

## Section 10. Laboratory Considerations

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## 10. Introduction

This section provides information and instructions for site clinical and laboratory staff related to the processing, storing, shipping and testing of MTN-038 laboratory specimens. Additional information for collection specimens from participant can be found in SSP Section 8 Clinical Considerations.

### 10.1. 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/>.

The tests to be performed at each visit during the MTN-038 study are listed in Table 10-1. Sites are responsible to ensure that specimen volumes do not exceed what is described in the informed consent process. The total blood volumes calculated in Table 10-1 include additional blood that may be collected for any clinically indicated testing. The MTN LC may request details of collection containers and volumes for this purpose, as shown in Table 10-2.

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

**Table 10-1: Overview of Laboratory Tests by visit for MTN-038**

		Visit 1 SCR	Visit 2 ENR (Day 0)	Visit 3 (Day 1)	Visit 4 (Day 7)	Visit 5 (Day 14)	Visit 6 (Day 28)	Visit 7 (Day 42)	Visit 8 (Day 56)	Visit 9 PUEV/Early Termination (Day 91)	Visit 10 Final Contact (Day 92)
URINE	Pregnancy test	X	X	*	*	*	X	*	X	X	*
	Urine dipstick/culture	*	*	*	*	*	*	*	*	*	*
BLOOD	HIV-1/2 testing	X	X	*	*	*	*	*	*	X	
	Plasma for archive		X								
	AST/ALT	X					*	*	*	X	
	CBC with differential and platelets	X	*	*	*	*	*	*	*	X	*
	Serum creatinine	X	*	*	*	*	*	*	*	X	*
	Hep B surface antigen	X									
	Syphilis serology	X		*	*	*	*	*	*	*	
	HSV 1/2 serology		X								
	TFV levels			X	X	X	X		X	X pre-removal; 4-HR post-removal	X
PELVIC	NAAT for GC/CT and trichomonas	X	*	*	*	*	*	*	*	*	
	Saline/KOH wet mount with pH for candidiasis and/or BV	*	*	*	*	*	*	*	*	*	*
	Pap test	X <sup>^</sup>									
	Vaginal swabs for microbiota		X pre-insertion				X		X	X	
	Vaginal Gram stain		X pre-insertion				X		X	X	
	CVF anti-HSV-2 activity		X pre-insertion				X		X		
	CVF TFV levels		X 1-, 4-HR post-insertion	X	X	X	X		X	X pre-removal; 4-HR post-removal	X
	CVF for biomarkers		X pre-insertion	X	X	X	X	X	X	X	
	CVL for PK						X		X		
	CVL for PD and biomarkers		X pre-insertion				X		X		
	Cervical biopsies for PK					X <sup>‡</sup>	X <sup>£</sup>				
Cervical biopsies for PK and PD								X <sup>‡</sup>	X <sup>£</sup>		
RECT	Rectal fluid TFV levels			X		X	X		X	X pre-removal; 4-HR post-removal	
<b>STUDY PRODUCT SUPPLY</b>											
	Removal and collection of study VR									X	
	X required; * If indicated and/or per local standard of care; ^ If participant [over age 21] is unable to provide documentation of a satisfactory Pap test within 3 years prior to enrollment ‡ randomized to biopsy collection either at Visits 5 and 8 <sup>£</sup> randomized to biopsy collection either at Visits 6 and 9										

Table 10-2 also shows where laboratory procedures may be performed: study site clinics or laboratories, approved commercial laboratories, and laboratories within the MTN Laboratory Center (MTN LC), including the MTN Pharmacology Core at Johns Hopkins University Clinical Pharmacology Analytical Laboratory (JHU CPAL). Regardless of whether tests are performed in clinic or laboratory settings, study staff that performs the tests must be trained in properly associated QC procedures prior to performing the tests for study purposes (i.e. training documentation should be available for inspection at any time).

**Table 10-2: Overview of Laboratory Testing Locations, Specimens, and Methods for MTN-038**

Test	Testing Location	Specimen Type	Tube or Container and tube size (recommended)	Kit or Method
Urine Pregnancy Test (hCG)	Local Lab/ In Clinic	Urine	Plastic screw top cup	Quidel Quickvue or SureVue Beckman Coulter ICON 25
Urine Dipstick and Culture*	Local lab/ In Clinic	Urine	Plastic screw top cup	Siemens Multistix® 10 SG or Uristix 4 or other MTN LC approved methodology
Complete Blood Count with Differential and Platelets	Local Lab	Consult Local Lab Requirements		Local methodology
Chemistries (AST, ALT, Creatinine)	Local Lab	Consult Local Lab Requirements		Local methodology
Syphilis Serology	Local Lab	Consult Local Lab Requirements		Local methodology
Hepatitis B (HBsAg)	Local Lab	Consult local lab requirements		Local Methodology
HIV-1/2 Testing	Local Lab/ In Clinic	Plasma, serum, or whole blood	EDTA or plain tube, 4-mL	FDA approved tests
HSV-1/2 IgG Serology	MTN LC	Serum	Plain or serum separator tube 4mL	MTN LC procedure
Plasma for Archive or Confirmation of Viral Load and HIV Resistance Testing	MTN LC	Plasma	EDTA at least 8-mL tube	MTN LC procedure MTN LC Virology
Plasma for Blood PK (TFV)	CPAL	Plasma	EDTA tube 10-mL	CPAL collection procedure
Vaginal NAAT for GC/CT	Local lab	Vaginal swab (supplied with kit)	Kit specific Transport tube	BD Probetec, Gen-Probe Aptima, or Cepheid GeneXpert
Vaginal NAAT for Trichomonas	Local lab	Vaginal swab (supplied with kit)	Kit specific Transport tube	Gen-Probe Aptima or Cepheid GeneXpert
Vaginal pH*	In clinic	Vaginal swab	N/A	S/P pH Indicator Strips
Vaginal Saline Wet Preparation (for BV and/or KOH wet mount)*	In clinic	Vaginal swab	tube with 6 drops of saline	MTN LC procedure
Vaginal swab for Microbiota q-PCR	MTN LC	Vaginal flocked swabs	2.0 mL Cryovials (Sarstedt, clear top)	MTN LC procedure
Vaginal swab for Microbiota Culture	MTN LC	Vaginal swab (supplied with kit)	Starplex Starswab Anaerobic (glass with gel) Transporter	MTN LC procedure

**Table 10-2: Overview of Laboratory Testing Locations, Specimens, and Methods for MTN-038**

<b>Test</b>	<b>Testing Location</b>	<b>Specimen Type</b>	<b>Tube or Container and tube size (recommended)</b>	<b>Kit or Method</b>
Vaginal Smear for Gram-stain	MTN LC	Vaginal swab	2 Slides with frosted end	MTN LC procedure
Cervicovaginal Fluid (CVF) Swab for PK	CPAL	Vaginal swab	2.0-mL Cryovial (Nalgene)	CPAL collection procedure
CVF swab for Biomarkers	MTN LC	Vaginal swab	2.0-mL Cryovial (Corning, orange top)	MTN LC procedure
CVF Swab for Anti-HSV-2 Activity	CONRAD-designated lab	Vaginal swab	Starplex Starswab Dry Swab (plastic, no gel) Transporter; 2.0-mL Cryovial (Sarstedt, clear top)	CONRAD- designated lab procedure
CVL for PK, PD & Biomarkers	CPAL & MTN LC	CVL using 10mL saline	15-mL tube	MTN LC procedure
Cervical Biopsy for PK	CPAL	Tissue	2.0 mL cryovial (Nalgene)	MTN LC procedure
Cervical Biopsy for PD	Local Lab	Tissue	Tube(s) containing Biopsy Transport Medium (check w/ Local PD Lab for transport preference)	MTN LC procedure
Pap Test**	Local Lab	Consult Local Lab Requirements		Local methodology
Rectal swab for PK	CPAL	Rectal swab	2.0-mL Cryovial (Nalgene)	CPAL collection procedure
Used Intravaginal Ring for PK residual assessment	CONRAD-designated Lab	Used IVR	Resealable foil pouch	CONRAD-designated lab procedure

\*Perform only if clinically indicated per local SOP.

\*\*Perform if participant is over age of 21 and does not have a documented satisfactory Pap within 3 years prior to Enrollment.

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.

Specimens that will be stored and shipped to the MTN LC or CPAL are highlighted in Table 10-3. These are the samples that will be entered into LDMS (section 10.4).

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 10-3: Overview of Specimens for Storage and Shipment**

<b>Specimen</b>	<b>Processing</b>	<b>Ship to</b>	<b>Shipping schedule</b>
<b>Plasma for Archive (at enrollment) or for Confirmation of Viral Load and HIV Resistance (at f/u)</b>	Prepare at least 2 – 3 aliquots of 1.5-mL plasma. Collect plasma within 4 hours if blood held at room temp or within 24 hours if held at 4°C. Store ≤ -70°C.	MTN LC	Store frozen at site until notified by MTN LC. However, if collected at a follow-up visit immediately contact MTN LC Virology Core.
<b>Plasma for PK (TFV)</b>	Within 8 hrs of blood collection, prepare two or more cryovials, ≥1.5-mL plasma in each. Store ≤ -70°C.	CPAL, MTN LC	Store frozen at site until conclusion of study or notified by MTN LC.
<b>Serum for HSV-1/2 Testing</b>	Within 8 hrs of blood collection, aliquot ≤1-mL serum into a cryovial with remainder in backup cryovial. Store ≤ -70°C	MTN LC	Store frozen at site until conclusion of study or notified by MTN LC.
<b>CVF Swab for PK (TFV)</b>	Record Pre- and Post-collection weights of swab (and the kit). Store swab in cryovial, ≤ -70°C within 2 hours of collection	CPAL	Store frozen at site until conclusion of study or notified by MTN LC.
<b>CVF Swab for anti-HSV-2 activity</b>	Collect sample using Starplex dry swab transporter (plastic, no gel). Cut or break (snap) swabs and place each into a 2-mL cryovial, store at ≤ -70°C.	MTN LC, Conrad designated lab	Store frozen at site until conclusion of study or notified by MTN LC.
<b>CVF Swab for Biomarkers</b>	Collect swab and place in 2-mL cryovial. Store at ≤ -70°C within 2 hours of collection.	MTN LC	Store frozen at site until conclusion of study or notified by MTN LC.
<b>CVL Supernatant for PK, PD, Biomarker</b>	Centrifuge and without disturbing pellet, aliquot into 6-9 cryovials with ≥1.0-mL each. Store ≤ -70°C within 2 hrs of collection.	MTN LC, CPAL	Store frozen at site until conclusion of study or notified by MTN LC.
<b>CVL Pellet</b>	After supernatant collected, centrifuge CVL primary tube once more, remove supernatant, add 0.5-mL normal saline to the pellet and transfer pellet into a single cryovial. Store ≤ -70°C within 2 hrs of collection.	MTN LC	Store frozen at site until conclusion of study or notified by MTN LC.
<b>Cervical Biopsies for PK</b>	Collect 2 biopsies, each placed in a cryovial. Record pre (without biopsy) and post (with biopsy) weights. Flash-freeze. Store at ≤-70°C.	CPAL	Store frozen at site until conclusion of study.
<b>Cervical Biopsies for PD</b>	Collect 2 biopsies, both placed in a tube of 4°C biopsy transport media, store on ice.	Local Lab	Immediately deliver to local lab for processing
<b>Vaginal Swab for Microbiota Culture</b>	2 swabs in Starplex transporter (glass with gel); store 4°C until shipped or transported to MTN LC.	MTN LC	Deliver on ice or ship on ice packs <u>the day of collection</u>
<b>Vaginal Swab for Microbiota q-PCR</b>	2 flocced swabs stored in separate cryovials. Store at ≤ -70°C within 2 hrs of collection.	MTN LC	Store frozen at site until conclusion of study.
<b>Vaginal Smear for Gram-stain</b>	Make 2 slides. Room temp. Label with LDMS label.	MTN LC	Place one slide in a case to be shipped with Starplex Starswab culture tube. Store 2nd slide (as backup) at site until all slides from first set are evaluated.
<b>Rectal Fluid Swab for PK (TFV)</b>	Record Pre-and Post-collecton weight of swab (and kit). Store ≤ -70°C within 2 hours of collection	CPAL	Store frozen at site until conclusion of study or notified by MTN LC.
<b>Used Intravaginal Ring for Residual TFV Assessment</b>	Clean with alcohol pads. Allow to air dry completely. Place VR in resealable foil pack. Store at -80°C.	MTN LC	-80°C storage at site until conclusion of study.

## 10.2. 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. Although PTIDs are pre-printed on these labels, study staff must write the specimen collection date on each label. The visit code also may be written on the label. Use an indelible ink pen (e.g., Sharpie) if information is handwritten such as the date or collection time point.

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 10-4 for tests that will be entered into LDMS and labeled with LDMS-generated labels. (*NOTE: Do not remove SCHARP label prior to placing the LDMS label on the tube.*)

## 10.3. 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.

If additional specimens need to be collected for the same test due to either laboratory error (lost, broken tube, clerical, etc.) or clinical error, a protocol deviation form may be required. The MTN 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 compromising specimen integrity
- Any situation that may indicate a protocol deviation

If site staff has any questions regarding time windows or collection processes, call MTN LC staff as soon as possible for guidance.

## 10.4. Use of LDMS

The Laboratory Data and Management System (LDMS) is a program that must be used by all sites for the storage and shipping of sample types listed in Table 10-3. LDMS is supported by the Frontier Science Foundation (FSTRF). 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 locally (frequency determined by site) and to export their data to FSTRF (at least weekly).

**LDMS Help:** Questions related to use of LDMS in MTN-038 may be directed to MTN LC or LDMS Technical (User) Support. LDMS User Support is available 24 hours a day, 7 days a week. Contact LDMS User Support at:

Email: [ldmshelp@fstrf.org](mailto:ldmshelp@fstrf.org)  
Phone: +716-834-0900, ext 7311  
Fax: +716-834-8432

**Discrepancy Reports:** Each site must export its LDMS data to Frontier Science (FSTRF) on a weekly basis. Exported data are used by the MTN Statistical and Data Management Center (SDMC) to generate a monthly specimen repository report and to reconcile data entered in LDMS with data entered on study case report forms (CRFs). Any discrepancies identified during the reconciliation are included

in a monthly discrepancy report for each site. 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 MTN 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 MTN LC and SDMC will discuss and document any items that, although resolved, appear 'irresolvable' in LDMS.

#### **10.4.1. LDMS Codes for Specimen Log In**

Table 10-4 should be used as a guide when logging in MTN-038 specimens for storage or shipping. Please use the LDMS codes listed in the table when logging in specimens for each test listed. LDMS tracking sheets for the various visits or sample types collected at a visit can be found in the Study Implementation Materials section on the MTN-038 webpage.



**Table 10-4 LDMS Specimen Management Guide to Logging in MTN-038**

Specimen	Primary Specimen	Primary Additive	Primary Volume <sup>£</sup>	No. of Aliquots	Aliquot Volume	Units	Aliquot Derivative	Sub Add/ Derivative	Other Spec ID	LDMS Monitoring Report
Plasma Archive	BLD	EDT	10.0 ML	2-4	1.5	ML	PL1/2	N/A	CON <sup>δ</sup>	--
Plasma for PK	BLD	EDT	10.0 ML	2-5	1	ML	PL1	N/A	PK	Collection Time; @V9: 0- and 4-HR Time points
Serum for HSV-1/2 Testing	BLD	NON	4.0 ML	1-2	1	ML	SER	N/A		--
Rectal Fluid Swab for PK	REC	NON	1 EA	1	Net Weight	MG	SWB	N/A	PK	Collection Time; @V9: 0- and 4-HR Time points
CVF for PK	CVF	NON	1 EA	1	Net Weight	MG	SWB	N/A	PK	Collection Time; @V2:1- and 4-HR Time points; @V9: 0- and 4-HR Time points
CVF for anti-HSV-2 activity	CVF	CTK	1 EA	2	1	EA	SWB	N/A	HSV-VG	--
CVF for Biomarkers	CVF	NON	1 EA	1	1	EA	SWB	N/A		--
Vaginal Smear for Gram Stain	VAG	NON	1 EA	2	1	EA	SLD	GRS		--
Vaginal Swab for qPCR	VAG	NON	1 EA	2	1	EA	FLS	N/A		--
Vaginal Swab for Quantitative Culture	VAG	CTK	1 EA	1	1	EA	SWB	N/A		--
CVL for PD, Biomarkers and PK	CVL	NSL	10.0 ML	6+	1	ML	FLD	N/A		Collection Time
				1	0.5	ML	CEN	NSL		
Cervical Biopsies for PK	CVB	NON	1 EA	2	Net Weight	MG	BPS	N/A	PK	Collection Time of first biopsy to match CRF
Cervical Biopsies for PD (entered by PD Lab)	CVB	BTM	2 EA	1, each primary	Net Weight	MG	BPS	N/A		Collection Time of first biopsy to match CRF
Used Vaginal Ring for TFV Residual Analysis	IVR	NON	1 EA	1	1	EA	IVR	N/A		--

<sup>£</sup>Based on recommended container, <sup>δ</sup>Other specimen ID (CON) used for follow-up visits only

### 10.4.2. Logging in Time for PK Samples

In this study, there will be visits with multiple PK time-points. For the sample types with multiple PK time-points, in addition to time of collection, the TIME and TIME UNIT fields are used to note the specific time point on your aliquot labels. In figure 10-1, the single tube of blood for PK is entered in the primary area (see yellow rectangle A), and the three aliquots of 1.8-mL plasma that are derived from the sample are entered in the lower section for the derivative (see blue rectangle B).

- The collection time, using the 24-hour clock notation, is entered in the Specimen Time area (Figure 10-1, red rectangle C). For this example, it is 16:00.
- During multiple PK time-point visits, the PK time-point information is entered in Time and Time Unit area (Figure 10-1, green rectangle D). This blood was for the 4-HR time point.
- Note that for this study, the collection time for each set of cervical biopsies for PK or PD will be based on the first biopsy collected (see LDMS entry instructions in Section 10.4.4 and Table 10-4). This is the single collection time that will be documented in LDMS for each set of two biopsies. This will be useful for LDMS-CRF reconciliation of LDMS monitoring reports.

Figure 10-1: LDMS Screen for Time Entry.

The screenshot displays the LDMS interface for entering specimen and derivative information. The top section shows a table with columns for Group, TYPE1, ID1, TYPE2, ID2, TYPE3, ID3, Visit, and Unit. Below this is a form for specimen entry with fields for Spec. Date, Exp. Date, and checkboxes for Remote and Imported. A yellow box labeled 'A' highlights the primary specimen entry table. A red box labeled 'C' highlights the 'Spec. Time' field, which contains '16:00'. A green box labeled 'D' highlights the 'Time' and 'Time Unit' fields, which contain '4.00' and 'Hours' respectively. A blue box labeled 'B' highlights the derivative entry table, which shows three aliquots of 1.8 mL plasma derived from the primary specimen.

Group	TYPE1	ID1	TYPE2	ID2	TYPE3	ID3	Visit	Unit
1 MTN	PID	302600006	PROTOCOL	013.0			2.00	Vol
2								
3								
4								
5								
6								

Specimen #	Global Spec ID	Primary	Additive	Volume	Units	Spec Time	Time	Time Unit
1 414V11002715	HCS012KK-00	BLD	EDT	8.00	ML	16:00	4.00	Hours

Specimen	Global Spec ID	Primary	Add	Der	Sub Add/Der	Volume	Units	Cond	Other Spec ID
1 414V11002721	HCS012KK-01	BLD	EDT	PL1	N/A	1.80	ML	SAT	
2 414V11002721	HCS012KK-02	BLD	EDT	PL1	N/A	1.80	ML	SAT	
3 414V11002721	HCS012KK-03	BLD	EDT	PL1	N/A	1.80	ML	SAT	

### 10.4.3. Entering weight measurements of rectal fluid and CVF swabs and cervical biopsies for PK in LDMS

Weight measurements of swabs and biopsies for PK are entered in the derivative or aliquot section of a primary sample (see Fig 10-2, blue rectangle, B). The VOLUME and UNIT field is used for displaying weight measurements with proper units. Once the net-weight of a sample is calculated by subtracting the

**Figure 10-2: LDMS Screen for Weight Entry**

The screenshot displays the LDMS interface for entering weight measurements. It is divided into two main sections, A and B, which are highlighted with yellow and blue boxes respectively.

**Section A (Primary Sample):** This section is highlighted in yellow. It contains a table with the following data:

Specimen #	Global Spec ID	Primary	Additive	Volume	Units	Spec Time	Time	Time Unit	Cond
1	414/V14006368	FC60756V-00	VGL	NON	1.00	EA	15:09		SAT

**Section B (Aliquot):** This section is highlighted in blue. It contains a table with the following data:

Specimen	Global Spec ID	Primary	Add	Der	Sub Add/Der	Volume	Units	Cond	Other Spec Id
1	414/V14006369	FC60756V-01	VGL	NON	SwB	N/A	66.5	MG	SAT

Section B also includes input fields for: # of Aliquots: 1, Vol: 66.5, Units: MG, Derivative: SwB, Sub Add/Der: N/A, and Other Spec ID.

pre-weight from the post-weight of the collection kit, the result can be entered into LDMS as shown in figure 10-2, red rectangle.

- In the primary sample area (section A), use table 10-4 to enter correct code for the sample. Make sure to place the correct collection time under Spec Time field. Click the 'add' button to the right. This will add the sample to field. Under Units, enter EA (for each) and enter '1' for Volume (See Figure 10-2).
- To enter the actual weights, make an aliquot in Section B for the primary sample by entering a '1' in the # of Aliquots field. For Volume, enter the net-weight and select 'MG' MG (milligrams) for UNITS. Enter the correct derivative and Sub-Add/Der codes, then click the add button (See Figure 10-2).
  - In the example in figure 10-2: Pre-weight Swab: 3073.2 mg, Post weight Swab: 3139.7 mg, Net weight of Swab is 66.5 mg (3139.7 -3073.2 = 66.5). Enter '66.5' under VOLUME and select 'MG' for Units, 'SwB' for Derivative, and 'N/A' for Sub-Add/Der, then press add.
  - For cervical biopsy, the # of aliquots entered will be "2"; therefore, two lines will show in section B. Ensure that the two different weights are entered.
- If net weight results in a negative value, investigate that the pre- and post-collection weights were not switched. Enter negative weights in the comment and storage comment fields for the aliquot(s). Complete a protocol deviation form and include corrective action.

#### 10.4.4. LDMS Entry for Vaginal Smear for Gram Stain, Vaginal Swabs for qPCR, and CVF Swab for Anti-HSV2 Activity, and Cervical Biopsies for PK

There are sets of non-liquid samples that will be entered as “1” for # of tubes (Fig 10-3, green rectangle) and “2” for # of aliquots. For Vaginal Smear for Gram Stain, the one swab that was used to inoculate the two slides is the primary sample. After the primary sample information is entered, then added, the two slides are entered as aliquots. In the example shown in figure 10-3, note that after the 2 aliquots were added, the entry of “1 EA” for the primary volume in the yellow section prompted a pop-up message warning the user that the total aliquot volume exceeds the primary volume. The message was ignored, LDMS entry continued, and the result was a main global spec ID number ending with -01 and -02 to distinguish the two aliquots (Fig. 10-3, blue rectangle, lines 1 and 2).

LDMS entry should be similar for the two flocked swabs used to collect microbiota qPCR and the (two) CVF Swab samples for anti-HSV2 activity, with each set of swabs collected simultaneously (not one at a time). The cervical biopsies for PK will be entered in a similar fashion, except each aliquot will have volume pertaining to weight. Note that to obtain global spec ID with -01 and -02 aliquots, similar results may be obtained using LDMS entry of “2 EA” for the primary volume, as long as “1” is entered as # of tubes for the primary (Figure 10-3, green rectangle).

Figure 10-3 LDMS Entry for Vaginal Smear for Gram Stain,

	Group	TYPE1	ID1	TYPE2	ID2	TYPE3	ID3	Visit	Unit
1	MTN	PID	335000159	PROTOCOL	024.0	ID3		6.00	Vst
2									
3									
4									
5									
6									

Spec. Date:	26/Jun/2014	Recd	Exp. Date:	0	<input type="checkbox"/> Remote	VQA
Rec. Date:	26/Jun/2014	Time: 15:30	Export ID:		<input type="checkbox"/> Imported	Culture Derivative
Import date:						

# of Tubes:	1	Primary Type:	VGL	Other Spec ID:		Spec. Time:	
-------------	---	---------------	-----	----------------	--	-------------	--

Specimen #	Global Spec ID	Primary	Additive	Volume	Units	Spec Time	Time	Time Unit	Conc
1	414V14006370	DC60756X-00	VAG	NON	1.00 EA				SAT

# of Aliquots:	2	Vol:	1	Units:	EA	Derivative:	SLD	Sub Add/Der:	GRS	Other Spec ID:	
----------------	---	------	---	--------	----	-------------	-----	--------------	-----	----------------	--

Specimen #	Global Spec ID	Primary	Add	Der	Sub Add/Der	Volume	Units	Cond	Other Spec Id
1	414V14006371	DC60756X-01	VAG	NON	SLD	GRS	1.00 EA	SAT	
2	414V14006371	DC60756X-02	VAG	NON	SLD	GRS	1.00 EA	SAT	

### 10.5. Urine Testing for Pregnancy, Urinary Tract Infection, and Urinalysis

#### 10.5.1. 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 to collect the portion of the urine flow that is required by the test
- If the urine is to be used for culture, instruct the participant to clean the labia prior to specimen collection and to collect a midstream urine sample.
- Instruct the participant to screw the lid tightly onto the cup after collection.

### 10.5.2. Pregnancy Testing

Pregnancy status is a critical participant safety consideration in MTN-038. The Beckman Coulter ICON 25, 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. All sites must maintain an adequate inventory of the pregnancy 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.

The pregnancy test is performed according to site SOPs and the package insert (i.e. a negative result is based on the recommended total time for test to be considered complete.) Do not perform any other urine pregnancy tests for confirmatory purposes. If the urine pregnancy test cannot adequately be interpreted because of interfering factors (e.g. excess blood or extreme cloudiness due to amorphous material), the sample can be centrifuged, 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 in which a participant becomes pregnant, study product use will be permanently discontinued. The participant will be terminated from the study.

### 10.5.3. Urinary Tract Infection

Urine Dipstick and/or Culture: Perform the tests according to the package insert for the dipstick and your local SOP for culture.

For initial diagnosis and treatment of a UTI use your local standard of care (if you use a dipstick for leukocytes and nitrites record the results on the Chemistry Panel CRF). If a culture is performed, however, the results are not recorded on the CRF.

**See also SSP Sections 8.6 and 9.3 for additional information.**

## 10.6. Blood Specimens for Chemistry, Hematology, HIV testing, Syphilis, HSV-1/2 Serology, Plasma Archive, and DPV Blood Levels

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.

### 10.6.1. Specimen Collection and Initial Processing

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

- Allow serum tubes (no additive or serum separator tubes) to clot, then centrifuge per site SOPs.
- Lavender top tubes (additive = EDTA) should be gently inverted at least eight times after specimen collection to prevent clotting. If whole blood for hematology testing and plasma 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. EDTA tubes will be used for plasma DPV PK levels, plasma archive at enrollment, and if applicable, plasma for confirmation of viral load and HIV resistance testing.

*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.*

### 10.6.2. Chemistry (Alanine transaminase, Aspartate aminotransferase, and Creatinine) and Hematology (CBC with Differentials and Platelets)

Testing will be performed per local standard of care.

- Tests performed for Chemistry
  - Liver Function:
    - Alanine transaminase (ALT),
    - Aspartate aminotransferase (AST).

- Renal Function
  - Creatinine
  - Creatinine Clearance, using the participant's weight and age in conjunction with the Cockcroft-Gault formula.
- MTN-038 Hematology tests (Complete blood counts (CBC) with five-part differentials)
  - Hemoglobin,
  - Hematocrit,
  - Platelets,
  - White Blood Cell Count and differential
  - Red Blood Cell Count

### 10.6.3. Hepatitis B Surface Antigen

This testing will be done on serum or EDTA plasma per local SOPs.

### 10.6.4. HIV Testing

EDTA plasma, whole blood (fingerstick or venipuncture) and serum can be used to test 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 10-1 in this section or appendix II of the MTN-038 protocol). Rapid tests, such as Oraquick, are considered immunoassays and can be used with whole blood (fingerstick or venipuncture). The first specimen drawn for immunoassay and confirmatory testing (performed by local clinical laboratory) is considered Sample 1.

If Sample 1 is HIV positive by the confirmatory test, a second specimen (Sample 2) is drawn for the local clinical laboratory to perform a second confirmation test. When Sample 2 is drawn, additional blood is drawn to collect plasma for shipment to the MTN Virology Core.

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

#### 10.6.4.1 HIV Rapid Test Result Interpretation

- If 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.
  - Go to 10.6.4.2 if SAMPLE 1 is Screening or Enrollment sample
  - Go to 10.6.4.3 if SAMPLE 1 is Follow-up Visit sample
- 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 protocol testing algorithm.

#### 10.6.4.2 HIV Confirmatory Test for Screening or Enrollment Visit

- Until a participant is randomized/enrolled, treat enrollment testing same as part of the screening process to determine participant eligibility.
- If the confirmatory test for SAMPLE 1 is negative, indeterminate or invalid, contact the MTN Virology Core: [mtnvirology@mtnstopshiv.org](mailto:mtnvirology@mtnstopshiv.org) for guidance.
- If the confirmatory test is positive for the screening visit, the participant is considered seropositive and is not eligible for enrollment.

#### 10.6.4.3 HIV Confirmatory Test for Follow-Up Visits

- If at a follow-up visit, the confirmatory test on SAMPLE 1 is negative, indeterminate or invalid, contact the MTN Virology Core for guidance:

- 412-383-8138
  - [mtnvirology@mtnstopshiv.org](mailto:mtnvirology@mtnstopshiv.org).
- If the confirmatory test is positive at a follow-up visit, a second sample of blood (SAMPLE 2) will be drawn for a second local confirmatory test.
- When Sample 2 is collected, draw an additional tube of blood to be shipped to the MTN Virology Core.
  - Draw enough whole blood to store a total of 5 mL of plasma to send to the virology core. If less than 5 mL of plasma is obtained, contact the MTN Virology Core for guidance.
- Processing of the MTN Virology Core sample is similar to Plasma for Archive:
  - Log aliquots into LDMS using "Other Spec ID" = CON.
  - Centrifuge per specifications in SSP 10.6.6 and aliquot ≥5 mL into 2-mL cryovials and freeze at ≤-70°C.
  - Alert the MTN Virology Core, 412-383-8138 or [mtnvirology@mtnstopshiv.org](mailto:mtnvirology@mtnstopshiv.org) when you are preparing the shipment.
    - The virology core will confirm for you when to ship and will send their current address and other shipping details.
    - The virology core may also request enrollment plasma to be shipped with plasma collected with Sample 2.
- Testing performed at the MTN Virology Core may include repeat confirmation testing, HIV RNA and/or HIV resistance testing.
  - For specific guidance on reporting of HIV results to participants, refer to SSP Section 11.1 HIV pre- and post-test Counseling and HIV/STI Risk Reduction Counseling.

#### 10.6.5. Syphilis Testing

Testing will be performed per local standard of care. For positive diagnosis of syphilis, Rapid Plasma Reagin (RPR) titer is required.

All testing must be done with FDA approved assays and by a CLIA certified laboratory.

Questions related to result interpretation concerning eligibility and enrollment in the study should be directed to the MTN-038 Protocol Safety Physicians ([mtn038safetymd@mtnstopshiv.org](mailto:mtn038safetymd@mtnstopshiv.org)).

#### 10.6.6. Plasma Archive

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

- 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 1300-1500×g 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, at least 3-mL total volume.
- If total volume is less than 0.5-mL, redraw as soon as possible.
- If less than 1-mL of plasma is 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.

### 10.6.7. Serum for HSV-1/2 Testing

Allow blood samples to clot at room temperature prior to centrifugation. Aliquot 1-mL aliquots of serum 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.
- Spin blood at 1300-1500 $\times$ g for 10 minutes, remove serum.
- Prepare one 1.0-mL aliquot in a 2-mL cryovial, and the remainder will be back-up.
- If samples are hemolyzed, process the sample and enter comments in LDMS.

### 10.6.8. Blood for PK of Tenofovir (TFV)

The rectal fluid PK swab and the vaginal PK swab are collected approximately within 30 minutes after the blood is drawn for PK. On multiple time-point days (PUEV), see section 10.7.6 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 1300-1500 $\times$ g for 10 minutes. The centrifugation must be completed, and the sample placed in the freezer within 8 hours of blood collection.
3. Use a pipette to aliquot 1.5-mL of the resulting plasma into 2-mL 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 sets of storage boxes. One set will be labeled as “primary plasma PK samples”, and the other as “back-up plasma PK 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.

#### SHIPPING:

- MTN LC will coordinate sample shipments throughout the course of the study if necessary and at its conclusion. Ship PK aliquots to JHU CPAL (LDMS Lab 194):  
Attn: Mark Marzinke / James Johnson  
Johns Hopkins School of Medicine  
Clinical Pharmacology Analytical Lab (CPAL) at Bayview  
5200 Eastern Ave  
MFL Center Tower Suite 6000 Rm. 621  
Baltimore, MD 21224  
(410)550-9703 or (410)550-9713  
Email: [mmarzin1@jhmi.edu](mailto:mmarzin1@jhmi.edu) and [jjohnso6@jhmi.edu](mailto:jjohnso6@jhmi.edu)
- All shipments will be on dry ice that will be sufficient for a 24-hour period and can be initiated Monday through Wednesday to ensure that samples arrive in the lab during the work week.
- The back-up samples will be retained at the site until advised by the MTN LC or MTN-038 leadership team. One purpose of the extra aliquots is to be available in case the shipment is not received in the proper condition (e.g. thawing of samples).

### 10.7. Vaginal Specimens for Gram Stain, pH, Saline/KOH Wet Mount, GC/CT and *Trichomonas* NAAT, Cervicovaginal Fluid for PK, Cervicovaginal Lavage for PK, PD, and Biomarkers, and Vaginal Ring for Remnant Drug Content Analysis

Refer to Pelvic Exam Checklist on the MTN-038 website under Study Implementation Materials for further information on the required sequence of specimen collection and diagnostic procedures to be performed during study pelvic exams.



### 10.7.1. Gram Stains of Vaginal Fluid

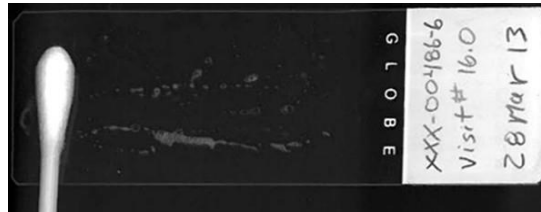
Dried vaginal fluid smears will be prepared for Gram staining and assessment for bacterial vaginosis at the MTN LC. The primary slide will be shipped to the MTN LC with the swab for microbiota culture, and the backup will be archived on site until written notification is received from the Statistical Center for HIV/AIDS Research & Prevention (SCHARP) that the slide may be discarded.

#### Materials for each collection

- 2 completed SCHARP labels
- Pencil
- 2 slides with frosted end, primary and backup (provided by MTN LC)
- 1 swab
- Slide holder for individual slide (provided by MTN LC) to send with culture
- Slide box (provided by MTN LC) to store backup slides until end of study or when requested

Instructions for slide preparation and shipping are provided below:

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



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



4. Allow the specimens to air-dry on the slides. Do not heat-fix.
5. Vaginal smears for gram stain are to be logged into LDMS (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. 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).

### 10.7.2. Vaginal pH and Wet Preps, if indicated for Bacterial Vaginosis (BV) and/or Yeast

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

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

Wet prep assessments used to diagnose BV and candidiasis are discussed in section 10.7.2.2 and summarized in Table 10-5.

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

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

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

#### 10.7.2.1 Vaginal Fluid pH, if indicated for BV

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

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

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

Materials:

- pH Indicator strip
- 1 swab

#### 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 STI Test Results CRF. It is not necessary to record pH values onto laboratory log sheets or other source documents prior to recording values onto CRFs.

Table 10-5 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)

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

Wet mount procedures for this study are only performed if indicated and consists of two different preparations: Potassium Hydroxide (KOH) and Saline. These procedures are for diagnosis of BV and candidiasis as summarized in Table 10-5.

#### Preparation and Examination of Wet Prep Slides

##### Materials:

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

##### Procedure:

1. Use a pencil to write the PTID and specimen collection date on one side of the frosted end of two microscope slides. Affix a SCHARP-provided PTID label to the other side of the slides (on the frosted end, under the pencil markings)
2. Immediately following collection from the lateral vaginal wall via swab (Dacron or cotton), smear vaginal fluid specimens onto each slide. Alternatively, the swab may be placed in a glass or plastic tube with approximately six drops (100 µL) sterile physiologic saline to allow for non-immediate slide preparation. In this case, vaginal fluid specimens should be smeared onto the two slides upon receipt from the collecting clinician.
3. Apply one drop of 10% KOH to one slide and immediately perform whiff test for a “fishy” amine odor. Then apply cover slip.
4. Apply one drop of sterile physiologic saline to the second slide, emulsify with the vaginal fluid specimen, and then apply cover-slip. Examine immediately at 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.

5. Examine the KOH slide at both 10X and 40X magnification for yeast and pseudohyphae.

**RESULTS:**

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

**10.7.3. Quantitative Vaginal Culture**

Vaginal swabs are collected for quantitative cultures at visits 2, 6, 8, and 9. The samples are delivered or shipped to the MTN LC the same day as collection. Shipping instructions follow.

Materials

Completed SCHARP Label

Starplex Starswab Anaerobic collection and (glass) transport kit (provided by MTN LC)

Shipping box

1. Use the Starplex Starswab Anaerobic collection and transporter kit. The kit comes with 2 sterile Dacron swabs and a glass transport tube.
2. Collect the specimen for culture by rotating 2 Dacron swabs several times over the lateral wall of the vagina. Do not collect culture swabs in the exact same area that another sample was collected (i.e: If the PK swab was collected first, then collect in a different location in the vagina preferably closer to the introitus). Insert the two swabs into the tube, slowly pushing the swabs half way into the gel, not to the bottom of the tube. Break off the shafts of the swabs (at perforation point) and secure the cap tightly.
3. The specimen may be kept at controlled room temperature for up to 4 hours. After four hours, the specimen must be refrigerated.
4. Deliver the Starplex tube and the LDMS specimen tracking sheet to the local LDMS laboratory.
5. Log the specimen into LDMS (Table 10-4) and label the Starplex tube with LDMS labels.
6. Use LDMS to generate a shipping manifest (i.e. batch file) for the cultures to be shipped to lab 414.
7. Ship the Starplex tube and one vaginal smear for Gram stain the same day of collection by overnight courier.
8. Into a biohazard specimen ziplock bag, place the Starplex tube with absorbent material (e.g. paper towels) and the case holding the corresponding Gram stain slide. If shipping multiple participant visits in same shipment, each participant visit should have its own specimen ziplock bag. Place the specimen ziplock bag(s), ice packs, and a copy of the manifest in a cardboard box lined with Styrofoam.
9. Use diagnostics packing code 650, UN3373 labels.
10. Confirm the address is correct (see below). The Research Institute is not open for weekend deliveries. Therefore, specimens collected on Friday must be sent to the hospital address for delivery on Saturday.

If sending **Monday through Thursday**, send to:

May Beamer  
Magee-Womens Research Institute  
204 Craft Ave, Room A530  
Pittsburgh, PA 15213  
Phone# 412-641-6041

If sending on **Friday** for Saturday delivery (**Select Saturday delivery**), and send to:

May Beamer, C/O Safety and Security  
Magee-Womens Hospital of UPMC  
300 Halket St.  
Pittsburgh, PA 15213

Phone # 412-641-4191 (contact number for Safety and Security)

\*\* [Be sure to select Saturday delivery on the Fed Ex label](#)

Notify the MTN LC via email ([hillierlab@mwri.magee.edu](mailto:hillierlab@mwri.magee.edu)) when the shipment has been picked up from the site by the courier/shipping company. Attach an electronic copy of the shipping manifest (i.e the LDMS batch file) to the email notification and include the following information: name of courier/shipping company, shipment tracking number, number of boxes shipped, date of shipment, and expected date of arrival.

#### 10.7.4. Vaginal swabs for q-PCR microbiota

Vaginal swabs are collected for detection of key microbiota using q-PCR at visits 2, 6, 8, and 9. The swabs are stored at  $\leq -70^{\circ}\text{C}$  and shipped to the LC at the end of the study.

##### Materials

Two Completed SCHARP labels

Two 2-mL Sarstedt Cryovials (provided by MTN LC)

Two flocked swabs (provided by MTN LC)

Rack for cryovials

Scissors

1. Affix the SCHARP labels to the cryovials.
2. Collect the specimen by rotating 2 flocked swabs several times over the lateral wall of the vagina. Do not collect swabs in the exact same area that another sample was collected (i.e: If the PK swab was collected first, then collect in a different location in the vagina preferably closer to the introitus).
3. Place the swabs in separate cryovials.
4. Break or cut shaft of swab at a minimum of 1cm beyond the swab and cap the vial.
5. Repeat with the second flocked swab as described above.
6. Record collection on the LDMS Tracking Sheet.
7. Deliver the tubes and an LDMS Specimen Tracking Sheet to the local LDMS laboratory.
8. Log the cryovial into LDMS (Table 10-4) and label each vial with a LDMS label. Avoid covering the entire PID on the original SCHARP label.
9. Store at  $\leq -70^{\circ}\text{C}$  within 2 hours of collection and record freezing time.
  - a. If delivered in dry ice, the clinic records the freezing time.
10. Batch ship samples to the MTN LC upon request at the end of the study.

#### 10.7.5. Testing for GC/CT (*Neisseria gonorrhoea* and *Chlamydia trachomatis*) and *Trichomonas* by NAAT

Testing for GC/CT and *Trichomonas* is performed at screening and when clinically indicated. Sites can choose to use the Cepheid GeneXpert or Gen-Probe Aptima. If the site does not have access to these tests, they can send the samples to the MTN LC for testing. Contact the MTN LC prior to sending specimens.

- If using GenProbe Aptima, both GC/CT and *Trichomonas* tests can be performed from one swab. Use only one collection kit.
- If using Cepheid GeneXpert, in order to ensure that sufficient sample volume is available for repeat testing, we recommend using two collection kits, one for GC/CT and the other for *Trichomonas*. If the testing lab does both, check with the lab.
- Affix a SCHARP-provided PTID label onto the manufacturer's transport tube.
- Use the swab provided in the manufacturer's collection kit to collect the sample.
- Immediately place the swab in the transport tube, break off the shaft of the swab, and cap the tube.
- Transport the specimen at ambient temperature to the local testing laboratory

### 10.7.6. Cervicovaginal Fluid (CVF) Swab for PK

Cervicovaginal fluid swabs for PK will be clinician collected. PK collection times need to be recorded on the LDMS sample tracking sheet. In addition to sample collection, this section discusses acceptable 'windows' on collection time points and action to be taken if collection is outside of this.

#### **Collection Timing and Target Times for Vaginal Swabs, Rectal Swabs, and Blood for PK**

##### Visits with multiple PK collection time-points:

- When to start the timer
  - At Enrollment,
    - The 1 and 4 HR collection times are determined by starting a timer upon VR insertion.
  - On Day 91,
    - 0 HR is collected before VR removal. Collect blood, then preferably the rectal swab for PK prior to vaginal swab for PK at this time.
    - The 4 HR time point collection is determined by starting a timer at time of VR removal.
    - If the VR is removed by the participant prior to the clinic visit, collect only one time point of blood and swabs for PK.
- When each time-point is due:
  - Blood will be drawn first, preferably followed by the rectal fluid swab sample, then the vaginal swab.
    - Clinicians should aim to start the blood draw exactly at the targeted collection time i.e. on the hour. Minor excursions from the target time may occur but should be no more than +/- 15 minutes.
    - The clinician will collect the rectal and vaginal swab for PK within 30 minutes of the blood draw.
- Make sure that specimen times are accurate, in case there are delays in sample collection. Correct recording will allow the interval of time to be correctly calculated.
- Missed or delayed blood draw time point:
  - There will be no bearing on the next time point.
    - Example: Although the 1 HR time point draw was 15 minutes late (drawn at 75 minutes), the 4 HR PK blood would still be drawn at the 4 HR mark.
  - At a given time-point, if the start of PK collection is more than +/- 15 minutes from the targeted time, missed entirely, or samples are collected more than 30 minutes apart from one another, notify the MTN-038 management team.

##### In the case that the VR is removed prior to a visit:

- Vaginal swab for PK should still be collected even if the VR has been out of the vagina for up to 7 days.
- This VR removal / re-insertion should be noted on LDMS tracking sheet and the Ring Adherence CRF.

#### **Procedure for CVF Sampling for PK assessment and weighing swab**

1. Each day of collection that requires weighing of rectal or vaginal swab and cervical biopsies for PK, perform QC of the analytical scale to accurately weigh samples to a weight of at least 0.1 milligrams. Do not turn off balance until weighing for the day is completed.
2. Materials for each time point:
  - 2 SCHARP labels with PTID, visit number, visit date, time point.
  - 2-mL Nalgene cryovials (provided by MTN LC)
  - Polyester-Tipped (Dacron) Swab (provided by MTN LC)
  - Ziplock biohazard sample bags
  - Gloves
  - Analytical scale

- Urine cup (without lid) or similar lightweight container, placed on middle of scale, to contain items to be weighed. (Some balances have an optional basket.)
  - A rack that will hold the cryovial
  - Scissors to cut swab shaft (optional)
  - Calculator
3. Handle items to be weighed with gloves.
  4. Place identically-labeled SCHARP label on each the cryovial and a biohazard sample ziplock bag.
  5. Perform pre-weight.
    - a. Zero the urine cup or similar container
    - b. Place the labeled 2-mL cryovial in the urine cup.
    - c. Place the packaged sterile Dacron swab upright in the urine cup. (Make sure it is not leaning on a part of the scale.)
    - d. Record this pre-weight on the LDMS Tracking Sheet.
    - e. Place the cryovial and the packaged Dacron swab in a biohazard sample ziplock bag with the matching label to the tube.
    - f. If multiple time points or multiple participants on that day, pre-weights for all time points may be obtained with careful observation of time-point labels.
  6. Make sure you have the correct participant time-point before the exam.
  7. In the exam room instruct the clinician that none of the items in the bag should be thrown into the garbage – only into the ziplock bag.
    - a. Prep for the clinician:
      - i. Have the rack ready.
      - ii. Unscrew the lid of the 2-mL cryovial and place the tube in the rack, the lid in the ziplock bag.
      - iii. Start the peel of the packaging of the swab to ensure sufficient separation.
    - b. The clinician will peel the packaging and remove the Dacron swab to collect vaginal fluid (slow count to 10).
      - i. The clinician will place the swab in the tube and the swab packaging into the ziplock bag.
    - c. Suggestions for cutting or bending to break the swab shaft. (!!!Potential to lose swab shaft!!!)
      - i. If cutting the shaft, for leverage, make sure shaft of swab is at the pivot point of the scissors, then cut. Do not use tip of blades to cut.
      - ii. If repeatedly bending to break the shaft with dominant hand, while doing so, it may be best to hold the top of the tube with the forefinger and thumb of the other hand.
    - d. Place the cut shaft in the ziplock bag.
    - e. Screw the lid back on the cryovial and place sample in the bag with the swab packaging and the swab shaft.
  8. Perform Post Weight:
    - a. Zero the urine cup or similar lightweight container.
    - b. Weigh the capped cryovial containing the absorbed swab tip, the swab packaging and the remainder of the swab shaft (Suggestion: Place the swab shaft into the packaging and have it upright during weighing.)
    - c. Make sure that the post-weight is larger than the pre-weight.
    - d. Record post-weight on the LDMS Tracking sheet.
      - i. If post-weight is less than pre-weight, no CVL or biopsy collected, and the participant is still available, check if participant is willing to provide another swab.
  9. On the LDMS tracking sheet, calculate the net weight by subtracting the pre- from the post-weight.
    - a. For a negative net weight, first check with personnel to make sure the pre- and post-weights



were recorded on the correct lines.

- i. Troubleshoot immediately to prevent recurrence of issue.
- ii. Notify and forward the daily QC report to MTN LC.
- iii. Document as a protocol deviation.

10. Log the samples into LDMS to create labels.

- a. In LDMS, enter the negative net weight in the comments section.

11. Affix the LDMS label over the SCHARP label and store  $\leq -70^{\circ}\text{C}$  within 2 hours of collection.

12. Record freezing time on the LDMS tracking sheet.

13. LC will coordinate shipments throughout if necessary and at the end of the study to JHU CPAL - see address listed in section 10.6.6.

#### **Shipping of PK swab samples**

- LC will coordinate sample shipments to JHU CPAL throughout course of study if necessary and at its conclusion. Ship PK swabs to JHU CPAL (LDMS Lab 194) – see address listed in section 10.6.8.
- All shipments will be on dry ice that will be sufficient for a 24-hour period and can be initiated Monday through Wednesday to ensure that samples arrive in the lab during the work week.

#### **10.7.7. CVF Swabs for Anti-HSV-2 Activity**

Cervicovaginal fluid swabs for Anti-HSV-2 Activity will be clinician collected. The collection kit that will be used is a Starplex Scientific Starswabs Dry Swabs (plastic) transporter. No weights will be necessary for these samples.

##### Materials

Clinic:

- Completed SCHARP label
- Starplex Scientific Starswabs Dry Swabs plastic transporter (provided by the MTN LC)

Lab:

- Two 2-mL Sarstedt cryovials (provided by the MTN LC)
- Scissors (optional)

1. Place a completed SCHARP label on the plastic transporter.
2. Remove the cap of the transporter, collect the sample, and recap.
3. Record the collection on the LDMS tracking sheet and deliver to the lab.
4. Enter the 1 primary sample with two aliquots into LDMS to create the LDMS labels.
5. Affix the LDMS labels to the cryovials.
6. Uncap the cryovials and place in a cryovial rack.
7. Remove the double-headed swab from the Starplex plastic transporter.
8. Position the swabs so that each swab head is in a cryovial.
9. To fit into the cryovial, raise the swab from the bottom, then cut or bend (similar to 10.7.6, 7c)
10. Store at  $\leq -70^{\circ}\text{C}$  in appropriately labeled storage box.
11. MTN LC will communicate shipping information when testing lab is ready to receive the samples.

#### **10.7.8. CVF Swabs for Biomarkers**

Cervicovaginal fluid swabs for biomarkers will be clinician collected. No weights will be necessary for these samples.

##### Materials

Completed SCHARP label

2-mL Corning orange top cryovial (provided by the MTN LC)

Dacron swab (supplied by the MTN LC)

Scissors

1. Complete a SCHARP label and affix it to the 2-mL cryovial.
2. Have one 2-mL cryovial ready, uncapped.
3. Remove Dacron swab from the packaging and collect the sample.
4. Place the swab head in the cryovial, raise it, and cut or bend the swab (similar to 10.7.6, 7c) to fit
5. Record the collection on the LDMS tracking sheet.
6. Log sample into LDMS to create the LDMS label.
7. Affix label onto cryovial and store at  $\leq -70^{\circ}\text{C}$ .
8. MTN LC will communicate shipping information when testing lab is ready to receive the samples.

### 10.7.9. Cervicovaginal Lavage (CVL) supernatant for PK, PD, and biomarkers and CVL pellet for biomarkers

#### Materials

Completed SCHARP label

15-mL conical

Normal saline

Implements to administer normal saline / collect CVL

Centrifuge

2-mL cryovials

Transfer pipets or pipettor

1. 10-mL of normal saline should be used to lavage the cervix, fornices, and vaginal walls. Using a syringe collect all of the CVL and place into a 15-mL conical tube. See SSP section 8.5.4 for CVL collection.
2. CVL specimens are kept on wet ice or refrigerated and should be processed within 2 hours of collection.
3. Record the collection on the LDMS Tracking Sheet.
4. For LDMS login, estimate the volume of the CVL to determine the number of 1-mL supernatant aliquots.
5. Create LDMS labels for the supernatant aliquots and the cell pellet. For the final aliquot, the cryovial will most likely have volume  $< 1\text{-mL}$ ; therefore, please store as a backup.
6. Centrifuge the 15-mL collection tube of CVL at  $800\times g$  for 10 minutes.
7. Add LDMS label to each cryovial. Without disturbing the cell pellet, pipet as many 1-mL aliquots of supernatant as possible into cryovials.
8. Re-spin the 15 mL conical tube containing cells for 10 minutes at  $800\times g$ .
9. Without disturbing the cell pellet, pull off additional supernatant and add to a supernatant vial.
10. Resuspend cell pellet in 0.5 mL normal saline and store in a cryovial with an already affixed LDMS label.
11. Store all supernatants and cell pellet at  $\leq -70^{\circ}\text{C}$  within 2 hours of collection.
12. If less than a total of 6 mL's (or less than 6 cryovials) of supernatant are recovered, contact the MTN LC.
13. Store one aliquot each into boxes labeled CVL for PK and CVL for PD, and the remaining aliquots into CVL for biomarker.
14. Store the pellet into boxes labeled CVL pellet.
15. Batch ship appropriate samples to MTN LC or JHU CPAL upon request.

Ship PD and biomarker aliquots and pellet samples to MTN LC (LDMS Lab 414)

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Ship PK aliquots to JHU CPAL (LDMS Lab 194) – see address listed in section 10.6.8.

#### **10.7.10. Preparation of Vaginal Ring (VR) for Remnant Content Analysis, Glycerin Content and Other Bioassays**

Collection of the ring will occur at day 91/ PUEV or early termination visit. The used VRs may contain vaginal secretions and therefore treated as a biohazard. After use, the VRs will be cleansed, placed into their original resealable foil pack, and stored at -80°C until further notice from the MTN LC. The clinical site will be supplied with a small supply of amber bags in case the resealable foil pack is misplaced. Additionally, biohazard labels will be provided for the site to affix to the foil pack (or amber bag, if necessary) after the VR is placed in it for storage.

For VRs that are defective or inserted briefly and removed for various reasons, consult the MTN 038 management team for the collection or destruction of the VR.

##### Important notes:

- Immediately before VR removal, blood, rectal fluid and CVF swabs, CVL and cervical biopsies should be collected.

If the VR is removed by the participant prior to the clinic visit and will not be reinserted, instruct the participant to place it in a container (white bag that was provided to the participant) and store at room temperature. At the clinic, the returned used VR will be prepared for residual drug analysis.

##### Materials:

- A disposable sterile container or a container that is autoclaved between each use
- Alcohol pads
- PPE: lab coat, gloves, face guard
- Paper towel
- Original ring packaging, which is a resealable foil pack (OR if misplaced, use amber bag with affixed biohazard label)
- Biohazard label for the resealable foil pack
- Completed SCHARP label for the foil packaging (or amber bag)

##### Removal of VR by clinician:

1. Wear lab coat, gloves, and protective face guards when performing this step.
2. The clinician will remove the used VR and place it in an empty sterile container.
3. Clinical staff will use alcohol pads to gently clean the VR until vaginal mucus and other fluids are not visibly present on the ring.
4. The VR will be allowed to air dry completely on paper towels or gauze.
5. After ensuring that the VR is dry, it is placed into the resealable foil pack (or amber bag).
6. Affix a biohazard label on the resealable foil pack. If using the amber bag, ensure a biohazard label is on it.
7. Add a completed SCHARP label to the foil pack (or amber bag).
8. Complete the LDMS tracking sheet.
9. Dispose of materials used during this procedure according to institutional biohazard policy.

##### Preparation of used VR for storage on-site:

1. Add a biohazard sticker if one is not already affixed to the resealable foil pack (or amber bag), making sure not to cover the identifier information (SCHARP label).
2. Use LDMS to log in the used VR.
3. Affix LDMS label to the foil pack.
4. Store the biohazard-labeled resealable foil pack (or amber bag) containing the used VR at -80°C.

5. At the end of the study, MTN LC will contact the sites to coordinate dry ice shipment to the appropriate organization.

### 10.8. Cervical Specimens: Pap Test and Biopsy for PK

Pap smears are only required if clinically indicated or if a participant is older than 21 and has not had a documented normal test within 3 years prior to Enrollment.

Two biopsy specimens, each from different areas in the cervix, will be collected, as described in the site SOP. See Section 8.5.5 for collection.

#### 10.8.1. Papanicolaou (Pap) Test (\*only if indicated)

If a Pap 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, testing and QC procedures must be performed and documented in accordance with study site SOPs.

#### 10.8.2. Cervical Biopsies for PK

*NOTE: Rectal and vaginal swabs are also collected at these visits; therefore, the analytical balance should still be on and QC completed to check accuracy to a weight of at least 0.1 milligrams.*

For scheduled cervical biopsies for PK, two biopsy samples will be collected. Each participant will be randomized for PK biopsy collection at:

- Visits 5 (day 14) and 8 (day 56) or
- Visits 6 (day 28) and 9 (day 91).

#### Materials

- 2 SCHARP labels with PTID, visit number, visit date, time point.
  - 2-mL Nalgene cryovials (provided by MTN LC)
  - Gloves
  - Analytical scale
  - Urine cup (without lid) or similar lightweight container, placed on middle of scale, to contain items to be weighed. (Some balances have an optional basket.)
  - A rack that will hold the cryovial
  - Implements to perform biopsy
  - Calculator
1. Complete two SCHARP labels and affix to two 2-mL cryovials (Nunc or Nalgene). Label the tube and the top of one cryovial "1", and the other tube and top pair "2", with "1" always being the first biopsy extracted.
  2. Handle the cryovials with gloves to avoid adding oils to the weight.
  3. Weigh each empty labeled cryovial and record pre-weight on the LDMS tracking sheet.
  4. Uncap the cryovials, directly transfer the first biopsy to the designated "1" pre-weighed cryovial, and cap. Record the collection time.
  5. The second biopsy is transferred to the "2" cryovial in a similar manner.
  6. Obtain the post-weight for each cryovial containing a biopsy and record on the LDMS tracking sheet.
  7. Calculate the net weight, which should be greater than zero.
  8. Immediately place the cryovials containing the PK biopsy in dry ice ethanol bath (dry ice with enough ethanol to make a slushy consistency) or liquid nitrogen.
  9. Document the time when the cryovial containing the biopsy was frozen on the LDMS tracking sheet.
  10. On the LDMS tracking sheet, calculate the net weight by subtracting the pre- from the post-weight.
    - a. For a negative net weight, first check with personnel to make sure the pre- and post-weights were recorded on the correct lines.
      - i. Troubleshoot immediately to prevent recurrence of issue.

- ii. Notify and forward the daily QC report to MTN LC.
  - iii. Document as a protocol deviation.
11. Log the samples into LDMS to create labels.
    - a. In LDMS, enter the negative net weight in the comments section.
  12. Affix the LDMS labels over the SCHARP labels and store  $\leq -70^{\circ}\text{C}$ . Record freeze time on LDMS tracking sheet.
  13. LC will coordinate shipments throughout if necessary and at the end of the study to JHU CPAL - see address listed in section 10.6.6.

### 10.8.3. Cervical Biopsies for PD (ex vivo challenge)

Two biopsy samples will be collected for tissue PD at Visit 8 (day 56) or visit 9 (day 91).

NOTE: *The local PD lab needs to begin processing the biopsy within 30 minutes of collection. Please communicate your MTN-038 schedule in order to receive the appropriate number of tubes containing biopsy transport medium from the PD lab and to ensure that someone will be available for processing.*

1. Communicate with the local PD lab prior to and on the day of collection.
2. Affix a completed SCHARP label to the tube(s) containing biopsy transport medium, which is stored at  $4^{\circ}\text{C}$ . (Note: A 15-mL conical containing 5-mL biopsy transport medium or two 2-mL cryovials containing 1-mL each of biopsy transport medium may be used. Check with local PD processing laboratory for transport preference.)
3. Biopsy transport medium will be provided weekly or as needed.
4. Both biopsies will be placed immediately into the one conical tube of transport media or into two separate cryovials containing transport media.
5. Immediately transport on ice to the local PD lab that validated the procedure.
  - Document chain of custody with a transmittal form (e.g. designated section on the LDMS tracking sheet.)
  - LDMS and the PD lab will determine which lab will do the LDMS entry.

### 10.9. Rectal Fluid Swab for PK

If done at the same visit, it is logistically best to collect prior to the cervical biopsy, which is a longer and a less predictable procedure.

- Each day of collection of rectal fluid for PK, perform QC that would be required for the analytical scale to accurately weigh samples to a weight of at least 0.1 milligrams. Do not turn off balance until weighing for the day is completed.
- Rectal and vaginal PK swabs must be collected within 30 minutes of PK blood draw.

#### Materials for each collection:

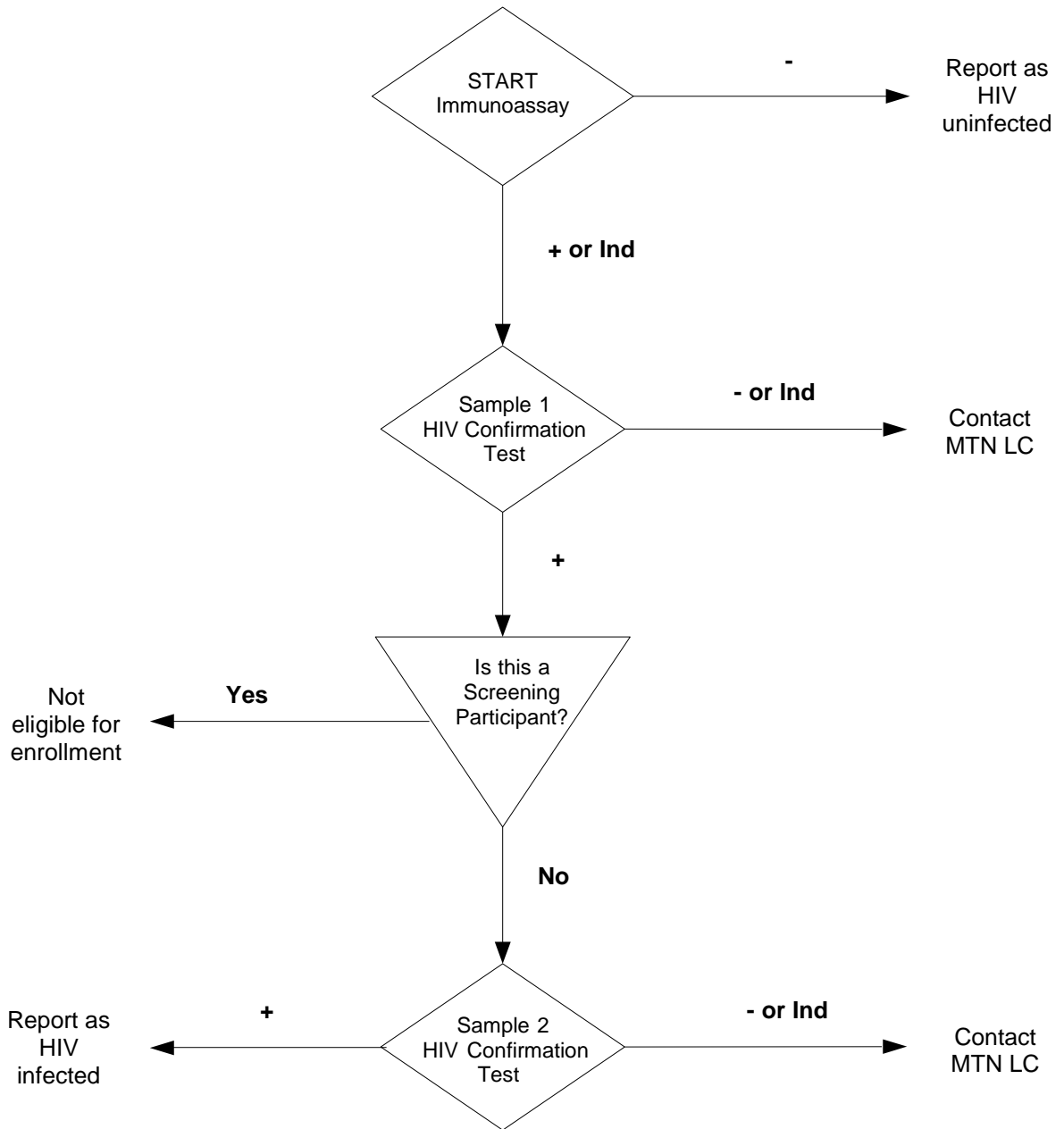
- 2 SCHARP labels with PTID, visit number, and visit date
- 2-mL Nalgene cryovial (provided by the MTN LC)
- Polyester-Tipped (Dacron) Swab (provided by the MTN LC)
- Zip-lock biohazard sample bag
- Plastic cup (without lid) or similar lightweight container, placed on middle of scale, to contain items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

#### Instructions

1. Place identically-labeled SCHARP labels on the cryovial and a biohazard sample bag.
2. Perform pre-weight. Handle items to be weighed with gloves.
  - a. Zero the cup or similar container on the scale.
  - b. Place the labeled 2-mL cryovial and packaged sterile Dacron swab upright in the cup. (Make sure it is not leaning on a part of the scale.)

- c. Record this pre-weight on the LDMS Tracking Sheet.
  - d. Place the cryovial and the packaged Dacron swab in a labeled biohazard sample bag.
3. Sample is collected while anoscope is in place. See section 8.5.6 for clinical details.
- NOTE: **All** of the items in the bag should return to the bag. Nothing will be thrown into the garbage.
- a. Remove swab from packaging. Do **NOT** discard the packaging. Place all of the packaging back into the bag.
  - b. Collect rectal fluid by holding the swab against the mucosa for 2 minutes.
  - c. Cut or break swab shaft by bending (similar to 10.7.6, 7c). **To cut:** Place the swab in the cryovial, slightly raise the swab, open the scissors wide so that the swab shaft is at the pivot point of the scissors, cut the shaft. Using the pivot point will prevent expulsion of the swab from the cryovial. **To break by bending:** Bend the shaft repeatedly with dominant hand. While doing so, it may be best to hold the top of the tube with the forefinger and thumb of the other hand. To avoid losing the shaft, which needs to be post-weighed, be sure to hold onto it. Do **NOT** discard the shaft.
  - d. Place the cut shaft in the specimen bag.
  - e. Screw the lid back on the cryovial and place sample in the bag with the swab packaging and the swab shaft.
  - f. Document the collection time (the time the swab was removed from the rectum) on to the LDMS tracking sheet.
4. Perform Post Weight:
- a. Zero the cup or similar lightweight container on the scale.
  - b. Weigh the capped cryovial containing the absorbed swab tip, the swab packaging and the remainder of the swab shaft (Suggestion: Place the swab shaft into the packaging and have it upright during weighing.)
  - c. Record post-weight on the LDMS Tracking sheet
5. On the LDMS tracking sheet, calculate the net weight by subtracting the pre- from the post-weight.
- a. For a negative net weight, first check with personnel to make sure the pre- and post-weights were recorded on the correct lines.
    - i. Troubleshoot immediately to prevent recurrence of issue.
    - ii. Notify and forward the daily QC report to MTN LC.
    - iii. Document as a protocol deviation.
6. Log the samples into LDMS to create labels.
7. In LDMS, enter the negative net weight in the comments section.
8. Affix the LDMS label over the SCHARP label and store  $\leq -70^{\circ}\text{C}$  within 2 hours of collection.
9. Record freezing time on the LDMS tracking sheet.
10. Log into LDMS (Table 10-3) and batch ship to JHU CPAL (LDMS Lab 194) upon request – see address listed in section 10.6.8.

Appendix 10-1: HIV ANTIBODY TESTING ALGORITHM



Ind: Indeterminate  
 MTN LC: MTN Laboratory Center