

Section 9. Laboratory Considerations

This section contains information on the laboratory procedures performed in MTN-011.

9.1 Overview and General Guidance

As transmission of HIV and other infectious agents can occur through contact with contaminated needles, blood, and blood products, 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 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 Network Laboratory (NL), including the MTN Pharmacology Core (at Johns Hopkins University). Table 9-1 and table 9-2 highlight specimen, storage and shipment requirements. Table 9-3 & 9-4 lists the tests to be performed at each visit for each group.

Regardless of whether tests are performed in clinic or laboratory settings, study staff that performs the tests must be trained in proper associated 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 NL 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 NL 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.

**Table 9-1
Overview of Laboratory Testing Locations, Specimens, and Methods for MTN-011 for
Groups 1 & 2**

Test	Testing Location	Specimen Type	Tube or Container and tube size (recommended)	Kit or Method
Urine pregnancy test (hCG), Female only	In clinic	Urine	Plastic screw top cup	One-Step or Combo hCG Quidel Quick Vue, or Fisher HealthCare Sure-Vue Urine hCG kit
Urine Culture ¹	local lab	Urine	Plastic screw top cup	Local methodology
Urine NAAT for GC/CT	Local lab	Urine	Kit specific Transport tube	BD Probetec or Gen-Probe Aptima
CBC and Platelet (female only)	Local Lab	Whole Blood	EDTA 4mL tube	Local methodology
HIV antibody screen and Western Blot	Clinic/Local Lab/ Network Lab	Plasma, serum, or whole blood	EDTA or plain tube 4mL	FDA approved tests
Blood PK Tenofovir (female only)	NL Pharmacology Core	Plasma	EDTA 10 mL tube	JHU collection procedure
Syphilis Serology	Local Lab	Serum or Plasma	EDTA tube, plain or serum separator, 4 ml	Local methodology
Hepatitis B (HBsAg)	Local Lab	Serum or Plasma	EDTA tube, plain or serum separator, 4 ml	Local methodology
Plasma Archive	Clinic/Local Lab	Plasma	EDTA 10mL tube	Network Lab procedure
Cervicovaginal Lavage (CVL) for Anti-HSV, PD, PK (tenofovir), Cell pellet, and/or semen biomarkers (Female only)	MTN Network Lab & NL Pharmacology Core	Fluid & pellet recovered from CVL (saline used)	15 mL Conical Vial	Network Lab Procedure
Trichomonas rapid test	Local lab or in clinic	Vaginal swab (supplied with kit)	Sterile tube with no additives	OSOM kit
Vaginal pH	In clinic	Vaginal swab	N/A	S/P pH Indicator Strips
Vaginal Biopsy for PK tenofovir	NL Pharmacology Core	Vaginal Biopsy	1.8 mL cryovial	JHU collection procedure
Vaginal saline wet preparation on females (for BV and/or KOH wet mount) ¹	In clinic	Vaginal swab	tube with 6 drops of saline	Network Lab procedure
Cervical Biopsy for PK	NL Pharmacology Core	Tissue	1.8 mL cryovial	JHU collection procedure
Cervical Cytobrush For PK or Flow Cytometry	NL Pharmacology Core	Cytobrush	50 ml Conical vial	Network Lab collection procedure
Pap Smear ²	Local Lab	Ecto & Endo-cervical cells	Slides	Local methodology
Rectal Tenofovir Level	NL Pharmacology Core	Rectal Sponge	5 ml cryovial	JHU collection procedure

Semen Analysis (Male only)	NL Pharmacology Core	Semen	Plastic screw top cup	JHU collection procedure
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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 Screening.

Table 9-2: Overview of Specimens for Storage and Shipment for Groups 1 & 2

Specimen and subsequent testing	Additive	Tube type or size recommended	Processing	Ship to:	Shipping schedule
Plasma for archive	EDTA	10 mL	Freeze plasma at $\leq -70^{\circ}\text{C}$ within 4 hours of draw	MTN Network Lab	Store frozen at site until notified
Plasma for blood PK (tenofovir)	EDTA	10 mL	Freeze plasma within 8 hours after collection	John Hopkins Pharmacology Lab	Store frozen at site until conclusion of study
Cervicovaginal Lavage (CVL) supernatant: PD	Saline (as a Cervicovaginal rinse)	2ml cryovial	Freeze supernatant within 8 hours of collection	MTN Network Lab	Store frozen at site until notified by MTN
Cervicovaginal Lavage (CVL) supernatant: Anti-HSV Activity	Saline (as a Cervicovaginal rinse)	2ml cryovial	Freeze supernatant within 8 hours of collection	MTN Network Lab	Store frozen at site until notified by MTN
Cervicovaginal Lavage (CVL) supernatant: PK (tenofovir)	Saline (as a Cervicovaginal rinse)	2ml cryovial	Freeze supernatant within 8 hours of collection	John Hopkins Pharmacology Lab	Store frozen at site until notified by MTN
Cervicovaginal Lavage (CVL) supernatant: : Semen biomarker	Saline (as a Cervicovaginal rinse)	2ml cryovial	Freeze supernatant within 8 hours of collection	John Hopkins Pharmacology Lab	Store frozen at site until notified by MTN
Cervicovaginal Lavage Cell Pellet (CVL pellet)	PBS	2ml cryovial	Freeze cell pellet within 8 hours of collection	MTN Network Lab	Store frozen at site until notified by MTN
Cervical Cytobrush for PK (tenofovir) Case Western	tRPMI	2 ml cryovial	Process within 2 hours of collection. Freeze lysate ASAP	John Hopkins Pharmacology Lab	Store frozen at site until notified by MTN
Vaginal Biopsy for PK (tenofovir)	None	1.8mL cryovial	Immediately freeze cryovial biopsy in dry ice ethanol bath	John Hopkins Pharmacology Lab	Store frozen at site until conclusion of study
Cervical Tissue Biopsy for PK (tenofovir)	None	1.8mL cryovial	Immediately freeze cryovial biopsy in dry ice ethanol bath	John Hopkins Pharmacology Lab	Store frozen at site until conclusion of study
Rectal PK Level (tenofovir)	None	5 mL cryovial	Freeze within 4 hours of collection	John Hopkins Pharmacology Lab	Store frozen at site until notified by MTN
Semen Analysis (Male only)	None	1.5 mL microvial	Liquefy, separate in 4°C centrifuge, freeze within 4 hours of collection.	John Hopkins Pharmacology Lab	Store frozen at site until notified by MTN

Table 9-3: Overview of Laboratory Tests by visit for MTN-011 Group 1

GROUP 1	Visit 1	Visit 2a	Visit 2b	Visit 3a	Visit 3b	Visit 4a	Visit 4b	Visit 5a	Visit 5b	Visit 6a	Visit 6b	Visit 7a	Visit 7b
	SCR: NO GEL/ NO SEX	ENR: NO GEL/ SEX	Post Coital Samples (♀)	GEL - 1/ SEX	Post Coita l Sam ples (♀)	GEL -1/No Sex (♀)	Sampl es (♀)	Gel - 24/ Sex	Post Coital Sampl es (♀)	Gel - 24/No Sex (♀)	Sampl es (♀)	Gel - 1/Sex/ Gel +1	Post- Coital Sampl es/ Final Clinic
Pelvic examination (FEMALE ONLY)	X	X	X	*	X	*	X	*	X	*	X	*	X
Laboratory													
hCG (FEMALE ONLY)	X	X		X		X		X		X		X	
Urine culture	*	*		*		*		*		*		*	*
Urine NAAT for GC/CT	X	*		*		*		*		*		*	*
CBC with platelets (FEMALE ONLY)	X												
HIV-1 serology	X												X
PK- blood (FEMALE ONLY)					X		X		X		X		X
Syphilis serology	X												
HBsAg	X												
Plasma archive		X											
CVL for PK & seman Biomarkers (FEMALE ONLY)			X		X		X		X		X		X
CVL for Anti-HSV , PD, Cell Pellet, & storage (female only)	X		X		X		X		X		X		X
Vaginal fluid pH (FEMALE ONLY)	X	X	X	X	X	X	X	X	X	X	X	X	X
Vaginal and cervical biopsies for PK (FEMALE ONLY)					X		X		X		X		X
Cytobrush flow cytometry (FEMALE ONLY)	X				X		X		X		X		X
Cytobrush PK(FEMALE ONLY)					X		X		X		X		X
Rapid Trichomonas test (FEMALE ONLY)	X	*		*		*		*		*		*	*
KOH wet mount for candidiasis (FEMALE ONLY)	*	*		*		*		*		*		*	*
Wet mount for BV (FEMALE ONLY)	*	*		*		*		*		*		*	*
Cervical specimen for pap smear (FEMALE ONLY)	*												
Rectal sponge for PK (FEMALE ONLY)					X		X		X		X		X
Semen sample (MALE ONLY)	X												

X required procedure, * if indicated, ♀= Female participants only

Note: At the Final Visit, participants should receive available test results, however if results are not available study staff should make arrangement to disclose those results.

Table 9-4: Overview of Laboratory Tests by visit for MTN-011 Group 2

Group 2	Visit 1	Visit 2	Visit 3a	Visit 3b	Visit 4	Visit 5	Visit 6	Visit 7a	Visit 7b	Visit 8	Visit 9
	SCR	ENR	Gel - 1/Sex	Post Coital Sampling (♀)	Provision of Product (♀)	Sampling (♀)	Provision of Product (♀)	Gel - 72/Sex	Post Coital Sampling (♀)	Provision of Product (♀)	Sampling / Final Visit
Pelvic examination (FEMALE ONLY)	X	X	*	X	*	X	*	*	x	*	x
Laboratory											
hCG (FEMALE ONLY)	X	X	X		X		X	X		X	X
Urine culture	*	*	*		*		*	*		*	*
Urine NAAT for GC/CT	X	*	*		*		*	*		*	*
CBC with platelets (FEMALE ONLY)	X										
HIV-1 serology	X										X
PK- blood (FEMALE ONLY)				X		X			X		X
Syphilis serology	X										
HBsAg	X										
Plasma archive		X									
CVL for PK & semen Biomarkers (FEMALE ONLY)				x		x			x		x
CVL for Anti-HSV ,PD, Cell Pellet, & storage (female only)	x			x		x			x		x
Vaginal fluid pH (FEMALE ONLY)	X	X	X	X	X	X	X	X	X	X	X
Vaginal and cervical biopsies (FEMALE ONLY)				X		X			X		X
Cervical cytobrush flow cytometry (FEMALE ONLY)	X			X		X			X		X
Cervical cytobrush PK (FEMALE ONLY)				X		X			X		X
Rapid Trichomonas test (FEMALE ONLY)	X	*	*		*		*	*		*	*
KOH wet mount for candidiasis (FEMALE ONLY)	*	*	*		*		*	*		*	*
Wet mount for BV (FEMALE ONLY)	*	*	*		*		*	*		*	*
Cervical specimen for pap smear (FEMALE ONLY)	*										
Rectal sponge for PK (FEMALE ONLY)				X		X			X		X
Semen sample (MALE ONLY)	X										

X required procedure, * if indicated, ♀= Female participants only

Note: At the Final Visit, participants should receive available test results, however if results are not available study staff should make arrangement to disclose those results

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 the specimens are collected should also be included on the label. Use an indelible ink pen (e.g., Sharpie) if information is handwritten such as the date.

Microscope slides used for evaluation of vaginal fluids also will be labeled with PTID labels provided by SCHARP. 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. Refer to Table 9.5 for tests that will be entered into LDMS 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 Network Lab must be notified 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 Network Lab staff 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 at all sites to track the collection, storage, and shipment of the sample types described in Table 9-5.

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-011 may be directed to Wayne Hall 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: +716-834-0900, ext 7311
Fax: +716-898-7711

Off-Hours Contact Information:

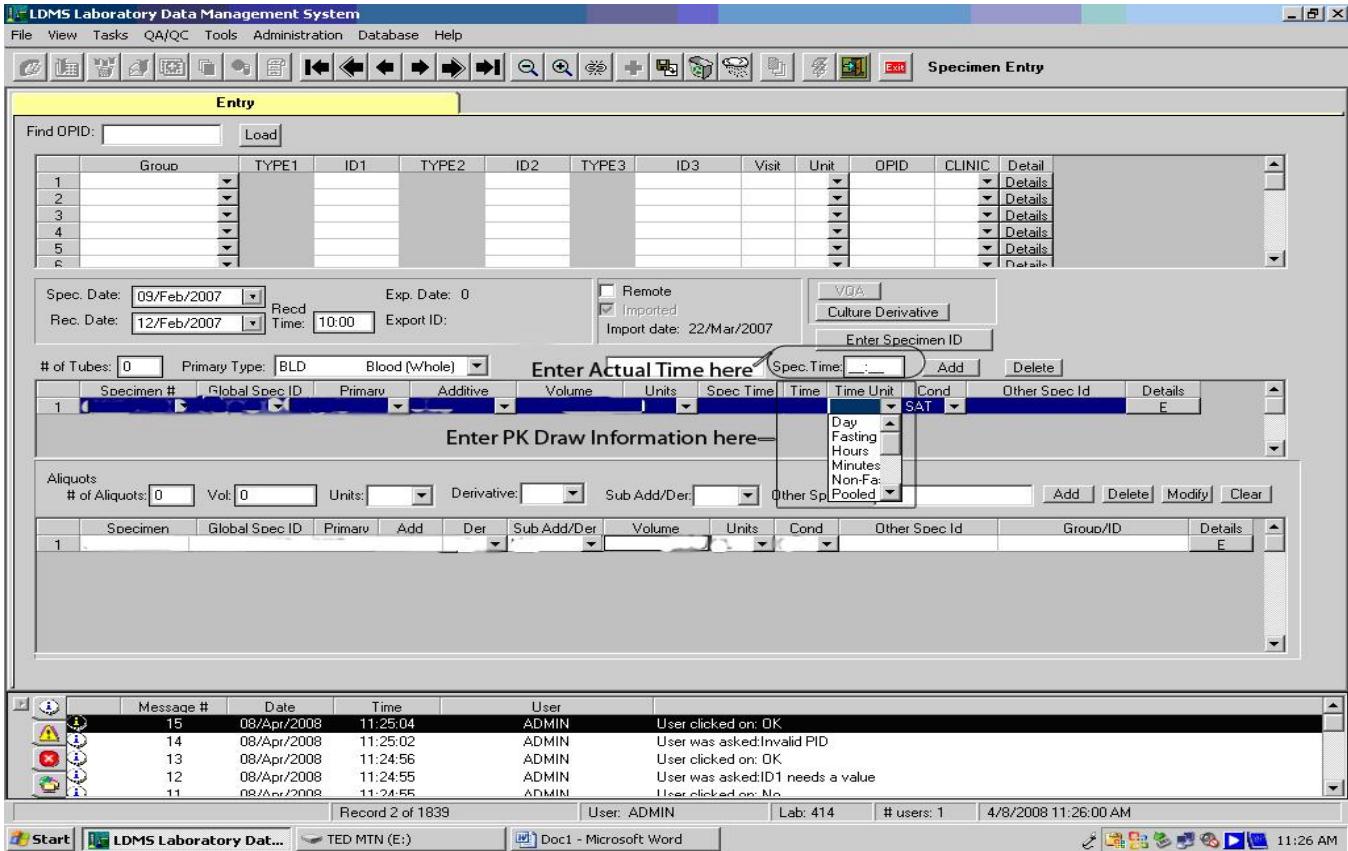
If you are locked out of your LDMS or are experiencing errors that prevent you from completing your LDMS lab work during off-hours, page LDMS User Support using the LDMS Web Pager utility. Alternatively, you may e-mail the paging system directly at ldmpager1@fstrf.org. Please allow at least 15 minutes 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 each site. Sites are expected to resolve all discrepancies within two weeks of receipt of the report. The MTN NL is responsible for reminding sites to adhere to the two week timeframe and for following up with sites that do not resolve discrepancies within two weeks. The MTN SDMC reviews the discrepancy reports for critical samples (e.g., plasma needed for confirmatory HIV testing) that appear to be missing, and works with the NL and site staff to undertake appropriate corrective action. All corrective action should be documented in paper-based clinic and/or laboratory records as appropriate, and entered in the details section of LDMS. The NL and SDMC will discuss and document any items that, although resolved, appear 'irresolvable' in LDMS.

Logging in PK Samples:

- Enter the actual time in the Specimen Time area (See Image 9-1)
- Enter the PK time point information in Time and Time Unit area (See Image 9-1)

IMAGE 9-1: LDMS Entry Screen



Weight measurements in LDMS:

The volume field in LDMS can be used for displaying weight measurements with proper units. Once the *net-weight* is attained by subtracting the pre-weight from the post-weight, the result can be entered into LDMS. In the primary sample field, enter the *net-weight* in the volume field and then change the units to milligrams. Here is an example: Pre-weight=3583.5 mg, Post weight=3621.1 mg; therefore, the net weight= 37.6 (3621.1-3583.5=37.6). Enter the 37.6 under 'volume', then change the unit to mg. Create an aliquot and enter the correct derivative code, volume (weight) and unit, then press the add prompt. Image 9-2 is an example of what this will look like in LDMS.

IMAGE 9-2: LDMS Weight Entry Screen

File Tasks QA/QC Tools Administration Database Help

Specimen Entry

Entry

Find OPID: Load

Group	TYPE1	ID1	TYPE2	ID2	TYPE3	ID3	Visit	Unit	OPID	CLINIC	Detail
1 MTN	PID	1111111111	PROTOCOL	001.0	ID3		Vis				Details
2											Details
3											Details
4											Details
5											Details
6											Details

Spec. Date: 19/Feb/2013 Exp. Date:
 Rec. Date: 19/Feb/2013 Recd. Time: 08:00 Export ID:
 Remote Imported
 Import date:
 VQA Culture Derivative Enter Specimen ID

of Tubes: Primary Type: VGL Other Spec ID: EXAMPLE Spec Time: 07:55 Add Delete

Specimen #	Global Spec ID	Primary	Addive	Volume	Units	Spec Time	Time	Time Unit	Cond	Other Spec Id	Details
1 414/13000419	VGL	NDN		37.60 MG		07:55			SAT	EXAMPLE	EDB

of Aliquots: 0 Vol: 0 Units: ML Derivative: Sub Add/Der: Other Spec ID: Add Delete Modify Clear

Specimen	Global Spec ID	Primary	Add	Der	Sub Add/Der	Volume	Units	Cond	Other Spec Id	Group ID	Details
1 414/13000420	VGL	NDN	TIS		N/A	37.60 MG		SAT	EXAMPLE	MTN/001.0	EDB

The table 9-5 should be used as a guide when logging in MTN-011 specimens. Please use the LDMS codes listed below when logging in specimens for each test listed. Tracking sheets can be found in the MTN-011 Study Implementation Materials on the MTN website.

Table 9-5: LDMS Specimen Management Guide to Logging in MTN-011 Specimens

Test	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALIQUOT DERIVATIVE	ALIQUOT SUB ADDITIVE/ DERIVATIVE	Aliquot volume	Units	INSTRUCTIONS FOR PROCESSING LAB
Plasma for Storage (archive)	BLD	EDT (purple top)	PL1/2	N/A	1.5	mL	Prepare as many 1.5 mL aliquots as possible with a total volume of aliquots \geq to 4ml. If sample is collected and held at room temp, freeze within 4 hours. If refrigerated after collection, freeze within 24 hours.
Plasma for PK (Tenofovir)	BLD	EDT	PL1/2	N/A	1.5-2 in 2ml cryovial	mL	Transport to lab ASAP. Centrifuge, split, and label two or more cryovials with a minimum of 1.5mL in each. Freeze within 8 hrs of blood collection.
Cervicovaginal Lavage (CVL) supernatant	CVL	NSL	FLD	N/A	1 ml in 2ml cryovial	mL	CVL supernatant for PD. Freeze at $\leq -70^{\circ}\text{C}$ within 8 hours of collection.
							CVL supernatant for PK. Freeze at $\leq -70^{\circ}\text{C}$ within 8 hours of collection.
							CVL supernatant for Semen Biomarker. Freeze at $\leq -70^{\circ}\text{C}$ within 8 hours of collection.
							CVL supernatant: 3 or more additional aliquots (used for backup or future testing marked 'extra CVL') & frozen at $\leq -70^{\circ}\text{C}$ within 8 hours of collection.

Test	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALIQUOT DERIVATIVE	ALIQUOT SUB ADDITIVE/ DERIVATIVE	Aliquot volume	Units	INSTRUCTIONS FOR PROCESSING LAB
Cervicovaginal Lavage (CVL) Cell Pellet	CVL	NSL	CEN	PBS	1 ml in 2ml cryovial	mL	CVL cell pellet: suspended in 0.5 mL of PBS & frozen at $\leq -70^{\circ}\text{C}$ within 8 hours of collection.
Cervical Cytobrush for Flow Cytometry (Pittsburgh)	CER	RPM	CTB	NON	Brush in 5ml tRPMI	Each	Keep on ice and deliver to Flow Cytometry ASAP to process within 2 hours from collection.
Cervical Cytobrush for PK (Case Western)	CER	RPM	CTB	MET	1 ml in 2ml cryovial	CEL	Store at least 1mL of lysate into an appropriately labeled 2 mL cryovial. Freeze Immediately at $\leq -70^{\circ}\text{C}$. In LDMS, use cell count for aliquot volume.
Vaginal Biopsy for PK (tenofovir)	VGL	NON	TIS	N/A	1 biopsy 1.8mL cryovials	mg	Pre (without biopsy) and post (with biopsy) weigh cryovials. Place each tissue in a cryovial and store at $< -70^{\circ}\text{C}$. Enter net weight in LDMS
Cervical Biopsy for PK	CVB	NON	TIS	N/A	1 biopsy 1.8mL cryovials	mg	Pre (without biopsy) and post (with biopsy) weight cryovials. Place each tissue in a cryovial and store at $< -70^{\circ}\text{C}$. Enter net weight in LDMS
Rectal PK Sponge (Tenofovir)	REC	NON	SPG	N/A	Sponge in 5 ml cryovial	mg	Perform 2 weights (Pre & post). Put on ice immediately and freeze at $\leq -70^{\circ}\text{C}$ within 4 hours of collection. Enter net weight in LDMS

Test	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALIQOT DERIVATIVE	ALIQOT SUB ADDITIVE/ DERIVATIVE	Aliquot volume	Units	INSTRUCTIONS FOR PROCESSING LAB
Semen for Biomarkers (Male only)	SEM	NON	FLD	N/A	0.5 mL in 1.5 ml cryovial	mL	Save the supernatant in as many 0.5 mL aliquots as possible. Mark one as primary, and the others as back-ups. Freeze within 4 hours of collection at $\leq -70^{\circ}\text{C}$

BLD: Whole Blood	NON: No Additive	SWB: Swab
CVB Cervical Biopsy	MET: Methanol lysate	REC: Rectal
CEN: Cell Pelet	NSL: Normal Saline	TIS: Tissue
CER: Cervix	PBS: Phosphate buffered saline	RPM: RPMI Transport Media
CTB: Cytobrush	PL1/2: Single or double spun plasma	VGL: Vagina
EDT: EDTA	SEM: Semen	CVL
FLD: Fluid Supernatant	SPG: Sponge	

9.5 Urine Testing for Pregnancy, Urinary Tract Infection, and CT/GC (Chlamydia trachomatis and Neisseria gonorrhoea) testing

The urine tests performed at each 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 performing multiple tests from one specimen, the correct order is pregnancy testing first, and then the urine dipstick (if clinically indicated).

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 participant not to clean the labia prior to specimen collection.
- Collect the first 20-30 mL of voided urine (not midstream urine) in a sterile collection cup if testing for CT/GC.
 - 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.

9.5.2 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 all sites. Perform the test according to site SOPs and the package insert. Do not perform any other urine pregnancy tests for confirmatory purposes.

Pregnancy status is a critical participant safety consideration in MTN-011. All sites must maintain an adequate inventory of the QuickVue One-Step test kits at all times. Inventory should be monitored closely and re-supply orders placed at least 8-12 weeks in advance of actual need (or longer if needed per site procurement policies and procedures). The 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.

Participants who become pregnant will be permanently discontinued from gel use and will be instructed to return all remaining product to site staff. The participant and her partner will be terminated from the study.

9.5.3 Urine Culture

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

9.5.4 Testing for Chlamydia trachomatis and Neisseria gonorrhoeae by NAAT

Testing for Chlamydia and Gonorrhea is done at screening and when clinically indicated. Sites can choose to use the BD Probetec or Gen-Probe Aptima. If the site does not have access to these tests, they can send the samples to the NL for testing. Contact the NL prior to sending specimens 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 (ProbeTec or Gen-Probe).

- Open the UPT kit and remove the UPT and transfer pipette. Label the UPT with the participants PTID number and date.
- Hold the UPT upright and firmly tap the bottom of the tube on a flat surface to dislodge any large drops from inside the cap.
- Fill the transport tube with urine to the level indicated by the black line on the tube. Do not under fill or overfill the tube.

- Transport to the local laboratory according to the specific manufacturers recommendations.
- Testing will be done at the local laboratories according to the site SOP.

9.6 Blood Specimens for HIV testing, Hematology, Hepatitis B, Syphilis, Plasma Archive, Blood Tenofovir

The blood tests performed at each study visit vary depending 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 tubes with a SCHARP-provided PTID label at the time of collection. After collection:

- Allow plain tubes (no additive or serum separator tubes) to clot, then centrifuge per site SOPs to yield serum for syphilis, HBsAg, and/or HIV testing.
- EDTA Tubes should be gently inverted at least eight times after specimen collection to prevent clotting. EDTA tubes are used for hematology, HIV testing, tenofovir, and plasma archive. If whole blood for hematology testing and plasma is to be taken from the same tube, hematological tests 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

EDTA plasma (whole blood and serum are also acceptable) will be tested for HIV using tests that have been validated at the study site. All HIV testing in laboratories must be done under Clinical Laboratory Improvement Amendment (CLIA) certification. All tests and associated QC procedures must be documented on local laboratory log sheets or other laboratory source documents.

HIV infection status will be assessed using an FDA-approved HIV immunoassay per the HIV testing algorithm (see appendix 9-1 in this section or appendix II of the MTN-011 protocol). If the screening test is negative, the participant will be considered HIV-seronegative. If the screening test is positive or indeterminate, an FDA-approved Western Blot (WB) test will be performed on the original screening

sample (Sample 1). If there is insufficient sample to perform WB, then additional blood must be recollected and must still be regarded as screening Sample 1 per the algorithm. If the WB is negative or indeterminate, contact the NL for guidance. If the WB is positive for the screening visit, patient is considered seropositive and will not be eligible for enrollment. If the WB is positive for any other visit, a second specimen (Sample 2) will be drawn for confirmatory testing. If the WB is negative or indeterminate, the site should contact the NL for further instructions.

Notify the NL immediately if any kit inventory or quality control problems are identified, so that appropriate action can be taken

Note: All HIV confirmation tests will require a Western Blot. If the local lab does not perform an HIV Western Blot test, then a sample will need to be sent to NL for confirmation testing to be done at the NL Virology lab in Pittsburgh.

9.6.3 Hematology Testing

Complete blood counts will be performed at all sites according to protocol at the Screening Visit.

Each of the following must be analyzed and reported:

- Hemoglobin
- Hematocrit
- Platelets
- White blood cell count
- Red blood cell count

These tests will be performed on EDTA whole blood per local site SOP's.

9.6.4 HbsAg test

Hepatitis B Surface Antigen (HbsAG) testing will be performed on serum or EDTA plasma per local SOPs.

9.6.5 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. 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 NL 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 NL 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-011 Protocol Safety Physicians (mtn011safety@mtnstopshiv.org).

RPR tests may be performed on either serum or plasma. Serum is the specimen of choice for syphilis confirmatory tests, however other sample types may be allowed according to the particular tests package insert. All testing and QC procedures must be performed and documented in accordance with study site SOPs.

9.6.6 Plasma Archive

For plasma archive, use collection tubes with EDTA anticoagulant. Aliquot plasma into 2 ml cryovials, store at $<-70^{\circ}\text{C}$, and batch onsite until the MTN NL study team requests shipping and/or testing.

- LDMS will be used to label and track the specimens.
- If sample is collected and held at room temp, freeze within 4 hours. If refrigerated or placed on ice after collection, freeze within 24 hours.
- Spin blood at room temperature in a centrifuge according to one of these techniques:
 - Single spun: Spin blood at 1500 RCF (g-force) for 10 minutes, remove plasma.
 - Double spun: Spin blood at 800 g for 10 minutes, place plasma in a tube to spin again at 800 g for 10 minutes, remove plasma.
- Prepare as many 1.5 mL aliquots as possible with a total volume of aliquots greater than or equal (\geq) to 4ml
- If total volume is less than 2.0 mL, redraw as soon as possible.
- If less than 4ml of plasma are available, store that plasma and inform the MTN NL for instruction.
- If samples are hemolyzed, store the aliquots as per normal and enter comments in LDMS.
- The MTN NL will send instructions to the site when shipping and/or testing is required.

9.6.7 Blood for Tenofovir PK

Collect blood into a labeled 10 mL EDTA Vacutainer tube using either an indwelling venous catheter or direct venipuncture.

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. The centrifugation must be completed and sample placed in the freezer within 8 hours of blood collection.
3. Use a pipette to aliquot approximately 1.5-2.0 mL of the resulting plasma into 2mL cryovials. One of these will serve as the primary sample; the others will serve as a back-up in case the primary samples are accidentally destroyed during shipment.
4. Prepare two storage boxes and label one as “primary samples” and the other as “back-up samples”. Transfer the tubes from each participant in chronological order into the storage boxes. All samples will be tracked in LDMS.

5. Store the boxes with samples at $\leq -70^{\circ}\text{C}$ until shipped to MTN Pharmacology Core.
6. Prior to shipping, prepare a shipment box (a foam chest) filled with dry ice sufficient for a 24 hour period with an appropriate shipping label.
7. Primary samples will be shipped to the MTN Network Lab in Baltimore, MD and assayed for tenofovir at conclusion of study unless informed otherwise. The back-up samples will be retained at the site until advised by the MTN-011 leadership group.

The shipping address for PK samples:

James Johnson
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
Email: jjohnso6@jhmi.edu

9.7 Cervicovaginal Lavage (CVL)

CVL aliquots will be collected, processed, and used for testing related to activity, PD, PK, semen biomarker, cell pellet and Anti-HSV 2.

9.7.1 Collection procedure for CVL

Potential Materials

- Drape sheet
- Gloves
- Sterile Normal Saline
- Sterile tubing (4-5 cm in length) (optional)
- Metal specimen rack
- Sterile specimen containers
- Sterile needle-less 30mL syringe
- Metal speculum
- 2ml pipette
- 15 mL conical centrifuge tube
- Study source documents
- Clock/timer
- Ice or cold packs
- Protective eyewear

Thermometer

Preparation Notes

- ✓ Prior to examination, have all necessary materials readily available on exam cart or counter near exam table.
- ✓ Check expiration of sterile saline prior to use.
- ✓ A training video is available at: <http://www.mtnstopshiv.org/node/773>.

Preparation:

1. Explain procedure to study participant.
2. Position patient for pelvic examination.
3. Wash hands thoroughly prior to procedure and put on gloves.
4. Examine external genitalia. Document and report findings on CRF.
5. Carefully insert the speculum about halfway into the vagina.
6. Open speculum gently to visualize anatomy/positioning. Close speculum and gently advance it. Repeat opening the speculum to guide insertion until part of cervix is visible.
7. Carefully open the speculum, without hitting the cervix, to position cervix into view.
8. Visually inspect cervix and vagina.

Sample Collection and Transport:

9. Draw 10cc of sterile into the 30 mL syringe.
10. Carefully insert tip of syringe into the vagina using care not to touch vaginal walls with syringe. With tip of syringe aimed at the cervix, dispense all 10 mL of saline onto the cervix. Gently tilt speculum if necessary to avoid leakage of saline.
11. Place tip of a 2ml pipette onto posterior blade of the speculum and draw fluid into pipette, using care not to touch the vagina or cervix.
12. Use the 10mL of saline to lavage the cervix, fornices and vaginal walls. Be sure to lavage each side wall at least twice. Only use the original 10cc of saline. Do not use any additional saline to perform lavage.
13. The saline must be in contact with the vaginal vault for at least 1 minute.
14. After at least one minute of contact, remove lavage fluid with 30mL syringe and sterile tubing or 2ml pipette.
15. Save lavage fluid for analysis. Transfer fluid to 15 mL conical centrifuge tube.
16. Once lavage procedure is complete, visually inspect cervix and vagina. Document and report findings on CRF.
17. Gently remove speculum.
18. Verify labeling of all specimens with study identifiers, visit code, date of collection.
19. Place specimen in refrigerator or on ice or cold packs immediately after collection.
20. Transport specimen to the laboratory on ice or cold packs.
21. Discard syringe, pipette and tubing in biohazard bag.
22. Remove gloves and wash hands thoroughly.

9.7.2 Processing of the CVL

The following steps are performed in conjunction with the collection of CVL:

1. CVL specimens are kept on ice or refrigerated and should be processed within 8 hours of collection.
2. All the CVL liquid will be spun at 800 x g for 10 minutes in the 15 mL conical collection tube.
3. Remove supernatant from the cell pellet and save fluid in cryovials.
4. Re-spin the 15 mL conical tube containing cells for 10 minutes at 800 x g.
5. Pull off and save any additional supernatant making sure not to remove any cells or debris.
6. 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, Anti-HSV, and semen biomarker testing. A minimum of 2 back-up aliquots is also required to be stored (mark as 'Extra CVL').
7. Freeze all aliquots at $\leq -70^{\circ}\text{C}$ within 8 hours of collection and track in LDMS.
8. If less than a total of 6 mL's (or less than 6 cryovials) of supernatant are recovered, contact the MTN NL.
9. Cell pellets will be suspended in 0.5 ml D-PBS in a plastic cryovial and frozen at $\leq -70^{\circ}\text{C}$ within 8 hours of collection.
10. The MTN NL will send instructions to the site when shipping is required.

Note: Study sites 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.

9.7.3 CVL PD (Pharmacodynamics)

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 for PD will be batched and shipped on dry ice to MTN NL at end of study:

Ship to:

Pamela Kunjara
Magee-Womens Research Institute
204 Craft Ave, Room A540
Pittsburgh, PA 15213
Phone# 412-641-6157
Email: pkunjara@mwri.magee.edu

9.7.4 CVL PK

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 NL Pharmacology Core at the end of the study to:

James Johnson
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
Email: jjohnso6@jhmi.edu

9.7.5 CVL Semen Biomarkers

CVL supernatant will be tested for Semen Biomarkers. Aliquots for Semen biomarkers will be batched and shipped on dry ice to the NL Pharmacology Core at the end of the study to:

James Johnson
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
Email: jjohnso6@jhmi.edu

9.7.6 CVL Cell Pellet

CVL Cell Pellet samples will be batched and shipped on dry ice to MTN NL at end of study:

Ship to:

Pamela Kunjara
Magee-Womens Research Institute
204 Craft Ave, Room A540
Pittsburgh, PA 15213
Phone# 412-641-6157
Email: pkunjara@mwri.magee.edu

9.7.7 CVL Anti-HSV 2

CVL supernatant will be tested for Anti-HSV 2 activity. Within 4 months of collection of the CVL, the samples are to be shipped on dry ice to Betsy Harold's lab:

Ship to:

Betsy Herold

Albert Einstein College of Medicine
1300 Morris Park Avenue
Bronx, NY 10461

718-430-2974 (Office)
718-430-2975 (Lab)
email: betsy.herold@einstein.yu.edu

9.8 Testing of Vaginal Specimens

Refer to Pelvic Exam checklist of this SSP manual for further information on the required sequence of specimen collection and diagnostic procedures to be performed during study pelvic exams.

9.8.1 Vaginal Biopsy

Refer to section 9.10 for Collection of Vaginal and Cervical Biopsies.

9.8.2 Vaginal Fluid pH

Vaginal fluid pH will be assessed as part of on-site evaluations 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 3 ways depending on if a speculum is used at that particular visit:

1. Obtained by the clinician during the pelvic examination
2. 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. It is not necessary to record pH values onto laboratory log sheets or other source documents prior to recording values onto case report forms.

9.8.3 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:

1. potassium hydroxide (KOH) prep
2. Saline prep

These procedures are for diagnosis of bacterial vaginosis and candidiasis as summarized in Table 9-6 below.

If wet prep slides are read in-clinic by clinical staff, results may be recorded directly on to 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 on laboratory log sheets or other laboratory source documents and then transcribed onto appropriate case report form.

CLIA regulations require semi-annual wet mount proficiency testing; therefore the MTN NL will administer a web-based proficiency test approximately every six months. The MTN NL 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 NL 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 NL for additional information and guidance on performing and documenting the proficiency testing. Also contact the MTN NL when new laboratory staff is hired, so that appropriate training can take place prior to such staff performing wet mounts for study purposes.

Table 9-6: 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 (<i>Gardnerella vaginalis</i> and/or anaerobic GNR) to be counted as clue cells.	Not applicable (clue cells are lysed by KOH)

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

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

- Use a pencil to write the PTID and specimen collection date on one side of the frosted end of two microscope slides. Affix a SCHARP-provided PTID label to the other side of the slides (on the frosted end, under the pencil markings) and write the specimen collection date in indelible ink (e.g. Sharpie pen) on each label.

- 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.
- 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, budding yeast, and pseudohyphae. Examine at 40X magnification to determine whether observed epithelial cells are clue cells and quantitate the cells. Clue cells are irregularly bordered squamous epithelial cells that are completely covered with bacteria (*Gardnerella vaginalis*). Clue cells must comprise at least 20 percent of the observed epithelial cells in order for the saline prep to be considered positive for clue cells.
- Examine the KOH slide at both 10X and 40X magnification for yeast and pseudohyphae.

9.8.4 Rapid Test for Trichomonas

This testing will be done using the OSOM Rapid Trichomonas test (manufactured by Sekisui Diagnostics formally Genzyme) with vaginal swabs per site SOPs approved by the MTN NL. The kit provides rayon 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.

9.9 Testing of Cervical Specimens

9.9.1 Cervical Biopsy

Refer to section 9.10 Collection of Vaginal and Cervical Biopsies.

9.9.2 Cytobrush

Cytobrush Processing for PK & Flow Cytometry

Supplies:

- Cytobrush: NL will supply
Use the Qiagen Digene brush (catalog number: 5126-1220 from USA — QIAGEN Inc.)
Note: Only use the brush from this kit and not the transport media supplied in the kit
- 50mL conical tube
- Trypan blue (0.4%): Cellgro Media tech #MT 25-900-CI 6 x 100mL
- D-PBS (without Ca+/Mg+): GIBCO Invitrogen Catalog # 14190-250 (10 x 500mL) or equivalent
- Transport Media (tRPMI):
 - Prepare tRPMI by making a 7.5% FBS solution of RPMI-1640: Prepare quantity that best fits laboratory needs. Here are 2 examples:
 1. Make 100mL: Adding 7.5mL of FBS into 92.5mL of RPMI-1640
 2. Make 500mL: Adding 37.5 mL of FBS into 462.5 mL RPMI-1640
 - Once tRPMI is prepared, store at 4°C and has a 30 day shelf life.
RPMI 1640: Invitrogen Catalog# 22400-105 10 x 500mL or 22400-089 1 x 500mL
Fetal Bovine Serum (FBS): Heat inactivated, Invitrogen Catalog #10082-147 500mL
- Hemocytometer or automated cell counter
- Refrigerated Centrifuge
- 70% ice cold methanol (Only for PK process)
- 2mL cryovial (Only for PK process)
- Petri dish

Specimen Collection Procedure:

1. Collect sample using cytobrush by inserting into the cervical os and perform 2 – 360° turns.
2. Immediately place cytobrush into appropriately labeled 50 ml screw cap conical vial containing 20mL of tRPMI.
3. Break off or use scissors to cut approximately 2 inches from the end of the shaft so the cytobrush will fit into the vial.
4. Keep on wet ice or refrigerate until processed.

Laboratory Processing Procedure:

5. Processing should occur within 2 hours from obtaining specimen.

6. Elute the cervical mononuclear cells into the tRPMI by agitation and rolling against the side of the tube. Pulse vortex on medium 1-2 seconds approximately 4 times.
7. Centrifuge the vial at 600 x g for 10 minutes at 4 °C in a refrigerated centrifuge.
8. Carefully remove the cytobrush, being careful not to disrupt the cell pellet.
9. Scrape the cytobrush against the side of a petri dish to dislodge the cells. Scrap it several times to ensure that there is no visible residue on the brush. Discard the cytobrush into a biohazard bag.
10. Pipette the cells from the petri dish and add these to the 50mL conical vial containing tRPMI. Using a pipette, wash the petri dish with D-PBS to recover any remaining cells.
11. Fill the 50mL conical vial containing the cells with D-PBS.
12. Centrifuge tube at 600 x g for 10 minutes at 4 °C.
13. Carefully pour off supernatant being cautious not to disrupt the cell pellet. If any fluid remains, the lip of the vial can be lightly blotted.
14. Add 1 ml D-PBS to cell pellet and suspend by vortexing.
15. Perform a cell count using an automated instrument (e.g. Orflo Moxi Z) or a manual count using a hemocytometer. For manual counts, use the dilution that will provide an accurate count. If possible, can use 10 µL aliquot of cells and add to 90µL trypan blue (0.4%) for simpler math commutation (using a factor of 10).
 - i. Record the total number of cells (including squamous cells*) and percent viable.

*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.
 - ii. The MTN NL will provide an excel sheet to record these results.

Pittsburgh:

The Flow Cytometry procedure will begin here with antibody staining

Case Western:

The PK process procedure continues at this point...

16. Centrifuge tube at 600 x g for 10 minutes at 4 °C in a refrigerated centrifuge.

17. Remove supernatant carefully using a P1000 and discard supernatant.
18. Add 1mL 70% ice cold methanol and lyse cells by briefly vortexing followed by mixing with a P1000.
19. As soon as lysis is complete, transfer at least 1mL of lysate into an appropriately labeled 2 mL cryovial.. . If more than 0.5mL of extra cytobrush lysate is available, place in another cryovial and mark 'extra cytobrush lysate'.
20. Freeze immediately and store at $\leq -70^{\circ}\text{C}$. At the end of the study, frozen PK samples can then be shipped to John Hopkins University on dry ice.

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9.9.3 Papanicolaou (Pap) Test (*only if indicated)

Pap smears are only required if clinically indicated or if a participant has not had a documented normal test within 12 months prior to screening. If a Pap smears is required, ecto- and endocervical cells will be collected after all tissues have been visually inspected and all other required specimens have been collected. The testing will be done at the site's local laboratory. Specimen collection, slide preparation, slide interpretation, and QC procedures must be performed and documented in accordance with study site SOPs.

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

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

report. Provide treatment as needed based on the results of the protocol-specified tests.

9.10 Collection of Vaginal and Cervical Biopsies

The Vaginal and Cervical biopsy should be the last part of the vaginal and cervical exam. One biopsy specimen, each from different areas of the cervix and the vagina, will be retrieved for Group 1 visits: 3b, 4b, 5b, 6b and 7b. They are also collected for Group 2 visits: 3b, 5, 7b, and 9. The biopsy will be collected as described in the site SOP, and will be using standard cervical biopsy instruments (Kevorkian, Tischler, etc) with a bite size measuring approximately 3 x 5 mm. Topical anesthetic will be not be used. Bleeding may be controlled through a combination of applied pressure, silver nitrate and/ monsel's solution. More information can be found in the MTN 011 SSP Section 8.7

9.10.1 Vaginal & Cervical Biopsies for PK analysis process procedure:

1. Label 2 1.8 ml cryovials (Nunc or Nalgene) with the appropriate sample/study identification information (1 for the vagina and 1 for the cervix).
2. Weigh the labeled cryovial using an analytical balance with a sensitivity rating of 0.1 milligrams or better. Document this pre-weight of each labeled cryovial on the LDMS tracking sheet.
3. Directly transfer each biopsy to its designated pre-weighed cryovial.
4. Obtain the post-weight for each cryovial containing a biopsy using an analytical balance and document on the LDMS tracking sheet.
5. Immediately freeze the cryovial containing the biopsy in dry ice ethanol bath (dry ice with enough ethanol to make a slushy consistency) or liquid nitrogen
6. Document the date and time when the cryovial containing the biopsy is frozen on the LDMS tracking sheet.
7. Store the labeled cryovials containing the frozen biopsies at $\leq -70^{\circ}\text{C}$.
8. All pre and post weights are also to be logged by the processing lab onto an excel weight worksheet supplied by network lab. The net-weights will be calculated by the formula in the worksheet and entered into the LDMS system.
9. At the end of the study, the PK biopsies can then be shipped to John Hopkins University on dry ice.

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9.11 Collection of Rectal Fluid

9.11.1 Rectal Specimen

This procedure outlines the collection of rectal fluid specimens using a collection sponge and should be followed for all rectal fluid specimens collected.

Materials:

Sponge: Merocel eye-wick Spears (Fisher Scientific # NC0093269)

5ml cryovial: Fischer Scientific Cat # 10-500-27 or equivalent.

Polyethylene (plastic) transfer pipettes: (Fisher Scientific # 13-711-20 or similar)

Anoscope (example – Owens and Minor cat# 1643082420)

PRE personal lubricant: Approved lubricant Good Clean Love. “almost naked” formulation.

Available from Drugstore.com. <http://www.drugstore.com/good-clean-love-all-natural-personal-lubricant-almost-naked/qxp156292?catid=181914&fromsrch=good%20clean%20love>

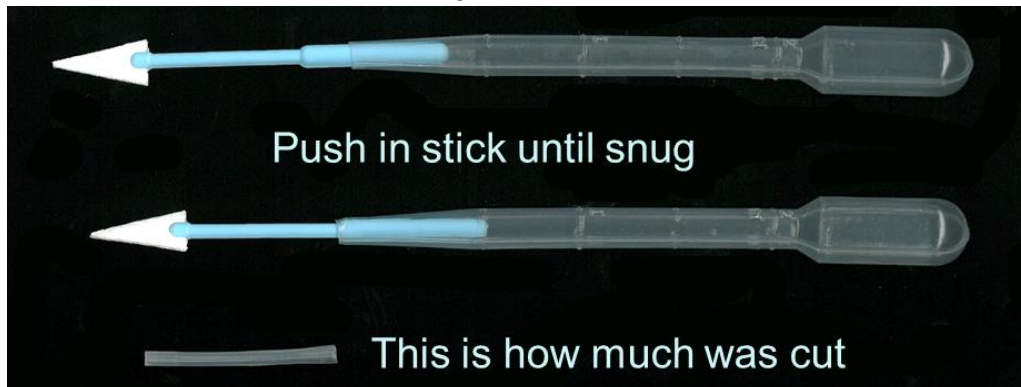
Analytical Scale with sensitivity: 0.0001 Grams (0.1 mg)

Preparation of Materials (1-2 hours prior to procedure):

1. While wearing gloves, remove sponge from package and label 5 ml cryovial.
2. Weigh the sponge (connected to the sponge stick) and 5 ml cryovial.
3. Record the *pre-weight* on the LDMS tracking sheet.
4. Prepare a sponge holder (also called an insertion tube) using a sterile plastic transfer pipette by cutting off the end approximately 1 inch from the tip. See Image 9-3.

NOTE: Make sure that the stem of the sponge will fit into the pipette snugly so that it will not dislodge during insertion or extraction from the rectal cavity.

Image 9-3



Rectal Sample Collection Procedure:

1. Use the PRE personal lubricant to lubricate the anoscope.
2. With subject placed in left lateral recumbent position slowly insert the anoscope with obturator in place through the anus and advance the instrument until the flange is

- flush with the subject's skin. Maintain pressure on flange to ensure continued placement of the anoscope.
3. Remove obturator; introduce the sponge (attached to the pipette sponge holder extension) through the anoscope into the rectum.
 4. Record the time onto the CRF (Rectal Sponge Collection Time).
 5. Hold (or leave) sponge in place for 5 minutes.
 6. Disengage sponge from holder (plastic pipette) and discard holder. Place the sponge in the 5ml cryovial from the initial weighing and cap **immediately** to avoid evaporation.
 7. Slowly remove anoscope.
 8. Weigh the cryovials with sponge (that includes the sponge stick) to record the *post-weight* on the LDMS tracking sheet.
 9. Place on ice immediately and freeze at $\leq -70^{\circ}\text{C}$ within 4 hours.
 10. Record the time that the sample was placed in the freezer on the LDMS tracking sheet.
 11. All pre and post weights are also to be logged by the processing lab onto an excel weight worksheet supplied by network lab. The net-weights will be calculated by the formula in the worksheet and entered into the LDMS system.
 12. At the end of the study, the Rectal Sponge PK samples can then be shipped to John Hopkins University on dry ice:

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9.12 Collection of Semen Sample from Males

9.12.1 Semen Sample Collection and Processing

1. Collect sample in a sterile collection cup with screw lid (refer to clinical consideration section 8.9.1 for collection details).
2. Record the appropriate sample/study identification information and the collection date/time on the CRF.
3. Allow liquefaction of the semen to occur. This typically occurs within 45 minutes of semen collection.
4. Volume measurement:
 - 4.1 Transfer all the liquefied semen to an appropriately labeled 15 mL conical tube using a 1000 μL , 200 μL and 100 μL microliter transfer pipettes. Start with the 1000 μL pipette and working down to the 100 μL pipette volume.

- Example: pipetted 5 portions with 1000 μ L, then 3 portions using 200 μ L, and 1 with the 100 μ L: 5.000 mL+ 0.600 mL + 0.100 mL= 5.7 mL
- 4.2 Compare the volume measurement to the volume gradients on the 15 mL conical tube to see if they agree.
 - 4.3 Record measured final volume onto the with 0.1 mL accuracy.
 5. Semen Fractionation:
 - 5.1 Record the semen separation start time.
 - 5.2 Centrifuge the 15mL conical tube containing the semen at 800 x g for 10 minutes at 4°C in a refrigerated centrifuge.
 - 5.3 Remove and save the supernatant in as many 0.5 mL aliquots as possible in 1.5 mL microvials. Mark one microvial as the primary sample, and the others will serve as a back-ups.
 - 5.4 Freeze within 4 hours of collection at \leq -70°C.
 - 5.5 Record the time the semen supernatant aliquots were frozen.
 6. At the end of the study, primary samples for PK can then be shipped to John Hopkins University on dry ice:

James Johnson
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
Email: jjohnso6@jhmi.edu

All primary samples for PK (Plasma PK, Biopsies, CVL PK, Cytobrush PK, and Rectal Sponge PK) can be sent together at the conclusion of the study or when requested by MTN NL. This shipment can also include CVL Semen biomarkers and Semen Supernatant.

**APPENDIX 9-1:
HIV ANTIBODY TESTING ALGORITHM**

