

## Section 9. Laboratory Considerations

This section contains information on the laboratory procedures performed in MTN-027.

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## 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 Laboratory Center (LC [formally known as Network Lab or NL]), including the MTN Pharmacology Core at the University of Colorado (Colorado Antiviral Pharmacology Laboratory [CAVP]). Table 9-1 and table 9-2 highlight specimen, storage and shipment requirements. Table 9-3 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 LC may request details of collection containers and volumes for this purpose.

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

Ideally, one method, type of test kit, and/or combination of test kits will be used for each protocol specified test throughout the duration of the study. If for any reason a new or alternative method or kit must be used after study initiation, site laboratory staff must perform a validation study of

the new method or test prior to changing methods. The MTN LC must be notified before the change and can provide further guidance on validation requirements.

Provided in the remainder of this section is information intended to standardize laboratory procedures across sites. Adherence to the specifications of this section is essential to ensure that 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-027**

Test	Testing Location	Specimen Type	Tube or Container and tube size (recommended)	Kit or Method
Urine pregnancy test (hCG)	In clinic	Urine	Plastic screw top cup	One-Step or Combo hCG Quidel Quick Vue, or Fisher HealthCare Sure-Vue Urine hCG kit
Dipstick	Local lab	Urine	Plastic screw top cup	Siemens Multistix or Uristix
Urine Culture <sup>1</sup>	Local lab	Urine	Plastic screw top cup	Local methodology
CBC with Diff & Platelet	Local Lab	Whole Blood	EDTA 4 mL tube	Local methodology
Chemistries (AST, ALT, Creatinine)	Local Lab	Serum or Heparinized plasma	Plain or serum separator 4 mL	Local methodology
Prothrombin Time Coagulation (PT and INR)	Local Lab	Sodium Citrated Plasma	Blue top, 4 mL tube	Local methodology
HIV Antibody Screen	Clinic/Local Lab	Plasma, serum, or whole blood	EDTA or plain tube 4 mL	Bio-Rad HIV-1,2+O EIA or other FDA approved test
HIV Confirmation	Local Lab	Plasma or serum	EDTA or plain tube 4 mL	Multispot or other FDA-approved test
Hepatitis testing for HBsAG and HCV	Local Lab	Serum	plain or serum separator, 4 mL	Local methodology
Blood PK Vicriviroc (MK-4176) & MK-2048 levels	Colorado Antiviral Pharmacology Lab (CAVP)	Plasma	EDTA 10 mL tube	CAVP collection procedure
Syphilis Serology	Local Lab	Serum or Plasma	EDTA tube, plain or serum separator, 4 mL	Local methodology
Plasma Archive	Clinic/Local Lab	Plasma	EDTA 10 mL tube	LC procedure
Vaginal Swab for biomarkers	LC	Vaginal Swab	2.0 mL cryovial	LC procedure
Vaginal Swab(s) for PK	CAVP	Vaginal Swab	2.0 mL cryovial	LC/CAVP procedure
Vaginal NAAT for Gonorrhea and Chlamydia	Local lab	Vaginal swab (supplied with kit)	Kit specific Transport tube	BD Probetec, Gen-Probe Aptima, or GeneXpert

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
Quantitative Vaginal Culture	LC	Vaginal swab	Port-a-Cul transport tubes by BD or BD culture swab Max V	LC procedure
Vaginal smear Gram-stain	LC	Vaginal Swab	Slides	LC procedure
Used Vaginal Ring for PK residual assessment	Merck Designated lab	Used VR	Biohazard labeled amber bag	LC/Merck procedure
Vaginal saline wet preparation (for BV and/or KOH wet mount) <sup>1</sup>	In clinic	Vaginal swab	Tube with 6 drops of saline	LC procedure
Cervical Cytobrush Flow Cytometry	MTN McGowan Lab	Cytobrush	50 mL Conical vial	MTN McGowan Lab collection procedure
Cervical Biopsy for PD (ex vivo challenge) Pitt only	LC	Tissue	2.0 mL cryovial	LC collection procedure
Cervical Biopsy for PK	CAVP	Tissue	2.0 mL cryovial	LC collection procedure
Rectal PK Levels	Merck Designated lab	Rectal Sponge	5 mL cryovial	LC collection procedure
Pap Smear <sup>2</sup>	Local Lab	Ecto & Endo-cervical cells	Slides	Local methodology

<sup>1</sup> Perform only if clinically indicated per local SOP.  
<sup>2</sup> Perform only if clinically indicated or for women ≥21 years old that do not have documentation of a satisfactory Pap within the past 3 years prior to Enrollment

**Table 9-2: Overview of Specimens for Storage and Shipment**

Specimen and subsequent testing	Additive	Tube type or size recommended	Processing	Ship to:	Shipping schedule
Plasma for Archive	EDTA	10 mL	Freeze plasma at ≤ -70°C within 4 hours of draw	LC	Store frozen at site until notified by LC
Plasma for Blood PK	EDTA	10 mL	Freeze plasma within 8 hours after collection	LC	Store frozen at site until conclusion of study
Cervical Cytobrush for Flow Cytometry (Pitt)	tRPMI	50 mL cryovial	Process within 2 hours of collection.	McGowan Lab	Inform lab when collected. Deliver ASAP
Cervical Cytobrush for Flow Cytometry (UAB)	tRPMI	50 mL cryovial	Process within 2 hours of collection.	McGowan Lab	Ship with ice packs to McGowan Lab First Overnight FedEx

Cervical Tissue Biopsy for PD (ex vivo challenge) (Pitt only)	Biopsy Transport Medium (BTM)	2 mL cryovial	Pre-cool conical tube with 1 ml of biopsy transport medium	Dezzutti Lab	Transport immediately (<30 min) to Dezzutti lab
Cervical Biopsy for PK	None	2 mL cryovial	Immediately freeze cryovial biopsy in dry ice ethanol bath	LC will coordinate shipment	Store frozen at site until notified by LC
Vaginal Dacron Swab for Vaginal Biomarkers	400uL PBS	1.5 mL Micro tube	Put swab in 400uL PBS, break off shaft	LC	Store frozen at site until notified by LC
Vaginal Dacron Swab for PK (self-collected)	None	2 mL cryovial	Freeze within 2 hours of collection	LC will coordinate shipment	Store frozen at site until notified by LC
Used Vaginal Ring for PK residual assessment	None	3"x5" amber Zippit pouch	Blot dry and place used VR in pouch	LC	Store 2-8 °C at site until notified by LC
Vaginal smear for Gram-stain	None	2 slides	Roll vaginal swab on slide, let air dry.	LC	Ship 1 slide the day of collection to LC. Store 2 <sup>nd</sup> slide at site until conclusion of study
Vaginal Swabs for Quantitative Vaginal Culture	Port-a-Cul (PAC)	2 swabs in transport tube	Place swab in Port-a Cul & break off shaft or use BD Max V culture kit	LC	Ship on ice packs to LC on the day of collection.
Rectal PK Level	None	5 mL cryovial	Freeze within 4 hours of collection	LC will coordinate shipment	Store frozen at site until notified by LC

**Table 9-3: Overview of Laboratory Tests by visit for MTN-027**

		SCR	ENR	D 1, 2	D 3	D 7	D 14	D 21	D 28	D 29, 30 & 31	D 35 Final Clinic	
Urine	<i>Collect Urine</i>											
	hCG	X	X	*	*	*	X	*	X	*	*	
	Dipstick UA	X	*	*	*	*	*	*	X	*		
	Urine culture	*	*	*	*	*	*	*	*	*		
Blood	<i>Collect Blood</i>											
	CBC with Diff and Plt	X	X						X		X	
	HIV-1 serology	X	X	*	*	*	*	*	*	*	X	
	HBsAg	X										
	Anti-HCV	X										
	PT Coag (INR)	X										
	Chemistries	X	X						X		X	
	Blood PK: Vicriviroc (MK-4176) & MK-2048 levels		x @ hr 1,2,4,6	X	X	X	X	X	X	x @ hr 0,1,2,4,6	X	X
	Syphilis serology	X	*	*	*	*	*	*	*	*	*	*
	Plasma archive		X									
<i>Collect pelvic specimens</i>												

		SCR	ENR	D 1, 2	D 3	D 7	D 14	D 21	D 28	D 29, 30 & 31	D 35 Final Clinic	
Vaginal	Vaginal fluid pH	*	X	*	X	*	*	*	X	X	X	
	Rapid Trichomonas test	X	*	*	*	*	*	*	*	*	*	
	KOH wet mount	*	*	*	*	*	*	*	*	*	*	
	Saline wet mount for BV	*	*	*	*	*	*	*	*	*	*	
	Vaginal NAAT for GC/CT	X	*	*	*	*	*	*	*	*	*	
	Quantitative Vag culture		X		X				X		X	
	Vaginal Gram stain		X		X				X		X	
	Self-collected PK-Vaginal Fluid Swab, UAB and Pitt <sup>☞</sup>		x @ hr 0,1,2,4,6	X	X	X	X	X	X	x @ hr 0,1,2,4,6	X	X
	Vag swab for biomarkers		X		X				X		X	
Cervical	Cervical Cytobrush		X@0hr						X@0hr <sup>☞</sup>			
	Cervical biopsy for PK								X@0hr <sup>☞</sup>			
	Cervical biopsy for PD (ex vivo challenge...Pitt site only)								X@0hr <sup>☞</sup>			
	Collect Pap test	*										
	<i>Collect Rectal specimen</i>											
Rec	<i>Rectal Sponge for PK</i>		☞@0hr						☞@0hr <sup>☞</sup>			
<b>STUDY PRODUCT</b>												
Provision of IVR			X									
Collect IVR									X		▲	
		SCR	ENR	D 1, 2	D 3	D 5, 7	D 14	D 21	Day 28	D 29, 30, & 31	D 35 Final Clinic	

X Required  
\* If clinically indicated  
▲ If Early Termination Visit and not already performed, collect IVR  
☞ At Day 28 (Visit 9.0), 'hour 0' samples are collected a short time after ring removal  
☞ To be collected on a subset of participants who opt in  
☞ Pitt will collect 3 swabs on first 8 participants on visit day 1 & 7. For all other visits, obtain 1 self-collected swab.

## 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 9.4 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 LC must be notified in the following cases

- Any time a participant must return to the clinic for specimen collection
- When PK specimens are missed
- 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 LC 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 9-4.

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).

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 (CRF). 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 LC is responsible for reminding sites to adhere to the two week timeframe and for following up with sites that do not resolve discrepancies within two weeks. The MTN SDMC reviews the discrepancy reports for critical samples (e.g., plasma 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.

Logging in Samples (see Figure 9-1):

- Enter the actual time of specimen collection in the Specimen Time area.
- Enter all specimen codes to populate the specimen receiving area (see section 'A')
- To make aliquots for each specimen in section 'A', fill out section 'B'

Figure 9-1: LDMS Entry Screen

The screenshot shows the LDMS Entry Screen with two main sections highlighted in red:

- Section A:** Contains fields for specimen details. Key fields include:
  - Spec. Date: 11/May/2015
  - Rec. Date: 11/May/2015
  - Spec. Time: 10:22
  - Spec. Time: 08:01 (Annotated with "Enter Actual Collection Time here")
  - Primary Type: BLD (Annotated with "Insert blood draw information here")
- Section B:** Contains fields for aliquot information. Key fields include:
  - # of Aliquots: 2
  - Volume: 1.50 ML (Annotated with "Insert aliquot information here")
  - Units: ML
  - Derivative: BLD
  - Sub Add/Der: PL1

### 9.4.1: 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 as shown in Figure 9-2. In the primary sample field (section A), enter the sample(s) that are weighted. Under volume *Units*, enter *each* (EA) in this field and enter '1' for *Volume*. Enter the exact time collected in the *Spec Time* field. Also, if there is a need to identify a particular specimen, (Ex: biopsy or if a blood PK sample), enter additional identifying information under *Other Spec ID*. To record the weights, make an aliquot in section B for each of the primary samples that were entered from section A. Enter the net-weight and change the units to milligrams. Here is an example of a biopsy weight: 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*, change the *Unit* to MG.



**Figure 9-2: LDMS Weight Entry Screen**

**A**

Spec. Date: [04/Feb/2015] Exp. Date: 0  
 Rec. Date: [04/Feb/2015] Recd. Time: [09:00] Export ID:  
 Remote  Imported  Import date:  
 VOA Culture Derivative  
 Enter Specimen ID

# of Tubes: 0 Primary Type: BLD Other Spec ID: Spec. Time: Add Delete

Specimen #	Global Spec ID	Primary	Add	Der	Sub Add/Der	Volume	Units	Cond	Other Spec ID	Details
1	414715001373	JC63894C-00	CVB	NON		1.00 EA		08:10	SAT	DB

**B**

# of Aliquots: 0 Vol: 0 Units: 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	414715001377	JC63894C-01	CVB	NON	BFS	N/A	3780	MG	SAT	Practice Group/1234567	RDB

Table 9-4 should be used as a guide when logging in MTN-027 specimens. Please use the LDMS codes listed below when logging in specimens for each test listed. Tracking sheets can be found in the Study Implementation Materials section on the MTN-027 webpage.

**Table 9-4 LDMS Specimen Management Guide for MTN-027 Specimens**

Test	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALIQUOT DERIVATIVE	ALIQUOT SUB ADDITIVE/ DERIVATIVE	Aliquot volume	Units	INSTRUCTIONS FOR PROCESSING
Plasma for Archive (plasma storage)	BLD	EDT	PL1/2	N/A	1.5-2.0	mL	Prepare as many 1.5 mL aliquots as possible with a total volume of aliquots $\geq$ 3 ml. If sample is collected and held at room temp, freeze within 4 hours. If refrigerated after collection, freeze within 24 hours.
Plasma for PK (Vicriviroc [MK-4176] & MK-2048)	BLD	EDT	PL1/2	N/A	1.5-2.0 in 2 mL cryovial	mL	Transport to lab ASAP. Centrifuge, split, and label two or more cryovials with a minimum of 1.5 mL in each. Freeze within 8 hrs of blood collection.

Test	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALIQOT DERIVATIVE	ALIQOT SUB ADDITIVE/ DERIVATIVE	Aliquot volume	Units	INSTRUCTIONS FOR PROCESSING
Cervical Biopsies for PK	<b>CVB</b>	<b>NON</b>	<b>BPS</b>	<b>N/A</b>	1 biopsy in each 2 mL cryovial	mg	Perform Pre (without biopsy) and Post (with biopsy) weights. Store at <-70°C. Enter weights on LDMS tracking sheet.
Cervical Biopsy for PD (ex vivo challenge) Pittsburgh only	<b>CVB</b>	<b>BTM</b>	<b>BPS</b>	<b>N/A</b>	2 mL Corning (orange cap) cryovial	Each	Place biopsy into a 2 mL cryovial tube containing 1 mL of chilled transport medium, take to Dezzutti lab immediately.
Cervical Cytobrush for Flow Cytometry (Pitt & UAB)	<b>CER</b>	<b>RPM</b>	<b>CTB</b>	<b>N/A</b>	Brush in 20 ml tRPMI	Each	Pitt: Keep on ice and deliver to Laboratory ASAP to process within 2 hours from collection. UAB: to ship stained on ice overnight to LC
Rectal PK Sponge	<b>REC</b>	<b>NON</b>	<b>SPG</b>	<b>N/A</b>	Sponge in 5 mL cryovial	mg	Perform pre & post weights. Put on ice immediately and freeze at ≤-70°C within 4 hours of collection. Enter net weight in LDMS
Vaginal Swab for biomarkers	<b>VAG</b>	<b>PBS</b> (400 µL PBS)	<b>SWB</b>	<b>N/A</b>	1 swab in 1.5 mL micro tube	Each	Place Dacron swab in a labeled cryovial containing 400 µL PBS. Immediately refrigerate or place vial on ice, then freeze at ≤-70°C within 8 hours.
Vaginal Smear for Gram Stain	<b>VAG</b>	<b>NON</b>	<b>SLD</b>	<b>GRS</b>	2 smears	Each	Make 2 slides. Re-label with LDMS label. Ship one slide to MTN LC and store other slide on-site.
Vaginal Swab for Quantitative Culture	<b>VAG</b>	<b>PAC</b>	<b>SWB</b>	<b>N/A</b>	2 swabs in Port-a Cul or use BD Max V culture kit	Each	Ship overnight on ice packs to MTN LC on the day of collection.

Test	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALIQUOT DERIVATIVE	ALIQUOT SUB ADDITIVE/ DERIVATIVE	Aliquot volume	Units	INSTRUCTIONS FOR PROCESSING
Self-collected Vaginal PK Swabs for <b>PITT</b>	<b>VAG</b>	<b>NON</b>	<b>SWB</b>	<b>N/A</b>	3 swabs	mg	First 8 participants on Visit days 1 & 7: Label (1, 2 & 3), Pre & post weigh each vial including swab, swab shaft & wrapper. Store at <-70°C. Enter weights on LDMS tracking sheet.
					1 swab	mg	Pre & post weigh each vial including swab, swab shaft & wrapper. Store at <-70°C. Enter weights on LDMS tracking sheet.
Self-collected Vaginal PK Swabs for <b>UAB</b>	<b>VAG</b>	<b>NON</b>	<b>SWB</b>	<b>N/A</b>	1 swab	mg	Pre & post weigh each vial including swab, swab shaft & wrapper. Store at <-70°C. Enter weights on LDMS tracking sheet.
Used Vaginal Ring for residual PK	<b>IVR</b>	<b>NON</b>	<b>IVR</b>	<b>NA</b>	1 pouch	Each	Store in amber bags at 2-8 °C.

**Table 9-5 LDMS Codes**

BLD: Whole Blood	GRS: Gram Stain	PL1/2: Single or double spun plasma
BPS: Biopsy	IVR: Used Intravaginal Ring	SWB: Swab
BTM: Biopsy Transport Medium	N/A: Not Applicable	SLD: Slide
CVB Cervical Biopsy	NON: No Additive	SPG: Sponge
CER: Cervix	PAC: Port-a-Cul or BD Max V (culture transport medium)	REC: Rectal
CTB: Cytobrush		RPM: RPMI Transport Media
EDT: EDTA	PBS: Phosphate buffered saline	VAG: Vaginal Swab

Questions related to use of LDMS in MTN-027 may be directed to LC 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](mailto:ldmshelp@fstrf.org)  
Phone: +716-834-0900, ext 7311 Fax: +716-898-7711

#### 9.4.2 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, the e-mail the paging system can be directly emailed at [ldmspager1@fstf.org](mailto:ldmspager1@fstf.org). Please allow at least 15 minutes to get a response before sending another e-mail to the paging system.

## 9.5 Urine Testing for Pregnancy, Dipstick Urinalysis, and Culture

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 first pregnancy testing, and then the urine dipstick (if required or clinically indicated).

### 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.
- Participant should not to clean her labia prior to specimen collection.
  - If testing is *only* for urinalysis, culture, and/or pregnancy test, then collect midstream urine.
- Instruct the participant to screw the lid tightly onto the cup after collection.

#### 9.5.1 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.

Pregnancy status is a critical participant safety consideration in MTN-027. All sites 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.

In the rare event, a participant becomes pregnant; study product use will be permanently discontinued. The participant will be terminated from the study.

#### 9.5.2 Dipstick Urinalysis

Only leukocytes (leukocyte esterase), nitrites, protein, and glucose on the dipstick are required. Any of the Siemens urine reagent test strips can be used. Perform this test according to site SOPs and the package insert. Assess and record UA results on the Safety Laboratory CRF (SLR-2). To avoid overgrowth of bacteria, refrigerate specimen before and during transport to laboratory.

At visits when both pregnancy testing and dipstick urinalysis are required, the same aliquot should be used for both tests, but the urinalysis should be performed after urine has been pipetted from the aliquot for the pregnancy test.

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

### 9.5.3 Urine Culture

Perform only if clinically indicated or by local standard of care. Instruct the participant to collect a midstream urine sample.

## 9.6 Blood Specimens: Collection and Processing for Chemistry, Hematology, Hepatitis, Coagulation, HIV testing, Syphilis, Plasma Archive, and PK Testing

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 immediately after a phlebotomy collection.

- Collect venous blood collection tubes (red or gold top SST) and invert 5-8 times, then allow samples to clot, then centrifuge per site SOPs to yield serum for chemistry, syphilis, HBsAg, anti-HCV, HIV (EIA) testing.
- Lithium Heparin (Light Green Top) may be the specimen of choice for chemistry tests (ALT, AST, and Creatinine). Invert 8 times directly after collecting specimen.
- EDTA tubes (purple top) should be gently inverted at least 8 times after specimen collection to prevent clotting. EDTA tubes are used for hematology, HIV testing, PK, 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.
- Sodium Citrate tube (Blue top) should be gently inverted at least 3-4 times after specimen collection to prevent clotting. The Na citrate tube will be used for testing the prothrombin time.

*Note: Use local laboratory guidelines for the type of tube required. 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.*

*Order of draw: Blue (sodium citrate) ⇒ Red (no additive) ⇒ Tiger or Gold (SST, gel, clot activator) ⇒ light green (Lithium Heparin) ⇒ Lavender (EDTA)*

### 9.6.2 Chemistry (ALT, AST, and Creatinine), Hematology (CBC with Diff and Platelets), and Coagulation.

Testing will be performed per the local standard of care.

- Chemistry Tests: Alanine transaminase (ALT), Aspartate aminotransferase (AST), and Creatinine. Renal function will be further evaluated using the participant's weight and age in conjunction with the Cockcroft-Gault formula. The Creatinine Clearance Calculator is located on the MTN-027 webpage (in the [Study Implementation Materials](#) section).
  - Cockcroft-Gault formula (for female):
$$= \frac{(140 - \text{age in years}) \times \text{weight in kg} \times 0.85}{72 \times \text{creatinine in mg/dL}} = \text{mL/min}$$
- Hematology tests are: Hemoglobin, Hematocrit, Platelets, White blood cell count (WBC), Red blood cell count (RBC) and differential.
- Hepatitis Testing: HBsAG and Anti-HCV
- Coagulation Test: Prothrombin Time to include an INR.

### 9.6.3 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. Successful ratings for proficiency testing such as College of American Pathologists (CAP) must be maintained.

HIV infection status will be assessed according to the HIV testing algorithm as presented in Appendix 9-1 in this section (or appendix II of the MTN-027 protocol). The first specimen drawn is considered Sample 1, and the confirmatory specimen drawn is considered Sample 2.

Test result interpretation is as follows:

- If the Sample 1 immunoassay result is negative, the participant will be considered HIV-seronegative.
- If the Sample 1 immunoassay result is positive or indeterminate, an FDA-approved confirmatory test should be performed on Sample 1. If there is insufficient sample to perform the confirmatory test, then additional blood must be drawn. This re-draw will still be regarded as Sample 1 per the algorithm.

#### **Screening Participants** (to include HIV testing for enrollment visit)

- Until enrolled, treat enrollment testing same as screening participants.

- If the confirmatory test is negative, indeterminate or invalid, contact the Virology LC for guidance at [mtnvirology@mtnstopshiv.org](mailto:mtnvirology@mtnstopshiv.org). It is not recommended for participants with discrepant HIV testing results to continue enrollment.
- If the confirmatory test is positive for the screening visit, the participant is considered seropositive and is not eligible for enrollment.

### **Follow-Up Participants**

- If the confirmatory test on sample 1 is negative, indeterminate or invalid, contact the Virology LC for guidance at [mtnvirology@mtnstopshiv.org](mailto:mtnvirology@mtnstopshiv.org).
- If the confirmatory test on sample 1 is positive at a follow-up visit, a second specimen (Sample 2) will be drawn for additional confirmatory testing and performed at the MTN Virology LC. Process the sample and aliquot the plasma in 1 mL aliquots and freeze at <-70°C. Contact the Virology LC to alert them of the samples and ship at least 3 aliquots immediately on dry ice. The Virology LC will provide test results to the site. In addition, draw extra blood if required for local standard of care.
  - If the confirmatory test on Sample 2 is positive, the participant is HIV positive.
  - If the confirmatory test on Sample 2 is negative, indeterminate or invalid, the Virology LC will provide guidance

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

### **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. These participants will not be eligible for enrollment in the study. MTN LC recommends for enrolled participants considered positive should include repeat non-treponemal assay tests at quarterly intervals following syphilis diagnosis to evaluate treatment effectiveness. If the RPR or VDRL titer does not decrease four-fold or revert to seronegative within three months after treatment, further investigation and/or treatment may be warranted.
2. Perform syphilis assessment using a specific FDA approved treponemal test (such as EIA, MHA-TP, TPHA, TPPA, or FTA-ABS) and confirming positive test results with a non-treponemal assay (RPR or VDRL). If the confirmatory non-treponemal assay is reactive at screening visit, appropriate clinical management action must be taken. These participants are not eligible for enrollment in the study. If the RPR or VDRL is negative, this may indicate prior treatment, late latent disease, or a false positive test. MTN LC recommends additional testing using an alternative treponemal test other

than the original treponemal test used for the original assessment so the participant can be correctly evaluated. (Of note, the FTA-ABS should not be used as the alternative confirmatory test due to performance issues). If the second confirmatory test is negative, the participant is not considered infected with syphilis. If the second confirmatory test is positive, the participant has had prior exposure to syphilis and depending on clinical scenario may or may not require treatment.

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

Questions related to result interpretation concerning eligibility and enrollment in the study should be directed to the MTN-027 Protocol Safety Physicians ([mtn027safetymd@mtnstopshiv.org](mailto:mtn027safetymd@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.

#### **9.6.5 Plasma Archive**

For plasma archive, use collection tubes with EDTA anticoagulant. Aliquot plasma into 2 ml cryovials, store at  $\leq -70^{\circ}\text{C}$ , and batch onsite until the MTN LC 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  $1500\times g^*$  (relative centrifugal force [RCF] in g) for 10 minutes, remove plasma.  
\*Follow manufactures directions for spinning tubes, many plastic tubes can only be spun at  $1300\times g$ .
  - Double spun: Spin blood at  $800\times g$  for 10 minutes, place plasma in a tube to spin again at  $800\times g$  for 10 minutes, remove plasma.
- Prepare as many 1.5-2.0 mL aliquots as possible with a total volume of aliquots greater than 3 ml.
- If total volume is less than 2.0 mL, redraw as soon as possible.
- If less than 3 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.

#### **9.6.6 Blood for PK: Vicriviroc (MK-4176) and MK-2048**

On single time-point days (visit days 1, 2, 3, 7, 14, 21, 29, 30, 31, & 35), The participant will self-collect the vaginal PK swab approximately within 1 hour after the blood is drawn for PK. On multiple time-point days (visit days ENR and 28), optimally the participant will self-collect the vaginal PK swab within 5 minutes after the blood PK sample is drawn. [See section 9.7.8 for details.](#)



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×g for 10 minutes (follow manufactures directions for spinning tubes, many plastic tubes can only be spun at 1300×g). 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.
6. LC will coordinate sample shipments throughout course of study if necessary and at its conclusion.
7. The back-up samples will be retained at the site until advised by the LC or MTN-027 leadership team.
8. All shipments will be on dry ice that will be sufficient for a 24 hour period and can be initiated Monday through Wednesday to insure that samples arrive in the lab during the work week.

## **9.7 Vaginal Samples: Gram Stain, Microbiology Culture, Vaginal Fluid pH, Vaginal Wet Mount, Trichomonas, CT/GC, Biomarkers, PK (secretions and IVR).**

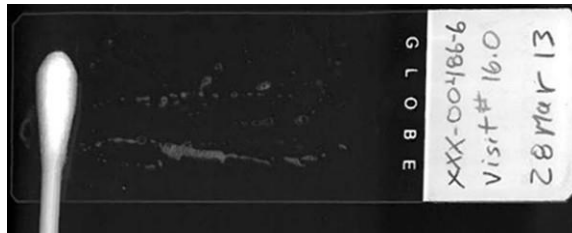
Refer to Pelvic Exam checklist of this SSP manual for further information on the required sequence of specimen collection and diagnostic procedures to be performed during study pelvic exams.

### **9.7.1 Gram Stains on Vaginal Fluid**

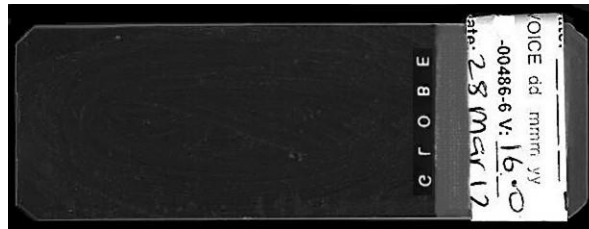
Dried vaginal fluid smears will be prepared for Gram staining and assessment for bacterial vaginosis at the MTN LC. Two slides (one designated as primary and the other as secondary) will be prepared at each required time point and both will be entered into LDMS. The primary slide will be shipped to the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

Instructions for slide preparation and shipping are provided below:

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



2. Immediately following specimen collection from the lateral vaginal wall via 3 turns of a swab (Dacron or cotton), roll the swab across each of the slides. (Be sure to collect the specimen from opposite the vaginal wall used for the wet mount specimen collection.) Do not place the swab in saline, transport medium, or any transport container prior to slide preparation.
3. A SCHARP-provided PTID label is to be placed on the underside of the slides (on the frosted end, under the pencil markings); write the specimen collection date in indelible ink (e.g. pen) on each label.



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



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

## 9.7.2 Microbiology: Vaginal Swab for Quantitative Culture

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

- Collect the specimen for culture by rotating two Dacron swabs\* several times over the lateral wall of the vagina. Do not collect culture swabs in the exact same area where another sample was collected (i.e. If the PK swab was collected first, then collect in a different location in the vagina preferably closer to the introitus). Insert swabs into one Port-A-Cul transport tube (labeled with a SCHARP label), submerging the swabs into the gel and breaking off the shafts of the swabs, and capping. If Port-a-Cul tubes are not available, use the BD culture Max V transporter (either the Port-A-Cul transport tubes or BD Max V will be provided by MTN LC.)
- The specimen may be kept at controlled room temperature for up to 4 hours. It must be refrigerated after that and shipped with ice packs.
- Deliver the culture transport system and the LDMS specimen tracking sheet to the local LDMS laboratory.
- Using the LDMS Tracking Sheet, log the slides into LDMS (specimen type = VAG) and label the Port-A-Cul tube or Max V culturette with LDMS labels.
- Use LDMS to generate a shipping manifest for the cultures to be shipped.
- Ship the culture transport system and the vaginal smear for gram stain the same day of collection by overnight courier.
- Place the culture transport system in a biohazard bag and secure in the leak-proof container with absorbent material. Place the container, ice packs, slides, and a copy of the manifest in a cardboard box lined with Styrofoam.
- Use IATA packing instructions code 650, UN3373 (Biological Substance, Category B).
- The Research Institute is not open for delivery on the weekend, the specimens taken on Friday must be sent to the hospital address for delivery on Saturday. Make sure correct address is used.

\*Use the swabs in the Max V kit when using the BD CultureSwab Max V (+) collection and transport system.

### Shipping instructions to MTN LC:

If sending Monday through Thursday, send to the Institute:

**Lorna Rabe**  
**Magee-Womens Research Institute**  
**204 Craft Ave, Room A530**  
**Pittsburgh, Pa. 15213**  
**Phone# 412-641-6042**

If sending on Friday for Saturday delivery, send to the hospital:

**Lorna Rabe, C/O Safety and Security**  
**Magee-Womens Hospital**  
**300 Halket St.**  
**Pittsburgh, Pa. 15213**

Phone # 412 641-4191 (this is the Safety and Security #)

Note: Check off Saturday delivery on the Fed Ex label.

Notify the MTN LC via email ([lrabe@mwri.magee.edu](mailto:lrabe@mwri.magee.edu) and [kstoner@mwri.magee.edu](mailto:kstoner@mwri.magee.edu)) when the shipment has been picked up from the site by the courier/shipping company and the tracking number. Attach the LDMS shipping manifest to the email notification.

### 9.7.3 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 2 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 Pelvic Exam CRF. It is not necessary to record pH values onto laboratory log sheets or other source documents prior to recording values onto the CRF.

### 9.7.4 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 STI Test Results CRF. 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 the CRF.

The MTN LC requires all wet mount readers are assessed by the LC for competency of the wet mount tests; therefore the MTN LC will administer a web-based proficiency test 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](mailto: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 notify the MTN LC when new laboratory staff is hired, so that appropriate training can take place prior to such staff performing wet mounts for study purposes.

**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 that are not observed in a saline prep may be seen in the 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 100X magnification for epithelial cells, budding yeast, and pseudohyphae. Examine at 400X 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 100X and 400X magnification for yeast and pseudohyphae.

### 9.7.5 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 LC. 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.

### 9.7.6 NAAT Gonorrhea and Chlamydia Testing

Sites can choose to use the BD Probetec, Gen Probe Aptima or Cepheid GeneXpert . If the site does not have access to any of these tests they can send the samples to the LC for testing. Contact the LC prior to sending specimens for GC/CT testing.

Collect vaginal sample (1 manufacturer's recommended swab) and transport to the local laboratory according to the specific manufacturer's recommendations. Testing will be done at the local laboratories according to the site SOP.

### 9.7.7 Vaginal Swab for Biomarkers

Biomarkers will be evaluated to determine the impact the intravaginal rings and drugs may have on innate immune mediators, cytokines, or other safety concerns. Vaginal fluids are collected from the posterior fornix using a Dacron swab with a plastic shaft for biomarker analysis at the MTN LC.

Procedure for Biomarker Vaginal Swab:

- Collect vaginal fluid using a Dacron swab from the posterior fornix.
- Place the swab in a labeled 1.5 ml micro tube containing 400  $\mu$ L PBS (1X Concentration), break off using finger over cryovial opening making sure swab does not fly out, discard swab shaft, and cap the vial.
- Immediately refrigerate or place vial on ice and freeze at  $\leq -70^{\circ}\text{C}$  within 8 hours of collecting the sample collection.
- UAB: The Dezzutti lab prefers that each participant be tested within approximately one month after completing their Day 35, unless instructed otherwise. This can be accomplished by shipping all Vaginal Biomarkers in storage every 2 months. All shipments will be on dry ice and can be initiated Monday through Wednesday to insure that samples arrive in the lab during the work week.

### 9.7.8 Vaginal Swab for PK: Self-collected Vaginal Swab

Vaginal fluid for PK assessment will be a self-collected swab by the participant.

- Vaginal fluid PK sampling should preferably not occur earlier than 8 hours after ring re-insertion. If the ring has been reinserted within 8 hours and sampling has to be completed, still collect the samples, and make a comment on the LDMS tracking sheet and Ring Adherence CRF, item 4.

Timing of samples and sample target times:

At Enrollment and Day 28:

- The timer begins upon ring insertion (ENR) or ring removal (Day 28).
  - All serial collections are based on this starting point.
- When each time point is due, blood will be drawn first, and then the participant will self-collect the PK swab. Ideally, this should occur within 5 minutes of the blood draw.
  - Make sure that specimen times are accurate. If there are delays in sample collection, then the interval of time can be correctly gauged.
  - Time '0' at the ENR visit: There is not a PK blood draw; hence, there will not be a time constraint for the participant to collect the self-collected swab at this time-point. Use additional time to instruct participant on self-collection.
- Missed or delay in blood draw time-point:
  - If for some reason that the blood draw for the time-point is delayed or missed, it would have **no** bearing on the next time point. Example: the '1 hour' time-point draw was 15 minutes late [drawn at 75 minutes], the 2 hour PK blood would still be drawn at the 2 hour (120 minute) mark. If a collection is missed entirely, notify the MTN-027 management team.

At single time points:

- The blood is drawn, and then the participant should collect the self-collected swab within 1 hour. The protocol states approximately within 1 hour.

Procedure for Participant-collected Vaginal Swab for PK:

Materials for each time-point:

- Gloves
  - SCHARP labels for each cryovial and ziplock bag with PTID, visit number, visit date, and time-point.
  - 1 or 3 Polyester-Tipped (Dacron) Swab(s)
  - 1 or 3 2 mL Nalgene cryovial(s)
  - Analytical scale (accurate to 0.1 milligrams)
  - 1 or 3 Ziplock (re-sealable) specimen bag(s)
  - Urine cup or similar lightweight container, placed on middle of scale, to contain items to be weighed.
  - Private area for participant to self-collect PK
  - Counter or table in room so participant can keep collection of swabs organized (important when they are collecting triplicates for Pitt Site).
  - A rack that will hold the cryovial (optional, and may be used if staff needs to cut or break swab shafts for participants)
1. Each day of collection of vaginal swab for PK, perform QC that would be required for the analytical scale to accurately weigh samples to a weight of at least 0.1 milligrams. Do not turn off balance until the pre- and post-weights for that day are completed. Use the same analytical scale for pre- and post- weights of a sample.

2. Ensure that new, clean, or sterilized supplies (gloves, swabs, vials, ziplock bags, etc.) are used for each sample. Always handle items to be weighed with gloves.
3. Before starting procedures, pre-label the cryovial(s) and ziplock bag(s) with a SCHARP label. If there are multiple time points or multiple participants on the same day, pre-weights for all time points may be obtained prior to participant visits with careful observation of matching PTID time-point labels for cryovials and specimen ziplock bag. Prepare additional kits (swab + cryovial placed in a bag) incase needed due to shaft being discarded or swab being contaminated (such as being dropped on the ground).

Pitt Site:

- For the first 8 participants on Visit days 1 & 7, the participant will be collecting 3 swabs, one after the other. Properly label cryovials and ziplock bags (making sure that 1, 2 or 3 is marked on each cryovial and bag, and to be collected in that order).
- All other participants and the other self-collections for the first 8 participants will only have to obtain 1 self-collected swab per visit or time point.

UAB

- All UAB participants will only have to obtain 1 self-collected swab per visit or time point.

4. Perform Pre-Weight measurement:
  - a. Tare the urine cup or similar container.
  - b. Place the labeled 2 mL cryovial including cap and a packaged sterile Dacron swab in the urine cup.
  - c. Record the total pre-weight on the LDMS Tracking Sheet.\*
  - d. Place the cryovial and the packaged Dacron swab into a specimen ziplock bag with the matching time-point label.
  - e. If more than one collection time is required (for visit ENR & day 28), delineate the collection hour that the swab is to be used on the bag (example: designate bag 'Collection Hour '0').
5. Self-collection of vaginal fluid: Performed at each designated time-point.

An Illustration can be found in the Study Implementation Materials section on the MTN-027 webpage.

  - a. Instruct the patient on how to collect the sample and emphasize that all items get returned back to the ziplock bag. Nothing is to be placed into the garbage.
  - b. Once in the patient room, the participant will insert the swab into the vagina approximately 2 inches (the length of the participants little finger). For 10-20 seconds, rotate (actually spinning swab) in a circular motion touching all walls to absorb as much fluid as possible. We recommend that the participant counts (thousand 1, thousand 2, up to 10) out loud while turning swab.
  - c. Immediately place swab into the cryovial.
  - d. Site has an option at this step:
    - ⊖ Have participant snap the shaft & recap the sample: Participant will lift tip of swab a few millimeters from bottom of cryovial, and then break off the plastic shaft (making sure their finger is holding down the shaft so it does not 'fly' out of cryovial).
    - ⊖ Have site staff break or cut the swab into the cryovial: The participant will place the cryovial with unbroken swab shaft into a rack that is placed on the



table, or can directly hand to the clinic staff for them to remove the swab shaft and cap the cryovial. This process is to be executed quickly to prevent sample evaporation.

- e. Make sure the cap is screwed down firmly!
- f. Place capped cryovial, detached swab shaft piece, and wrapper back into zip-lock bag to be post-weighed.
- g. Do not throw away the shaft that has been broke off or the empty wrapper (place everything in the zip-lock bag and close).

Note: if swab shaft is discarded or breaks in multiple pieces that do not get placed in the bag, then repeat the collection starting with a new collection kit (replacement kit that already has been pre-weighed) and then recollecting sample.

**6. Perform Post-Weight:**

- a. Tare the urine cup or similar lightweight container.
- b. Place the cryovial containing the absorbed swab tip, the swab packaging and the remainder of the swab shaft into the urine cup or similar.
- c. Record post-weight on the LDMS Tracking sheet.\*  
NOTE: Ensure it is larger than pre-weight!
- d. Dispose of the cutoff shafts, wrapper, and ziplock bag.

**7. Within 2 hours, place the cryovials in the freezer at  $\leq -70^{\circ}\text{C}$ .**

**8. LDMS processing Lab:**

- Enter pre-weights and post-weights in to EXCEL sheet to calculate vaginal swab fluid for PK weight =Post-weight minus Pre-weight.
- Write or confirm (if site staff already wrote the net weight) that the correct vaginal PK net weight is on the LDMS tracking sheet.
- Enter results into the LDMS system (see LDMS section 9.4).

**9. Shipping of PK swab samples**

- LC will coordinate sample shipments throughout course of study if necessary and at its conclusion.
- The back-up samples will be retained at the site until advised by the LC or MTN-027 leadership team.
- All shipments will be on dry ice that will be sufficient for a 24 hour period and can be initiated Monday through Wednesday to insure that samples arrive in the lab during the work week.

\* In addition to the tracking sheet, a site log sheet is recommended but not mandatory for recording results. This would be useful if the tracking sheet was lost, became illegible, or values needed double checked.

### **9.7.9 Testing of Intravaginal Ring (IVR), Remnant content analysis**

Used rings will be analyzed for residual levels of Vicriviroc (MK-4176) and MK-2048, and will be collected at visit day 28. The used rings may contain vaginal secretions and therefore treated as a biohazard. The rings will remain in the amber pouch (to protect ring from sun) and stored at room temperature until further notice from the MTN LC. Rings that are defective or inserted briefly and removed for various reasons may be destroyed at the site via biohazard procedures.

Step 1\*: Wear lab coat, gloves, and protective face guards when performing this step. The clinician will remove the used ring and place in a clean container\* with tap water. Move the ring around in the water or swirl the container to remove vaginal material. Take the ring out of the water and blot dry with paper towels or gauze. The ring should be dry before storing in pouch. Dispose of blotting materials and contaminated water according to your institution biohazard policy.

Important notes:

\*Hour 0 Blood and Vaginal swab for PK should be collected immediately *before* ring removal.

\*Use a disposable container or a reusable container that was cleaned using 10% bleach solution for 20 minutes or sterilized.

If the ring is removed by the participant prior to the clinic visit and will not be reinserted, then the used ring is still prepared for residual drug analysis. After the used ring is taken out of the participant's bag (container they returned the ring in), follow directions starting with step 1.

Step 2: Site staff will place the ring into a new 3"X5" amber Zippit pouch (see figure 9-3) that was provided by LC to store the rings. Label the pouch with the participant ID number and visit number. Add a biohazard sticker if one is not already attached to the pouch, making sure not to cover the identifier information.

Step 3: Store the used ring within the biohazard labeled amber pouch between 2-8 °C.

Step 4: The use of LDMS is required to log in all used rings.

Step 5: At the end of the study, LC will contact site to coordinate shipment.

**Figure 9-3: 3"X5" amber Zippit pouch**



## **9.8 Cervical Specimens for PK, PD, Cytobrush and Pap Test.**

### **9.8.1 Cervical Biopsy Collection**

Cervical biopsies will be performed at both UAB and Pitt sites. The UAB site will collect one cervical biopsy only, for PK testing. The Pittsburgh site will collect two cervical biopsies, one for PK testing and one for PD testing. Biopsies 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 not be used. Bleeding may be controlled through a combination of applied pressure, silver nitrate and/ monsel's solution.

### **9.8.2 Cervical Biopsy for PD (ex vivo challenge, Pitt site only)**

One fresh biopsy will be collected at visit day 28 using forceps to insert into a 2 mL Corning (orange cap) cryovial with 1 mL of cold biopsy transport medium (kept at 4°C). Release tissue directly into transport media by gently shaking tube until biopsy is dislodged from forceps. Immediately transport to MTN Dezzutti lab. If Pitt CRS staff cannot take to Dezzutti laboratory, call extension 1-6157 for biopsy pick-up. If there is no answer, call pager#: 412-917-9343.

### **9.8.3 Cervical Biopsy for PK (Pitt & UAB):**

One biopsy will be collected for a tissue PK level at Visit day 28.

1. Label one 2 mL cryovial (Nunc or Nalgene) with the appropriate sample/study identification information.
2. Weigh the labeled cryovial using an analytical scale with a sensitivity rating of 0.1 milligrams or better. Document this pre-weight of the labeled cryovial on the LDMS tracking sheet.
3. Directly transfer the biopsy to its designated pre-weighed cryovial.
4. Obtain the post-weight for the cryovial containing a biopsy using an analytical scale and document on the LDMS tracking sheet.
5. Immediately freeze the cryovial containing the PK biopsy in dry ice ethanol bath (dry ice with enough ethanol to make a slushy consistency) or liquid nitrogen.
6. Store the labeled cryovials containing the frozen biopsies at  $\leq -70^{\circ}\text{C}$ .
7. All pre and post weights are also to be logged by the processing lab onto an excel weight worksheet supplied by LC. The net-weights will be calculated by the formula in the worksheet and entered into the LDMS system.
8. LC will coordinate shipments throughout if necessary and at the end of the study. All shipments will be on dry ice and can be initiated Monday through Wednesday to insure that samples arrive in the lab during the work week.

## 9.8.4 Cytobrush for Flow Cytometry

### 9.8.4.1 Supplies:

- Medscand Cytobrush Plus:
  - The Cytobrush Plus can be ordered from Cooper Surgical at 800-243-2974, order # C0104. They come in a box of 100 (10 bags of 10 brushes/bag). If sites have trouble obtaining this item, contact the MTN LC.
- 50mL conical tube
- Trypan blue (0.4%): Cellgro Media tech #MT 25-900-CI (6 x 100mL)
- D-PBS (without Ca<sup>++</sup>/Mg<sup>++</sup>): 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 2-8°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
- 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 20 mL 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×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. Scrape 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 50 mL conical vial containing the cells with D-PBS.
12. Centrifuge tube at 600xg 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 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 90 µL 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 LC will provide an excel sheet to record these results.
- iii. In LDMS, record the cell count under aliquot volume, and change the unit to ‘cells’.

***The Flow Cytometry procedure will begin here with antibody staining; see McGowan Research Laboratory SOP (MRL-202.007).***

#### 9.8.4.2 Transport of samples: UAB

1. Once samples have been stained and fixed according to McGowan Research Laboratory SOP, **snap the cap** of each of the 12 labeled Falcon tubes tightly (Primary container).
2. Wrap a strip of Parafilm around the top of each cap to prevent any leakage.
3. Place the Falcon tubes into a (secondary container) screw top shipping receptacle\* with absorbents. This container must be leak proof.
4. Place container with specimens into a Styrofoam shipper\* inside an outer box that is UN Certified.
5. The outer packaging must be of good quality and strong enough to withstand handling during transport. Be sure to place enough cool packs to keep samples between 2-8°C during transport.
 

Note: Culture and gram stain samples can be shipped in the same box as the cytobrush, next to the screw top container.
6. Place shipping manifest in box with samples. Email LDMS electronic batch file and manifest to LC.
7. Go onto the Federal Express website: <http://www.fedex.com/us/>. Enter the shipping information and package details. Under "service type," be sure to choose: **FIRST OVERNIGHT.**

8. Notify Wayne Hall of the Hillier Lab, [hallwb@mwri.magee.edu](mailto:hallwb@mwri.magee.edu) and McGowan laboratory [mtnflowcytometry@mtnstopshiv.org](mailto:mtnflowcytometry@mtnstopshiv.org) by email when samples are to be sent. Include the Fed Ex tracking number with all shipment notices.
9. Ship samples to the following address:  
**Magee-Womens Research Institute  
c/o McGowan Laboratory  
204 Craft Avenue, B530  
Pittsburgh, Pa, 15213**

### 9.8.5 Papanicolaou (Pap) Test (\*only if indicated)

Pap smears are performed only if clinically indicated or for women  $\geq 21$  years old that do not have documentation of a satisfactory Pap within the past 3 years prior to Enrollment. If a Pap smear 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 Rectal Fluid

### 9.10.1 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)

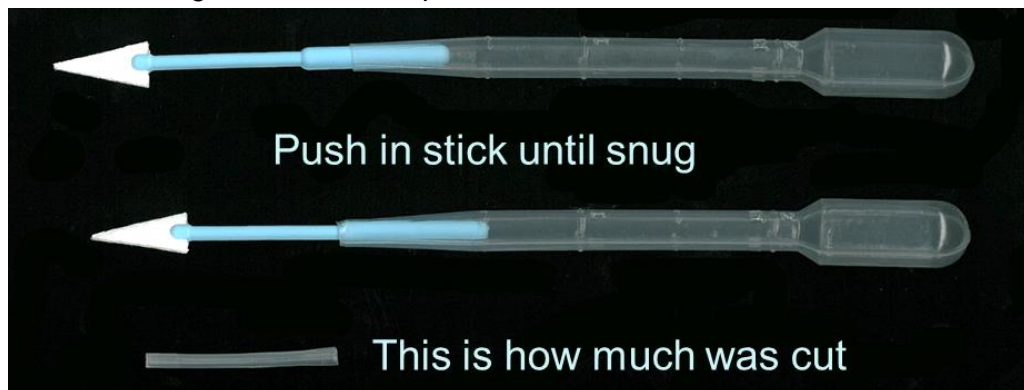
PRE personal lubricant: Approved lubricant Good Clean Love. “almost naked” formulation. Available from Drugstore.com. <http://www.drugstore.com/good-clean-love-all-natural-personal-lubricant-almost-naked/qxp156292?catid=181914&fromsrch=good%20clean%20love>  
Analytical Scale with sensitivity: 0.0001 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 Figure 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.

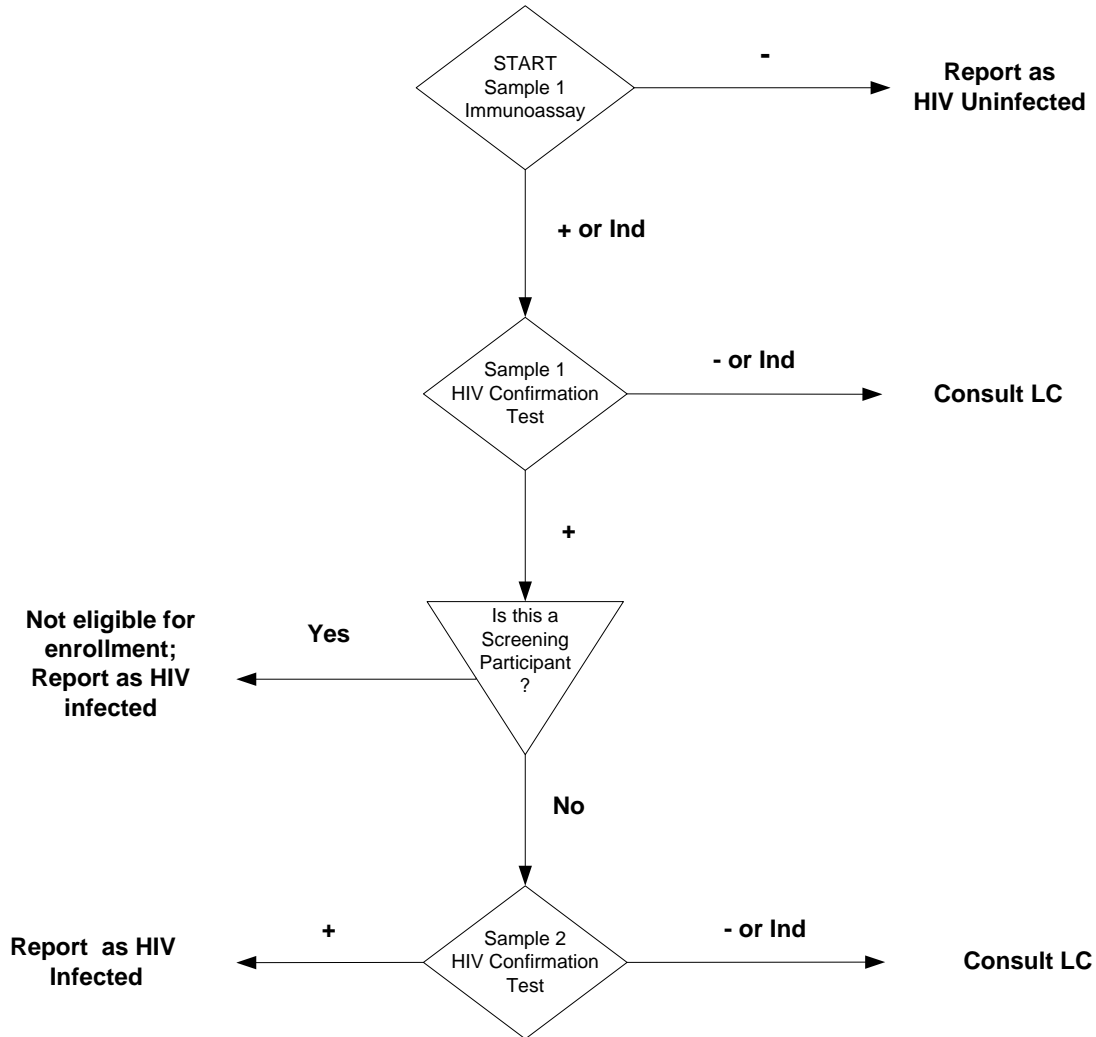
Figure 9-4: Rectal specimen collection device



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 LDMS Specimen Tracking Sheet (Rectal Sponge Collection Time).
5. Hold (or leave) sponge in place for 2 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. 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.
11. At the end of the study, the LC will contact site to coordinate shipment.

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



Ind: Indeterminate test results  
LC: Laboratory Center