Vaginal Biopsies for PK	JHU CPAL	2 Biopsies	1.8 mL cryovial	JHU Protocol
Vaginal Biopsies for Gene Expression Array	MTN LC	1 Biopsy	1.8mL Cryovial with RNA*later*	MTN LC Protocol
Vaginal fluid for PK	JHU CPAL	Vaginal Swab	1.8mL Cryovial	JHU Protocol
CVL for PK, PD, and Biomarkers	MTN Network Lab & JHU CPAL	Fluid & Pellet Recovered from CVL (Saline Used)	50 mL Conical Vial	MTN LC Protocol
Cervical Cytobrush for PK	JHU CPAL	Cytobrush	5 ml Cryovial	JHU Protocol
Cervical biomarkers	MTN LC	Cervical Swab	Cryovial with 400ul PBS	MTN LC Protocol
Rectal NAAT for Gonorrhea and Chlamydia	Local, Regional or MTN LC	Rectal Swab	Kit Specific Transport tube	GenProbe Aptima or GeneXpert
Rectal fluid for PK, PD, and biomarkers	MTN Network Lab & JHU CPAL	Rectal Sponges	5ml Cryovial	MTN LC Protocol
Rectal Biopsies for PK	JHU CPAL	4 Biopsies	1.8 mL Cryovial	JHU CPAL Protocol
Rectal Biopsies for Gene Expression Array	MTN LC	2 Biopsies	1.8mL Cryovial with RNA*later*	MTN LC Protocol
Rectal Biopsy for Histology	MTN LC	1 Biopsy	Cryovial with 1.5ml 10% Formalin	MTN LC Protocol
Rectal Biopsy for Proteomics	MTN LC	1 Biopsy	1.8mL Cryovial	MTN LC Protocol

1. Perform only if clinically indicated per local SOP.
2. Perform only if clinically indicated or if participant does not have a documented satisfactory Pap within the 12 months prior to Enrollment.

Volumes may vary depending on the site's testing platforms. Please confirm with the testing lab to determine minimum volume requirements. The site is 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.
CPT 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:
Leftover Urine from hCG (See Section 9.5.2)	None	Sterile Cup	Store refrigerated and freeze within 8 hours of collection.	Batch to MTN LC
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. Process within 8 hours of collection.	Batch to JHU CPAL
PBMCs for PK	CPT (Na Citrate)	3x8mL	Isolate and lyse PBMCs per JHU Protocol. Process and freeze within 8 hours of collection.	Batch to JHU CPAL
Vaginal Gram Stain	None	Slide	Store at room temp.	Batch to MTN LC
Vaginal and Cervical Biomarkers	PBS	Cryovial	Store refrigerated and freeze within 8 hours of collection.	Batch to MTN LC
Vaginal Fluid for PK	None	Cryovial	Freeze within 2 hours of collection.	Batch to JHU CPAL
Vaginal biopsy for PK	None	1.8mL Cryovial	Flash freeze within 2 hours of collection	Batch to JHU CPAL
Vaginal biopsy for Gene Expression	RNAlater	1.8mL Cryovial	Store at 4ºC overnight (16-24 hours) then transfer to ≤-70°C.	Batch to MTN LC
CVL for PK	Saline	2mL Cryovial	Freeze supernatant within 8 hours of collection.	Batch to JHU CPAL
CVL for PD and Biomarkers	Saline	2mL Cryovial	Process and freeze within 8 hours of collection.	Batch to MTN LC
Cervical Cytobrush for PK	PBS	5 ml cryovial	Process within 2 hours of collection.	Batch to JHU CPAL
Rectal Sponge for PK	None	Merocel Sponge in 5mL Cryovial	Freeze within 2 hours of collection	Batch to JHU CPAL
Rectal Sponge for PD and Biomarkers	None	Merocel Sponge in 5mL Cryovial	Freeze within 2 hours of collection	Batch to MTN LC
Rectal Biopsies for PK	None	1.8mL Cryovial	Flash freeze within 2 hours of collection	Batch to JHU CPAL

Specimen and Subsequent Testing	Additive	Tube type or size recommendation	Processing and Storage	Ship to:
Rectal Biopsy for Mucosal gene expression	RNA*later*	1.8mL Cryovial	Store at 4ºC overnight (16-24 hours) then transfer to ≤-70°C.	Batch to MTN LC
Rectal Biopsy for Histology	10% Formalin	2.0mL tube	Store at room temperature	Scheduled shipment to MTN LC
Rectal Biopsy for Proteomics	None	1.8mL Cryovial	Flash freeze and store at ≤-70°C within 2 hours of collection	Batch to 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 Sharpie pen).

Microscope slides used for evaluation of vaginal/cervical 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. 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 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 LC 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 lab related protocol deviation

If site staff has any question regarding time windows or collection processes, call LC staff (Pam Kunjara 412-641-6393) or e-mail 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 to track storage and shipping of laboratory specimens. It is supported by the Frontier Science Foundation (FSTRF). LDMS must be used at the site to track the collection, storage, and shipment of specimens listed in Table 9-3. Detailed instructions for use of LDMS are provided at: https://www.fstrf.org/ldms (may require a password).

The site is 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. The site 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-014 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) 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: +716-834-0900, ext 7311
Fax: +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.

The site must export its LDMS data to Frontier Science (FSTRF) on a weekly basis. Exported data are used by the MTN Statistical and Data Management Center (SDMC) to generate a monthly specimen repository report and to reconcile data entered in LDMS with data entered on study case report forms (CRFs). Any discrepancies identified during the reconciliation are included in a monthly discrepancy report for each site. The site is expected to resolve all discrepancies within two weeks of receipt of the report. The MTN LC is responsible for reminding the site to adhere to the two week timeframe and for following up to 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 014 Specimens

The table below should be used as a guide when logging in MTN 014 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 014 study implementation materials.

Test	Primary	Additive	Primary Volume	No. of Aliquots	Aliquot Volume	Units	Derv	Sub Add/ Derv	Other Spec ID
Leftover Urine from hCG (See Section 9.5.2)	URN	NON	5.0 ML	5	1.0	ML	URN	N/A	
Plasma Archive	BLD	EDT	10.0 ML	4-5	1.0	ML	PL1/2	N/A	
Plasma for PK	BLD	EDT	10.0 ML	4-5	1.0	ML	PL1	N/A	PK
PBMCs for PK	BLD	CPS	24.0 ML	1	Cell count	CEL	CIO	MET	
Vaginal Gram Stain	VGL	NON	2	2	1	EA	SLD	GRS	
Vaginal Biomarkers	VAG	PBS	0.80	2	0.4	ML	VAG	N/A	
Vaginal Fluid for PK	VAG	NON	1 EA	1	1	EA	VAG	N/A	
Vaginal Biopsies PK	VGL	NON	2	2	Varies by weight	MG	BPS	N/A	
Vaginal Biopsies Gene Expression	VGL	RNL	1	1	1	EA	BPS	N/A	
CVL for PK, PD and Biomarkers	CVL	NSL	6-12 ML	6-12	1	ML	FLD	N/A	
				1	1	ML	CEN	PBS	
Cervical cytobrush PK	CER	PBS	3.5 ML	1	1	ML	CTB	MET	
Cervical Biomarkers	CXS	PBS	0.80	2	0.4	ML	CXS	N/A	
Rectal Sponge for PD and biomarkers	REC	NON	2 EA	2	Weight	MG	SPG	N/A	PD
Rectal Sponge for PK	REC	NON	1 EA	1	Weight	MG	SPG	N/A	PK
Rectal Biopsies for PK	FSR	NON	4 EA	4	Weight	MG	BPS	N/A	PK
Rectal Biopsy for Gene Expression	FSR	RNL	2 EA	2	1	EA	BPS	N/A	
Rectal Biopsy for Histology	FSR	FOR	1 EA	1	1	EA	BPS	N/A	
Rectal Biopsy for Proteomics	FSR	NON	1 EA	1	1	EA	BPS	N/A	PRO

BLD: Whole Blood
BPS: Biopsy
CEL: PBMCs, viable
CEN: Fresh Cells from a Non-Blood Specimen Type
CER: Cervix
CIO: Cells in other solution, Non-viable
CPS: Cell Preparation Tube SCI
CTB: Cytobrush
CVL: Cervical Vaginal Lavage
CXS: Cervical Swab
EDT: EDTA
FLD: Fluid Portion from a Non-Blood Specimen Type
FSR: Rectal biopsy by flexible sigmoidoscopy
GRS: Gram Stain
MET: Methanol
NON: None
NSL: Normal Saline
PBS: Phosphate Buffered Saline
PL1: Single spun Plasma
PL2: Double spun Plasma
REC: Rectal
RNL: RNAlater
SLD: Slide
SPG: Sponge
URN: Urine
VAG: Vaginal Swab
VGL: Vagina

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. At study visits when urine testing is required, a single specimen will be collected and then aliquoted for each test when possible. When doing multiple tests from one specimen, an aliquot of urine should first be obtained for pregnancy testing and/or dipstick urinalysis and the remaining specimen should be reserved for chlamydia and gonorrhea testing if indicated.

Collect urine specimens before collecting any pelvic specimens.

9.5.1 Urine 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.
- Collect the first 15-60 ml of voided urine in a sterile collection cup (not mid-stream).
  - Note: If only testing for urine culture and/or pregnancy, then collect midstream urine.
- Instruct the participant to screw the lid tightly onto the cup after collection.
- At visits when pregnancy testing is required, and dipstick urinalysis is indicated, aliquot 5-10 ml for these tests and store the remaining urine at 2-8° C or introduce the urine immediately into the UPT for subsequent chlamydia and gonorrhea testing. *For participants who have consented to Long-Term Storage and Future Testing ONLY:* Leftover urine collected is to be stored in as many 1.0mL aliquots as possible and frozen at ≤-70˚C.

9.5.2 Urine Pregnancy Testing

The Quidel QuickVue One-Step hCG urine, Quidel QuickVue Combo hCG urine/serum pregnancy or Fisher Healthcare Sure Vue Urine hCG test must be used at the site. Perform the test according to site SOPs and the package insert. Do not perform any other urine pregnancy tests for confirmatory purposes.

Pregnancy status is a critical participant safety consideration. The site must maintain an adequate inventory of test kits at all times. Inventory should be monitored closely and re-supply orders placed at least 8-12 weeks in advance of actual need (or longer if needed per site procurement policies and procedures). The date and time of pregnancy testing must be documented.

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.

For participants who have consented to Long-Term Storage and Future Testing ONLY: Leftover urine collected for hCG is to be stored in as many 1.0mL aliquots as possible and frozen at ≤-70˚C. No preservative is required. These stored aliquots will be logged into LDMS and reported on the SCHARP Specimen Storage CRF.

- Urine must be kept refrigerated.
- Aliquot urine into cryovials at 1.0ml volume each.
- Freeze within 8 hours of collection.
- The MTN LC will send instructions to the site when shipping and/or testing is required.

9.5.3 Dipstick Urinalysis

Perform only if indicated as part of local standard of care. At visits when dipstick urinalysis is indicated, dip the urinalysis test strip into an aliquot of urine. At visits when both pregnancy testing is required and

dipstick urinalysis is indicated, 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.

Bayer/Siemens urine test strips must be used at the site. The site may choose test strips that have all tests necessary at a specified visit. Perform this test according to site SOPs and the package insert. Inventory should be monitored closely and re-supply orders placed at least 8 to 12 weeks in advance of actual need (or longer if needed per site procurement policies and procedures). Notify the LC immediately if any kit inventory or quality control problems are identified, so that appropriate action can be taken.

9.5.4 Urine Culture

Perform only if indicated as part of local standard of care. Midstream urine is to be collected in a sterile collection cup with no additive and processed according to local standards.

9.5.5 Chlamydia and Gonorrhea Testing in Urine

Perform only if clinically indicated at Enrollment and at follow-up visits. Urine is preferred if the participant has been on product within the past 24 hours.

Instructions for transferring urine into the Urine Preservation Tube (UPT)

1. Collect first void urine.
2. Open the UPT kit (either BD ProbTec or GenProbe Aptima) 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. 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. If testing with ProbeTec the urine does not need to be transferred into the UPT if the urine is kept at 2-8°C and processed within 7 days of collection, or kept at -20°C and processed within 2 months of collection.

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 be stored up to 45 days at 2-15°C or up to 3 days at 16-30°C.

9.6 Blood Testing

The blood tests performed depends 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 if applicable.

9.6.1 Specimen Collection and Initial Processing

The order of blood draw for multiple tube collections should be Citrate tube, SST and/or serum tube, Heparin tube, then EDTA tube.

Label all required primary tubes with a SCHARP-provided PTID label at the time of collection. After collection, complete the following:

- Allow serum tubes (i.e., no additive such as red top or serum separator tubes) to clot, then centrifuge per site SOPs to yield serum.
- Gently invert tubes with anticoagulant (i.e., EDTA, heparin) at least eight times after specimen collection to prevent clotting. If whole blood and plasma are to be taken from the same tube, the whole blood testing 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.

9.6.2 Plasma Archive

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.

- 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.
- If total whole blood volume is less than 2.0 mL, redraw as soon as possible.
- 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.
- Prepare as many 1.0 mL aliquots as possible with a total volume of aliquots greater than or equal (≥) to 4ml
- If less than 4 mL of plasma are available, store that plasma and inform the MTN LC for instruction.
- If samples are hemolyzed, store the aliquots as per normal and enter comments in LDMS.
- The MTN LC will send instructions to the site when shipping and/or testing is required.

9.6.3 HIV Testing

Although the HIV algorithm (Appendix II of the MTN 014 protocol) allows for EIA testing, rapid testing is recommended in order to obtain immediate results confirming participant eligibility and continuation of study participation.

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-014 protocol). If the test is negative, the participant will be considered HIV-seronegative. If the test is positive or indeterminate and this patient has already been enrolled into the study, an FDA-approved Western Blot (WB) test will be performed on the original sample. If there is insufficient sample to perform WB, then additional blood must be collected. If the WB is negative or indeterminate, contact the LC for guidance. Notify the LC immediately if any kit inventory or quality control problems are identified, so that appropriate action can be taken.

If the site no longer has local access to HIV WB testing, plasma samples may be shipped overnight on dry ice to the MTN Virology Core. Please notify the MTN LC (pkunjara@mwri.magee,edu) and Virology Core (ump3@pitt.edu) via e-mail with tracking number and details of each specimen prior to shipping. These samples will not be entered into LDMS.

Ship samples to MTN Virology Core:
Urvi Parikh/ Kristen Cummings
University of Pittsburgh
3550 Terrace Street

S804 Scaife Hall
Pittsburgh, PA 15261
Phone # 412-648-3103
Fax # 412-648-8521

9.6.4 Syphilis Testing

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

1. Rapid plasma reagin (RPR) 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 results must have a titer reported. For reactive RPR tests observed during screening, a confirmatory test is performed and appropriate clinical management action must be taken prior to enrollment in the study. If the second confirmation test is positive, they cannot be enrolled at this time. 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 IgG 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 prior to enrollment in the study. If the RPR or VDRL is negative, this may indicate that the participant may have been previously treated, has an advanced latent disease, or the original test was a false positive. MTN LC recommends additional testing preferably using different antigens than the original treponemal IgG test so the participant can be correctly evaluated. If the second confirmation test is negative, the participant is not considered infected with syphilis. If the second confirmation test is positive, they cannot be enrolled at this time. 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.

   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-014 Protocol Safety Physicians (mtn014safetymd@mtnstopshiv.org).

9.6.5 Blood Chemistries

The following chemistry tests will be performed per local SOPs:

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

9.6.6 Hematology Testing

Complete blood counts will be performed on EDTA whole blood per local site SOPs at the site. Each of the following must be analyzed and reported:

- Hemoglobin
- White blood cell count with platelets

9.6.7 INR/PT

Testing will be performed on whole blood collected in light blue tubes (Na Citrate) per local SOP

9.6.8 Tenofovir Levels in blood

Plasma Tenofovir

Collect blood into a labeled 10 mL EDTA Vacutainer tube using either an indwelling venous catheter or direct venipuncture. Document collection time on 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 store at ≤-70°C until shipped to JHU CPAL).
5. One aliquot will be shipped to the JHU CPA and assayed for Tenofovir at conclusion of study unless informed otherwise. The remaining samples will be retained at the site until advised by the MTN LC.

Peripheral Blood Mononuclear Cells (PBMC) for Intracellular Tenofovir

- Draw three 8 mL Cell Preparation Tubes (CPT) with Sodium Citrate (BD Cat# 362761 is recommended) at each PK time point. Document collection time on LDMS tracking sheet.
- Specimens must be kept upright at room temperature (22-25°C) and processed within 8 hours. The following instructions were obtained from the Johns Hopkins SOP.

1. Invert CPT tubes gently to mix anticoagulant thoroughly.
2. Spin at 1800 x g for 20 minutes using a refrigerated centrifuge set at 20-25°C. Centrifuge temperatures may rise when spinning for extended amounts of time. Document start time of centrifugation on site developed processing worksheet. A worksheet template can also be provided by the MTN LC.
3. Gently invert the CPT tube 2 times, without disturbing the underlying gel, to suspend the PBMCs in the plasma. Transfer the cells, using a disposable transfer pipette, from the CPT tube to an appropriately labeled 15 mL conical tube.
4. Add PBS, using a serological pipette, to bring the total volume of the conical tube containing the cells up to the 12 mL mark on the tube. Cap the 15 mL conical tube and mix by gently inverting.
5. Centrifuge the conical tube at 400 x *g* for 15 min at 4°C. Remove and discard supernatant.
6. Resuspend each pellet in 3 mL PBS, pool all suspensions into a single 15 mL conical tube and make up the volume to 10 mL with PBS.
7. Transfer 0.2mL for cell count. Perform a viable cell count during step 8.
8. Centrifuge conical tube at 400 x g for 15 minutes at 2-8°C.
9. Remove and discard as much of the supernatant as possible without disturbing the cell pellet using a pipette.
10. Add 1.0mL of fresh cold 70% methanol (7 parts methanol and 3 parts distilled water). Vortex lightly to lyse cells completely. **NOTE:** Prepare fresh 70% methanol lysing solution each day and store at 2-8°C for at least 2 hours before use.
11. Transfer all contents to cryovial ~ 1.0ml.
12. When logging into LDMS (Table 9-3), although there were 3 CPT specimens collected, enter the primary specimen as 1 sample with a volume of 24ml since they are pooled at processing. Use LDMS to label and track all aliquots.
13. Place PBMC lysate directly into ≤-70°C freezer. Record time frozen on processing worksheet. This should be completed within 8 hours from collection.
14. The MTN LC will send instructions when shipping and/or testing is required.

The shipping address for PK samples:

Johns Hopkins University
Division of Clinical Pharmacology
600 N. Wolfe Street, Osler 523

Baltimore, MD 21287
Lab Phone#: (410) 955-9710 or (410) 614-9978

9.7 Testing of Vaginal and Cervical Specimens

Refer to Protocol Section 7 and the Genital Exam checklist located on the MTN-014 Study Implementation Materials webpage for further information of the required sequence of specimen collection and diagnostic procedures to be performed during study pelvic exams.

9.7.1 Vaginal Fluid pH

Vaginal fluid pH will be assessed as part of on-site evaluations. Indicator Strips (pH range 3.6 to 6.1) must be used as follows:

- During pelvic examination, vaginal fluids should be collected via swab and then swabbed onto the pH strip. Avoid contact with cervical mucus, which has a higher pH. Do not insert the pH strip into the vagina to collect the vaginal fluid.
- 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.

9.7.2 Vaginal Fluid Wet Mount Testing

When clinically indicated wet mount will be performed. Wet mount procedures for this study consist of two different preparations — saline prep and potassium hydroxide (KOH) prep — for diagnosis of bacterial vaginosis and candidiasis, as summarized in Table 9-4.

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

Prior to study initiation, clinicians or qualified clinic staff should be proficient in reading wet mounts for clue cells and KOH preps for yeast. CLIA regulations require semi-annual proficiency testing; therefore the MTN LC will administer a web-based proficiency testing approximately every six months. The MTN LC will post wet mount slides on the MTN web pages for this purpose every 6 months; results will be entered directly on the website (contact: Lorna Rabe: lrabe@mwri.magee.edu). The MTN LC will report results back to the Laboratory Manager and also specify any corrective action that may be needed based on the results. Contact the MTN LC for additional information and guidance on performing and documenting the proficiency testing. Also contact the MTN LC when new applicable clinical or laboratory staff is hired, so that appropriate training can take place prior to such staff performing wet mounts for study purposes.

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
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)

Assessment	Saline Prep	KOH Prep
Trichomonads	Use OSOM Rapid Trichomonas test (see below)	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 site SOPs as follows:

Immediate examination of wet mount in clinic:

- Use a pencil to write the PTID and specimen collection date on one side of the frosted end of two microscope slides. Affix a SCHARP-provided PTID label to the other side of the slides (on the frosted end, under the pencil markings) and write the specimen collection date in indelible ink (e.g. Sharpie pen) on each label.
- Immediately following collection from the lateral vaginal wall via swab, smear vaginal fluid specimens onto each slide. Alternatively, the swab may be placed in a glass or plastic tube with approximately six drops (100 µL) sterile physiologic saline to allow for non-immediate slide preparation. In this case, vaginal fluid specimens should be smeared onto the two slides upon receipt from the collecting clinician.
- Apply one drop of 10% KOH to one slide and immediately perform whiff test for a "fishy" amine odor. Then apply cover slip.
- Apply one drop of sterile physiologic saline to the second slide, emulsify with the vaginal fluid specimen, and then apply cover slip. Examine immediately at 10X magnification for epithelial cells, motile trichomonads, budding yeast, and pseudohyphae. Examine at 40X magnification to determine whether observed epithelial cells are clue cells and quantitate the cells. Clue cells are irregularly bordered squamous epithelial cells that are completely covered with bacteria (mainly *Gardnerella vaginalis*). Clue cells must comprise at least 20 percent of the observed epithelial cells for the saline prep to be considered positive for clue cells.
- Examine the KOH slide at both 10X and 40X magnifications for yeast and pseudohyphae.

Non-immediate wet mount examination in laboratory:

- Immediately following collection of vaginal fluid from the lateral vaginal wall via swab, place the swab in a glass or plastic tube with approximately 6 drops (100µl) sterile isotonic saline. Snap off the shaft of the swab and cap the tube.
- Deliver the tube to the laboratory for testing as described above for immediate examination. Testing must take place within 4 hours.

9.7.3 Rapid Test for Trichomoniasis

This testing will be done using the OSOM Rapid Trichomonas test with vaginal swabs per site SOPs approved by the MTN LC. The kit provides Dacron swabs for this test.

- 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.

9.7.4 Vaginal Gram Stain

Dried vaginal fluid smears will be prepared for Gram staining and assessment for bacterial vaginosis at the MTN LC. Two slides will be prepared at each required time point and both will be entered into LDMS. One will be shipped to the MTN LC and the other will be archived on site until written notification is received from the MTN SDMC that the slide may be discarded. Instructions for slide preparation and shipping are provided below.

- Use a pencil to write the PTID and specimen collection date on one side of the frosted end of one microscope slide. Affix a SCHARP-provided PTID label to the other side of the slides (on the frosted end, under the pencil markings) and write the specimen collection date in indelible ink (e.g. Sharpie pen) on each label.
- Immediately following specimen collection from the lateral vaginal wall via swab, roll the swab across each of the 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 the slides and an LDMS Specimen Tracking Sheet to the local LDMS laboratory.
- Using the LDMS Specimen Tracking Sheet, log the slides into LDMS (Table 9-3) 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 both slides in the slide box locations assigned in LDMS at room temperature.
- The laboratory will be notified by the MTN LC to ship one of the two slides collected for each participant and visit.
- The duplicate slide will be archived on site until written notification is received from the MTN SDMC that the slide may be discarded.

Ship to:

Pam Kunjara
Magee-Womens Research Institute
204 Craft Ave, Room A540
Pittsburgh, PA 15213
Phone: 412-641-6393/6157
E-mail address: pkunjara@mwri.magee.edu

9.7.5 Papanicolaou Test (Pap smear)

Pap smears will be performed when clinically indicated. At visits when Pap smears are needed, ecto- and endocervical cells will be collected after all tissues have been visually inspected and all other required specimens have been collected. Specimen collection, slide preparation, slide interpretation, and QC procedures must be performed and documented in accordance with study site SOPs.

- At some study sites, Pap smear results may include notations of findings associated with certain STIs (e.g., trichomoniasis). Because Pap smear methods are not adequately sensitive and specific for STIs (including HPV), Pap smear findings associated with STIs should not be used to diagnose any STIs during follow up.

9.7.6 Chlamydia and Gonorrhea Testing in Vaginal swabs

Note: Testing for chlamydia and gonorrhea is done at screening only. If participant has used product within the past 24 hours, collect urine instead of vaginal swab. (Section 9.5.5)

Instructions for collection and transport for testing with Gen-Probe:

1. Carefully insert the swab into the vagina about 2 inches (5 cm) past the introitus and gently rotate the swab for 10 to 30 seconds. Make sure the swab touches the walls of the vagina so that moisture is absorbed by the swab and then withdraw the swab without touching the skin.

2. While holding the swab in the same hand, unscrew the cap from the tube. Do not spill the contents of the tube. If the contents of the tube are spilled, use a new specimen collection kit.
3. Immediately place the swab into the transport tube so that the score line is at the top of the tube.
4. Carefully break the swab shaft at the score line against the side of the tube.
5. Immediately discard the top portion of the swab shaft.
6. Tightly screw the cap onto the tube.
7. Transport at room temperature to the laboratory.

Instructions for collection and transport for testing with ProbeTec
1. Insert the collection swab into the vagina and rotate for 15-30 seconds.
2. If using the ProbeTec Dry Transport Kit withdraw the swab and immediately place the swab with the cap attached into the transport tube. Make sure the cap is tightly secured to the tube.
3. If using the ProbeTec Amplified DNA Assay Collection kit that contains the CT/GC Diluent tube place the swab into the diluent tube, break the shaft of the swab at the score mark, and tightly recap the tube.

Instructions for collection and transport for testing with GeneXpert:
1. Collect specimen using the Xpert collection swab.
2. Label the pink-capped transport tube with the participants PTID number and date.
3. Remove the swab and insert into the vaginal according to the procedure specified in the Genital Exam Visit Checklist and rotate gently through 360 degrees and remove.
4. Immediately place the swab in the transport tube, break off shaft of swab and cap.
5. Cap tightly and invert or gently shake the tube 3-4 times to elute material from the swab. Avoid foaming.
6. The specimen can now remain at 2-30°C for 60 days.

9.7.7 Vaginal Fluid for PK

One vaginal swab will be used to absorb cervicovaginal secretions for the analysis method that will be performed at the Clinical Pharmacology Department in Johns Hopkins University School of Medicine.
- Collect vaginal fluid using a Dacron swab from the posterior fornix.
- Place swab in a 1.8 cryovial (no additive) marked PK Swab, break off stick, and cap.
- Within 2 hours, place the sample tubes in the freezer at ≤ -70˚C.
- At the end of the study, ship on dry ice to the JHU CPAL (Section 9.6.8)**.**

9.7.8 Vaginal Swabs for Biomarker Analysis

At each pelvic exam, vaginal fluids are collected from the posterior fornix using a Dacron swab with a plastic shaft for biomarker analysis at the MTN LC.
- Two separate Dacron swabs will be taken.
- Collect vaginal fluid using a Dacron swab from the posterior fornix.
- Place the swab in a SCHARP labeled cryovial containing 400 µL PBS.
- Break shaft of swab at a minimum of 1cm beyond the swab and cap the vial.
- Repeat with the second Dacron swab as described above.
- Store refrigerated or on ice for up to 8 hours prior to delivery to the lab.
- Deliver the tubes and LDMS Specimen Tracking Sheet to the local LDMS laboratory within 8 hours.
- Log the cryovial into LDMS (Table 9-3) and label the vial with a LDMS label.
- Freeze at ≤ -70ºC.
- Batch ship at end of study to MTN LC. See Section 9.7.4 for shipping address.

9.7.9 Endocervical Swabs for Biomarker Analysis

At each pelvic exam, endocervical cells will be collected using a Dacron swab with plastic shaft for biomarker analysis at the MTN LC.

- Remove cervical mucus with a large swab to expose the cell layer (discard swab).
- Two separate Dacron swabs will be taken.
- Collect endocervical cells by inserting a Dacron swab approximately 1 cm into the endocervical canal and rotating two full turns.
- Withdraw the swab, place it in a labeled cryovial containing 400 µL PBS.
- Break shaft of swab at a minimum of 1cm beyond the swab and cap the vial.
- Repeat with the second Dacron swab as described above.
- Store refrigerated or on ice for up to 8 hours prior to delivery to the lab.
- Deliver the tubes and an LDMS Specimen Tracking Sheet to the local LDMS laboratory within 8 hours.
- Log the cryovial into LDMS (Table 9-3) and label the vial with a LDMS label.
- Freeze at ≤ -70ºC.
- Batch ship at end of study to MTN LC. See Section 9.7.4 for shipping address.

9.7.10 CVL for PK, PD and biomarker

CVL aliquots will be collected, processed, and used for testing related to PD, PK, biomarker, and cell pellet. CVL specimens are kept on ice or refrigerated and should be processed within 8 hours of collection.

1. All the CVL liquid will be spun at 800 *x g* for 10 minutes in the 15 mL conical collection tube.
2. Remove supernatant from the cell pellet and save fluid in cryovials.
3. Re-spin the 15 mL conical tube containing cells for 10 minutes at 800 *x g*.
4. Pull off and save any additional supernatant making sure not to remove any cells or debris.
5. Store all supernatant in as many 1 mL aliquots as possible in 2mL cryovials, assuring there are at least 1 aliquot each for PD, PK, and semen biomarker testing. A minimum of 3 back-up aliquots is also required to be stored (mark as 'Extra CVL').
6. Freeze all aliquots at ≤-70°C within 8 hours of collection and track in LDMS.
7. If less than a total of 6 mL's (or less than 6 cryovials) of supernatant are recovered, contact the MTN LC.
8. Cell pellets will be suspended in 0.5 ml PBS in a plastic cryovial and frozen at ≤ -70°C within 8 hours of collection.
9. The MTN LC will send instructions to the site when shipping is required.

Note: Study site should schedule PK visits to avoid menses. If a participant is menstruating when CVL is scheduled, collect the CVL and include a comment on the CRF and LDMS tracking sheet.

Tenofovir levels will be evaluated on CVL samples taken throughout the study. The CVL supernatant aliquots for PK will be batched and shipped on dry ice to the JHU CPAL at the end of the study. See Section 9.6.8 for shipping address.

The pharmacodynamics (PD) of tenofovir will be studied to determine the effectiveness of the drugs by evaluating the anti-HIV-1 activity present in the genital tract. Biomarkers may also be evaluated to determine the impact the gel and drug may have on innate immune mediators, cytokines, or soluble factors. CVL supernatant aliquots and cell pellet for PD will be batched and shipped on dry ice to MTN LC at end of study. See Section 9.7.4 for shipping address.

9.7.11 Cytobrush for PK

Cytobrush: use the Qiagen Digene brush (catalog number: 5126-1220 from USA — QIAGEN Inc.). If the site has trouble obtaining this item, contact the MTN LC.

1. Collect sample using cytobrush by inserting into the cervical os and perform 2 - 360° turns.
2. Immediately place cytobrush into appropriately labeled 5 ml screw cap vial containing 3.5 mL of PBS.
3. The shaft of the cytobrush can be broken off at this step to cap the sample.
4. Keep on wet ice or refrigerate until processing for storage.
5. Processing should occur within 2 hours of specimen collection.
6. Elute the cervical mononuclear cells into the PBS by agitation and rolling against the side of the tube. Pulse vortex on medium 1-2 seconds approximately 4 times.
7. Clip off the cytobrush head from the support and centrifuge the tube at 400 x *g* for 10 minutes at 4°C.
8. Carefully remove the cytobrush head and vortex on a medium setting for 2 seconds.
9. Centrifuge tube at 400 x *g* for 10 minutes at 4°C.
10. Remove supernatant carefully using a P1000 (1000 microliter pipette) and discard.
11. Add 2 ml PBS to cell pellet and suspend by vortexing.
12. Remove 50 µL aliquot to count cells using a hemacytometer with trypan blue exclusion.
    a. Record the total number of cells (including squamous cells*) and percent viable.
    b. The MTN LC will provide an excel sheet to record these results.
13. Centrifuge tube at 400 *x g* for 10 minutes at 4 °C
14. Remove supernatant carefully using a P1000 and discard supernatant.
15. Add 1mL 70% ice cold methanol and lyse cells by briefly vortexing followed by mixing with a P1000 pipettor.
16. As soon as lysis is complete, transfer lysate into appropriately labeled cryovial and freeze at ≤-70˚C immediately.
17. Batch ship to the JHU CPAL at end of study. See Section 9.6.8for shipping address.

*Note: squamous cells are expected to be rare on this specimen and will appear similar to squamous cells in urine. They will be larger than cervical mononuclear cells and will have a “fried egg” appearance. These should be counted in the same fashion as cervical mononuclear cells.

9.7.12 Vaginal Biopsies for PK

1. Tare the weighing balance and ensure balance has been calibrated within the past year.
2. Weigh each cryovial using an analytical balance – use the same analytical balance throughout the procedure. Document the weight of the labeled cryovial (pre-weight) on the LDMS Tracking Sheet.
3. Number cryovials depending on how many biopsies are received with appropriate participant information (e.g. PTID).
4. Transfer biopsy to a pre-weighed cryovial. Store only ONE biopsy per cryovial. Ensure biopsy sits at bottom of cryovial.
5. Weigh the cryovial containing the biopsy (post-weight). Document the weight of the cryovial containing the biopsy on the LDMS Tracking Sheet.
6. Freeze the cryovial containing the biopsy in Liquid Nitrogen or a dry ice-alcohol bath within 2 hours of collection.
    - For liquid nitrogen emerge cryovial for 15 minutes. Transfer immediately to ≤-70°C
    - For dry ice-alcohol bath set up dry ice-alcohol (ethanol or isopropanol may be used) slurry in an ice bucket or suitable tray. Emerge cryovial in slurry making sure that the slurry level is above the tissue. Freeze for 15 minutes. Transfer immediately to ≤-70°C

7. Log into LDMS (Table 9-3) and label specimen with LDMS label.
8. Store the labeled cryovial containing the biopsy in a ≤-70°C freezer. Document the date/time the cryovial containing the biopsy was placed in the freezer.
9. Specimens may be batched and shipped on dry ice. Once the LC notifies the site to ship, use LDMS to create a shipping manifest.
10. Ship specimens to the JHU CPAL Monday through Wednesday for overnight delivery. See section 9.6.8 for shipping address.

9.7.13 Vaginal biopsies for Gene Expression Array

1. Take one cryovial containing 1.5 mL of RNA*later* (Ambion, Invitrogen Cat #AM7020) and label it with PID, study visit and date.
2. Place one biopsy into the cryovial and submerge the tissue in the RNA*later* solution.
3. Store each vial containing one biopsy in RNA*later* at 4ºC overnight (16-24 hours).
4. Complete the LDMS tracking sheet and submit to lab for LDMS entry.
5. Log the specimen into LDMS (Table 9-3) and label specimen with LDMS label.
6. Transfer vials from 4ºC to ≤-70°C. Each biopsy must be stored at ≤-70°C for a minimum of 24 hours prior to shipping.
7. Batch and ship specimens to the MTN LC on dry ice. The MTN LC will notify the site when to send the specimens. See Section 9.7.4 for shipping address.

9.8 Rectal Specimens

The tests performed on rectal specimens 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.

Rectal samples should be collected in the following order.
1. Rectal swab for GC/CT
2. Rectal fluid sponges for PD and PK
3. Rectal sponge for biomarkers
4. Biopsies for PK, mucosal gene expression array, histology and proteomics.

Table 9-2 gives a brief summary of how these rectal samples should be handled.

*If at any time the collection of biopsies is limited, submit for assays in order of importance – PK, Mucosal Gene Expression Array, Histology, and then Proteomics.

9.8.1 Rectal NAAT for Gonorrhea and Chlamydia

Note: Testing for Chlamydia and Gonorrhea is done at screening.
Product gel may cause interference during testing. Please be careful to avoid contact with gel when collecting specimen.

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

If the site does not have access to these tests, they can send the samples to the LC for testing. Contact the LC (PKunjara@mwri.magee.edu) for shipping instructions and timeline for GC/CT testing.

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

Instructions for collection and transport of rectal swabs for GC/CT testing with Gen-Probe:
1. Collect specimen using the Gen-Probe Aptima Unisex Swab (blue swab).
2. Label the transport tube with the participants PTID number and date.
3. Remove the swab from the plastic transport tube and insert into the rectum according to the procedure outlined in the SSP for Clinical Considerations (Section 8) and rotate gently through 360 degrees and remove.
4. Immediately place the swab in the transport tube, break off shaft of swab and cap. The specimen can now remain at 2-30°C for 30 days.

5. Place the transport tube in a biohazard zip-lock bag and transport to the local laboratory for testing. If samples are to be tested by the MTN LC, please ship samples bimonthly or sooner on ice pack. Refer to section 9.7.4 for shipping instructions.

Instructions for collection and transport of rectal swabs for GC/CT testing with GeneXpert:

1. Collect specimen using the Xpert collection swab.
2. Label the pink-capped transport tube with the participants PTID number and date.
3. Remove the swab and insert into the rectum according to the procedure outlined in the SSP for Clinical Considerations (Section 8) and rotate gently through 360 degrees and remove.
4. Immediately place the swab in the transport tube, break off shaft of swab and cap.
5. Cap tightly and invert or gently shake the tube 3-4 times to elute material from the swab. Avoid foaming.
6. The specimen can now remain at 2-30°C for 60 days.
7. Place the transport tube in a biohazard zip-lock bag and transport to the local laboratory for testing. If samples are to be tested by the MTN LC, please ship samples bimonthly or sooner on ice pack. Refer to section 9.7.4 for shipping instructions.

9.8.2 Rectal fluid for PK, PD and Biomarkers

1. Tare the weighing balance and ensure balance has been calibrated within the past year.
2. Remove three sponges (Merocel eye-wick Spears Fisher Scientific # NC0093269) from the box, wear gloves at all times when handling sponges.
3. If collecting multiple sponges (1 for PK, 1 for PD, and 1 for biomarkers), using a permanent marker, identify each of the sponges by numbering the sponge shaft or using another unique identifier.
4. Place each sponge into an appropriately labeled 5mL cryovial, labeled with a unique patient identifier.
5. Number the exterior of each cryovial with the same number used to label the sponge shaft.
   NOTE: ALWAYS REPLACE SAME SPONGE INTO THE SAME VIAL
6. Weigh the dry sponge + labeled cryovial and document the weight (pre-weight) on the LDMS Tracking Sheet.
7. The clinician will collect specimen using the pre-weighed sponges according to the procedures outlined in the SSP for Clinical Considerations.
8. Place the sponges back into the original weighed cryovial (by matching the number of the sponge to the tube) and ensure that the cap is fully tightened.
9. Transport the cryovials so that they can be weighed using the same balance that was used in the preparation of the sponges. Weigh the sponge + labeled tube and document the weight (post-weight) on the LDMS tracking sheet.
10. Cryovials must be transported on ice to the LDMS laboratory to allow storage within 2 hours of collection.
11. Log into LDMS (Table 9-3) and label specimen with LDMS label.
12. Place in a ≤-70°C freezer for storage until shipment is requested by the LC.
13. Record the time that the sample is introduced to the freezer on the LDMS Tracking Sheet.
14. Rectal sponges for PD and biomarkers will be batched and shipped to the MTN LC on dry ice at end of study. Please see section 9.7.4 for shipping address.
15. Rectal sponge for PK will be shipped to the JHU CPAL on dry ice at end of study. Please see Section 9.6.8 for shipping address.

9.8.3 Rectal Biopsies for PK

1. Number cryovials 1-4 depending on how many biopsies are collected with appropriate patient information.
2. Weigh each cryovial using an analytical balance – use the same analytical balance throughout the procedure. Document the weight of the labeled cryovial (pre-weight) on the LDMS Tracking Sheet.
3. Place each biopsy in a pre-weighed cryovial. Store only ONE biopsy per cryovial. Ensure biopsy sits at bottom of cryovial.

4. Weigh the cryovial containing the biopsy (post-weight). Document the weight of the cryovial containing the biopsy on the LDMS Tracking Sheet.
5. Log into LDMS (Table 9-3) and label specimen with LDMS label.
6. Freeze the cryovial containing the biopsy in Liquid Nitrogen or a dry ice-alcohol bath within 2 hours of collection.
    - For liquid nitrogen emerge cryovial for 15 minutes. Transfer immediately to ≤-70°C
    - For dry ice-alcohol bath set up dry ice-alcohol (ethanol or isopropanol may be used) slurry in an ice bucket or suitable tray. Emerge cryovial in slurry making sure that the slurry level is above the tissue. Freeze for 15 minutes. Transfer immediately to ≤-70°C
7. Store the labeled cryovial containing the biopsy in a ≤-70°C freezer. Document the date/time the cryovial containing the biopsy was placed in the freezer.
8. Specimens may be batched and shipped on dry ice. Once the LC notifies the site to ship, use LDMS to create a shipping manifest.
9. Ship specimens to the JHU CPAL Monday through Wednesday for overnight delivery. See section 9.6.8for shipping address.

9.8.4 Rectal Biopsies for Mucosal Gene Expression Array

1. For each biopsy prepare one cryovial containing 1.5 mL of RNA*later* (Ambion, Invitrogen Cat #AM7020) and label it with PID, study visit and date.
2. Place one biopsy into each cryovial and submerge the tissue in the RNA*later* solution.
3. Store each vial containing one rectal biopsy in RNA*later* at 4ºC overnight (16-24 hours).
4. Complete the LDMS tracking sheet and submit to lab for LDMS entry.
5. Log the specimen into LDMS (Table 9-3) and label specimen with LDMS label.
6. Transfer vials from 4ºC to ≤-70°C. Each biopsy must be stored at ≤-70°C for a minimum of 24 hours prior to shipping.
7. Batch and ship specimens to the MTN LC on dry ice. The MTN LC will notify the site when to send the specimens. See section 9.7.4 for shipping address.

9.8.5 Rectal Biopsy for Histology

1. Place one biopsy into a microtube filled with 10% formalin for shipping. These can be kept at room temperature.
2. Complete the LDMS tracking sheet and submit to lab for LDMS entry.
3. The tissue processing, staining and evaluation will be performed at the MTN LC.
4. Log specimens into LDMS (Table 9-3), label specimen with LDMS label.
5. Batch ship the specimens within 5 days of collection to the MTN LC at room temperature. See section 9.7.4 above for shipping instructions.

9.8.6 Rectal Biopsy for Proteomics

1. Place one biopsy into a cryovial and snap freeze at ≤-70°C within 2 hours of collection.
2. Complete the LDMS tracking sheet and submit to lab for LDMS entry.
3. Log the specimens into LDMS (Table 9-3) and label specimens with LDMS label.
4. Batch and ship specimens to the MTN LC on dry ice. The MTN LC will notify the site when to send the specimens. See section 9.7.4 for shipping address and e-mail notification.