

## 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-036 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-036/IPM 047 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.

	Visit 1 SCR	Visit 2 ENR (Day 0)	Visits 3-4 (Days 1, 2)	Visits 5-7 (Days 3, 7, and 14)	Visit 8 (Day 28/ Week 4)	Visit 9 (Day 56/ Week 8)	Visit 10 PUEV/Early Termination (Day 91/ Week 13)	Visit 11 Final Contact (Day 92, 93 or 94)	
<b>LABORATORY TEST</b>									
URINE	Pregnancy test	X	X	*	*	X	X	*	
	Urine dipstick/culture	*	*	*	*	*	*	*	
BLOOD	HIV-1 testing	X	X	*	*	*	*	X	
	Plasma for archive		X						
	AST/ALT	X				*	*	X	
	CBC with differential and platelets	X	*	*	*	*	*	X	
	Syphilis serology	X		*	*	*	*	*	
	DPV levels		1-, 2-, & 4-HR PK	X	X	X	X	Pre-ring removal, 1-,2-, & 4-HR PK	X
PELVIC	NAAT for GC/CT and trichomonas	X	*	*	*	*	*	*	
	Saline/potassium hydroxide wet mount with pH for candidiasis and/or bacterial vaginosis	*	*	*	*	*	*	*	
	Pap test	X <sup>^</sup>							
	Vaginal swabs for microbiota -culture		X			X	X	X	
	Vaginal swabs for microbiota - qPCR		2			2	2	2	
	Vaginal Gram stain		X			X	X	X	
	CVF DPV levels		1-, 2-, & 4-HR PK	X	X	X	X	Pre-ring removal, 1-,2-, & 4-HR PK	X
	CVL for PK, PD, and biomarkers		Pre-ring insertion			X	X	Post-4HR PK collections	
	Cervical biopsies for PK					2		2 (Pre-ring removal)	
RECTAL									
		4 HR PK		X	X		Pre-ring removal	X	
<b>STUDY PRODUCT SUPPLY</b>									
	Removal and collection of study 25-mG VR				X	X	X		
	Removal and collection of study 100- or 200-mG 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

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

**Table 10-1: Overview of Laboratory Tests by visit for MTN-036/IPM 047**

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

**Table 10-2: Overview of Laboratory Testing Locations, Specimens, and Methods for MTN-036/IPM 047**

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	Quidel Quickvue or SureVue Beckman Coulter ICON 25
Urine Dipstick and Culture*	Local lab	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)	Local Lab	Consult Local Lab Requirements		Local methodology
Syphilis Serology	Local Lab	Consult Local Lab Requirements		Local methodology
HIV serology	Clinic/Local Lab	Plasma, serum, or whole blood	EDTA or plain, 4-mL	FDA approved tests
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 (DPV)	CPAL	Plasma	EDTA at least 8-mL tube	CPAL collection procedure
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 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
Cervicovaginal Fluid for PK	CPAL	Vaginal swab	2.0-mL Cryovial	CPAL collection procedure
CVL for PK, PD & biomarkers	CPAL & MTN LC	CVL using 10mL saline	15-mL tube	MTN LC procedure
Vaginal swab for microbiota culture	MTN LC	Vaginal swab	Starplex transporter	MTN LC procedure

Vaginal swab for microbiota q-PCR	MTN LC	Vaginal flocked swabs	2.0 mL Cryovials	MTN LC procedure
Vaginal Smear for Gram-stain	MTN LC	Vaginal Swab	2 Slides	MTN LC procedure
Pap Test**	Local Lab	Consult Local Lab Requirements		Local methodology
Cervical Biopsy for PK	CPAL	Tissue	2.0 mL cryovial	MTN LC procedure
Rectal swab for PK	CPAL	Rectal swab	2.0-mL Cryovial	CPAL collection procedure
Used Intravaginal Ring for PK residual assessment	IPM-designated Lab	Used IVR	Biohazard labeled 3"x5" amber Zippit pouch	MTN LC 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.

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

Specimen	Processing	Ship to	Shipping schedule
<b>Plasma for Archive (at enrollment) or for Confirmation of Viral Load and HIV Resistance (at f/u)</b>	Prepare at least two 1.5-mL aliquots. If sample is collected and held at room temp, freeze $\leq -70^{\circ}\text{C}$ within 4 hours. If refrigerated after collection, freeze $\leq -70^{\circ}\text{C}$ within 24 hours.	MTN LC	Store frozen at site until notified by MTN LC. However, if a follow-up visit plasma for HIV confirmation, contact MTN LC Virology Core.
<b>Plasma for Blood PK (DPV)</b>	Centrifuge and aliquot into two or more cryovials with a minimum of 1.5-mL in each. Freeze within 8 hrs of blood collection.	CPAL, MTN LC	Store frozen at site until conclusion of study or notified by MTN LC.
<b>Vaginal Swab for PK</b>	Record Pre- and Post-collection weight of swab. Freeze at $\leq -70^{\circ}\text{C}$ within 2 hours of collection	CPAL	Store frozen at site until conclusion of study or notified by MTN LC.
<b>CVL Supernatant for PK, PD, Biomarker</b>	Centrifuge and aliquot into 8-10 cryovials with a minimum of 1.0-mL in each. Freeze 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>	Centrifuge, add normal saline, transfer pellet into a single cryovial. Freeze within 2 hrs of collection.	MTN LC	Store frozen at site until conclusion of study or notified by MTN LC.
<b>Vaginal Swab for Microbiota Culture</b>	2 swabs in Starplex transporter, store on ice	MTN LC	Ship on ice packs <u>the day of collection</u>
<b>Vaginal Swab for Microbiota q-PCR</b>	2 flocked swabs stored in cryovials. Freeze at $\leq -70^{\circ}\text{C}$ 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 confirmed as received and evaluated.

<b>Cervical Biopsies for PK</b>	Collect 2 biopsies, each placed in a cryovial. Perform Pre (without biopsy) and Post (with biopsy) weights. Flash-freeze. Store at $\leq -70^{\circ}\text{C}$ .	CPAL	Store frozen at site until conclusion of study.
<b>Rectal Fluid Swab for PK</b>	Record Pre-and Post-collecton weight of swab. Freeze at $\leq -70^{\circ}\text{C}$ within 2 hours of collection	CPAL	Store frozen at site until conclusion of study or notified by MTN LC.
<b>Used Intravaginal Ring for PK Residual Assessment</b>	Rinse the used ring. Blot dry. Place IVR in amber pouch. Store at $-20^{\circ}\text{C}$ .	MTN LC	$-20^{\circ}\text{C}$ storage at site until conclusion of study.

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.

## 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).

Table 10-4 LDMS Specimen Management Guide to Logging in MTN-036/IPM 047 Specimens*									
Sample	Primary Specimen	Primary Derivative	Aliquot Derivative	Aliquot Sub additive/ derivative	Other Specimen ID	# of Aliquots	Aliquot Volume	Units	Time or Weights
Plasma for Archive or Confirmatory Test	BLD	EDT	PL1 (single spin) PL2 (double spin)	N/A	EPA (optional for enrollment)	2-5	≥1.5 mL in 2-mL cryovials	mL	--
Plasma for PK (DPV)					CON (follow-up)				
Vaginal Smear for Gram Stain	VAG	NON	SLD	GRS	--	2	2 smears	Each	--
Vaginal Swabs for qPCR	VAG	NON	FLS	N/A		2	1	Each	
Vaginal Swabs for Culture	VAG	CTK	SWB	N/A		1	1	Each	
CVL Supernatant	CVL	NSL	FLD	N/A		6-9	≥1.0	mL	
CVL Pellet	CVL	NSL	CEN	NSL		1	0.5	mL	
CVF for PK	VAG	NON	SWB	N/A	--	1	variable	mG	See 10.4.2 10.4.3
Cervical Biopsies for PK	CVB	NON	BPS	N/A	--	2	variable	mG	See 10.4.2 10.4.3

**Table 10-4 LDMS Specimen Management Guide to Logging in MTN-036/IPM 047 Specimens\***

Sample	Primary Specimen	Primary Derivative	Aliquot Derivative	Aliquot Sub additive/ derivative	Other Specimen ID	# of Aliquots	Aliquot Volume	Units	Time or Weights
Rectal Fluid Swab for PK	REC	NON	SWB	N/A	--	1	variable	mG	See 10.4.2 10.4.3
Used Vaginal Ring for PK Residual Assessment	IVR	NON	IVR	N/A	--	1	1 pouch	Each	--

\*List of Codes and their definitions:

<b>BLD:</b> Whole Blood	<b>IVR:</b> Used Intravaginal Ring	<b>PL1/2:</b> Single or double spun plasma	<b>BPS:</b> Biopsy
<b>VAG:</b> Vaginal Swab	<b>EDT:</b> EDTA	<b>SLD:</b> Slide	<b>GRS:</b> Gram stain slide
<b>CVB:</b> Cervical Biopsy	<b>NON:</b> No Additive	<b>SWB:</b> Swab	<b>N/A:</b> Not Applicable
<b>FLD:</b> Fluid	<b>CTK:</b> Culture transporter	<b>CEN:</b> Centrifuge	<b>NSL:</b> Normal saline
<b>FLS:</b> Flocked Swab	<b>REC:</b> rectal	<b>TIS:</b> tissue	

**LDMS Help:** Questions related to use of LDMS in MTN-036/IPM 047 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-036/IPM 047 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-036/IPM 047 webpage.

#### 10.4.2. Logging in Time for PK Samples

In this study, there will be multi PK time-point visits, and in LDMS, in addition to time of collection, the TIME and TIME UNIT field 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.

**Figure 10-1: LDMS Screen for Time Entry.**

Group	TYPE1	ID1	TYPE2	ID2	TYPE3	ID3	Visit	Unit
1	MTN	PID	302600026	PROTOCOL	013/0	ID3		2.00
2								
3								
4								
5								
6								

Spec. Date: 15/Nov/2011    Exp. Date: 0    Remote    VQA  
 Imported    Culture Derivative

# of Tubes: 1    Primary Type: BLD    Other Spec ID:    Enter Specimen ID    Spec Time: 16:00

Specimen #	Global Spec ID	Primary	Additive	Volume	Units	Spec Time	Time	Time Unit
1	414V11002715	HC6012KK-00	BLD	EDT	8.00	ML	16:00	4.00 Hours

# of Aliquots: 3    Vol: 1.8    Units: ML    Derivative: PL1    Sub Add/Der: N/A    Other Spec ID:

Aliquot #	Specimen	Global Spec ID	Primary	Add	Der	Sub Add/Der	Volume	Units	Cond	Other Spec ID
1	414V11002721	HC6012KK-01	BLD	EDT	PL1	N/A	1.80	ML	SAT	
2	414V11002721	HC6012KK-02	BLD	EDT	PL1	N/A	1.80	ML	SAT	
3	414V11002721	HC6012KK-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:

In the derivative area for the primary sample, The VOLUME and UNIT field is 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 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.



Figure 10-2: LDMS Screen for Weight Entry

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

Spec. Date: 26/Jun/2014 Exp. Date: 0  
 Rec. Date: 26/Jun/2014 Recd Time: 15:30 Export ID:

Remote  Imported

Enter Specimen ID:

# of Tubes: 1 Primary Type: VGL Other Spec ID:  Spec. Time: 15:09

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

**A**

# of Aliquots: 1 Vol: 66.5 Units: MG Derivative: Sw/B Sub Add/Der: N/A Other Spec ID:

	Specimen	Global Spec ID	Primary	Add	Der	Sub Add/Der	Volume	Units	Cond	Other Spec Id
1	414V14006369	FC60756V-01	VGL	NON	Sw/B	N/A	66.50	MG	SAT	M

**B**

#### 10.4.4. LDMS Entry for Vaginal Smear for Gram Stain

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. An example is shown in figure 10-3. Note that after the 2 aliquots are added, a pop-up message will warn the user that the total aliquot volume exceeds the primary volume. Ignore the message and continue.

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 Vol
2									
3									
4									
5									
6									

Spec. Date: 26/Jun/2014 Exp. Date: 0  
 Rec. Date: 26/Jun/2014 Recd Time: 15:30 Export ID:

Remote  Imported

Enter Specimen ID:

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

	Specimen #	Global Spec ID	Primary	Additive	Volume	Units	Spec. Time	Time	Time Unit	Cond
1	414V14006370	DC60756V-00	VAG	NON	1.00	EA				SAT

**A**

# 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	DC60756V-01	VAG	NON	SLD	GRS	1.00	EA	SAT	M
2	414V14006371	DC60756V-02	VAG	NON	SLD	GRS	1.00	EA	SAT	M

**B**

## **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-036/IPM 047. 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 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 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 Local Laboratory Results 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, 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.

- Light blue top tubes (additive = Na Citrate) are used for coagulation determinations. These tubes should be gently inverted at least 4 times after specimen collection to prevent clotting.

*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 Hematology (CBC with Diff and Platelets))

Testing will be performed per local standard of care.

- Tests performed for Chemistry
  - Liver Function:
    - Alanine transaminase (ALT),
    - Aspartate aminotransferase (AST).
- MTN-036/IPM 047 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. 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-036/IPM 047 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 for the collection and storage of plasma, which will be shipped to the MTN Virology Core for more testing.

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

#### 10.6.3.1 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 10.6.3.2 if SAMPLE 1 is Screening or Enrollment sample
  - Go to 10.6.3.3 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.

### 10.6.3.2 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.

### 10.6.3.3 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 for SAMPLE 1 at a follow-up visit, a second sample of blood (SAMPLE 2) will be drawn for the local clinical laboratory to perform a second confirmatory test.
- In addition to SAMPLE 2, blood tube for plasma is drawn for shipment to the MTN Virology Core.
  - Draw enough whole blood to store a total of 5-mL plasma to send to the MTN Virology Core. If less than 5-mL plasma is obtained, contact the MTN Virology Core for guidance.
- The processing of plasma for shipment to MTN Virology Core is similar to Plasma for Archive (SSP 10.6.5):
  - Centrifuge per specifications in SSP 10.6.5 and aliquot  $\geq 5$ -mL total plasma into 2-mL cryovials and freeze at  $\leq -70^{\circ}\text{C}$  until shipped to MTN Virology Core.
  - Log aliquots into LDMS using "Other Spec ID" = CON.
  - Alert the MTN Virology Core, 412-383-8138 or [mtnvirology@mtnstopshiv.org](mailto:mtnvirology@mtnstopshiv.org) when you are preparing the shipment.
    - The MTN Virology Core will confirm when to ship and provide shipping address and other details.
    - The MTN Virology Core may request that the enrollment plasma aliquots (Plasma Archive) for the same participant are included in the shipment of their CON plasma.
- 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.

### 10.6.4. Syphilis Testing

Serum is the specimen of choice for treponemal assays (EIA, MHA-TP, TPHA, TPPA, or FTA-ABS) and the non-treponemal VDRL assay. RPR tests may be performed on either serum or plasma. All testing must be done with FDA approved assays and by a CLIA certified laboratory.

Syphilis testing for MTN-036/ IPM 047 will be performed using the reverse sequence syphilis screening algorithm:

- At screening, syphilis assessment is done using a specific FDA approved treponemal test (such as EIA, MHA-TP, TPHA, TPPA, or FTA-ABS).
  - If negative, the participant is eligible for enrollment.
  - If positive, confirm with a non-treponemal assay (RPR or VDRL).
    - If the confirmatory non-treponemal assay is reactive at screening or enrollment visit, the participant is not eligible for the study.
    - If the confirmatory non-treponemal assay is negative, follow up with a second treponemal assay that has different antigens than the original treponemal assay. (See note below).
      - If the second assay is negative, the participant is considered eligible for the study.
      - If the second assay is positive, the participant is not eligible and appropriate clinical management should be taken. This scenario indicates that the participant has had prior exposure to syphilis and, depending on the clinical

scenario, may or may not require treatment. If needed, consult with the Protocol Safety Physicians.

- For enrolled participants that are being tested “as indicated” at a follow up visit:
  - Follow the same testing algorithm as for screening but if any test is positive consult with the Protocol Safety Physicians for guidance on clinical care and product hold.

**NOTE:** 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).

Questions related to result interpretation concerning eligibility and enrollment in the study should be directed to the MTN-036 Protocol Safety Physicians ([mtn036safetymd@mtnstopshiv.org](mailto:mtn036safetymd@mtnstopshiv.org)).

#### 10.6.5. MTN-036/IPM 047 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×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.

#### 10.6.6. Blood for PK of Dapivirine (DPV)

On single time-point days (visit days 1, 2, 3, 7, 14, 28, 56 and Final Contact), the rectal fluid swab (if collected on same day) and the vaginal PK swab are collected approximately within 30 minutes after the blood is drawn for PK. On multiple time-point days (Enrollment and 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 1500×g for 10 minutes. The centrifugation must be completed and sample placed in the freezer within 8 hours of blood collection.
3. Use a pipette to aliquot approximately 1.5 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 course of study if necessary and at its conclusion. All shipments will be on dry ice and can be initiated Monday through Wednesday to

ensure that samples arrive in the lab during the work week. 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  
4940 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-036/IPM 047 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

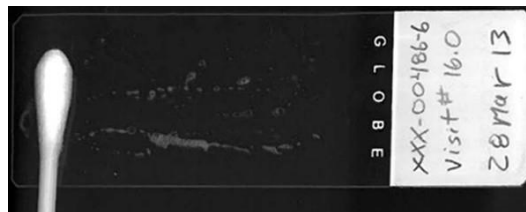
Refer to Pelvic Exam Checklist on the MTN-036 website under Study Implementation Materials for further information on the required sequence of specimen collection and diagnostic procedures to be performed during study pelvic exams.

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

Instructions for slide preparation and shipping are provided below:

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



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



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

#### 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 SHARP labels, 3 if using optional tube
- 2 frosted end slides
- Glass or plastic tube, optional
- Sterile physiologic saline
- 10% KOH
- Dacron Swab
- 2 cover slips
- Microscope, 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, 8, 9, and 10 and sent to the MTN LC the same day as collection via overnight FedEx. Shipping instructions follow.

- Use the Starplex Starswab Anaerobic collection and transporter kit. The kit comes with 2 sterile Dacron swabs and a glass transport tube.
- 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 and secure the cap tightly.
- The specimen may be kept at controlled room temperature for up to 4 hours. After four hours, the specimen must be refrigerated.
- Deliver the Starplex tube and the LDMS specimen tracking sheet to the local LDMS laboratory.
- Log the specimen into LDMS (Table 10-4) and label the Starplex tube with LDMS labels.
- Use LDMS to generate a shipping manifest (i.e. batch file) for the cultures to be shipped to lab 414.
- Ship the Starplex tube and the vaginal smear for Gram stain the same day of collection by overnight courier.
- 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.
- Use diagnostics packing code 650, UN3373 labels.

- 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 (**Do check Saturday delivery on the Fed Ex label**), 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 check 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, 8, 9, and 10. The swabs are stored at <-70°C and shipped to the LC at the end of the study.

- Label 2 cryovials with SCHARP labels.
- 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).
- Place the swabs in separate cryovials.
- Break or cut shaft of swab at a minimum of 1cm beyond the swab and cap the vial.
- Repeat with the second flocked swab as described above.
- Freeze within 2 hours of collection. They can be placed on dry ice at the clinic if transport to the lab is delayed. .
- Deliver the tubes and an LDMS Specimen Tracking Sheet to the local LDMS laboratory.
- 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.
- Freeze at  $\leq -70^{\circ}\text{C}$ .
- 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) by NAAT

Testing for chlamydia, gonorrhoea 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 for GC/CT testing.

- 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 you must use two collection kits, one for GC/CT and the other for *Trichomonas*. This is to ensure that sufficient sample volume is available in case repeat testing is

necessary.

- Use the manufacturer's vaginal collection swab and transport tube.
- Affix a SCHARP-provided PTID label onto the transport tube.
- Swab the lateral wall of the vagina.
- Immediately place the swab in the transport tube, break off the shaft of the swab, and cap the tube.
- Transport the specimen at ambient temperature to the local laboratory

#### 10.7.6. Cervicovaginal Fluid (CVF) Swabs 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, 2, and 4 HR collection times are determined by starting a timer upon VR insertion.
    - At 4 HR collect rectal swab prior to vaginal swab.
  - On Day 91,
    - 0 HR is collected before VR removal. Collect rectal swab for PK prior to vaginal swab for PK at this time.
    - The 1, 2, and 4 HR time points are 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, followed by rectal fluid swab sample (if applicable), 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 as close as possible to the blood draw, and no more than 30 minutes apart.
- 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 gauged.
- 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 2 HR PK blood would still be drawn at the 2 HR (120 minutes) 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-036/IPM 047 management team.

###### Follow-up Visits with single PK collection time point

- The blood is drawn first, and within 30 minutes, the rectal swab (if required) should be collected, followed by the vaginal swabs
- Days 28 and 56: Participants receiving the 25 mg VR, collect specimen prior to VR removal. For Day 28, collect rectal swab prior to vaginal swab for PK.

###### 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 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 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
  - Polyester-Tipped (Dacron) Swab
  - Ziplock biohazard sample bags
  - 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.
  - a. 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.
  - b. 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.
  - c. 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.
  - d. 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.
  - e. Place the cut shaft in the ziplock bag.
  - f. Screw the lid back on the cryovial and place sample in the bag with the swab packaging and the swab shaft.

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

#### **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 10.6.6.
- The back-up samples will be retained at the site until advised by the LC or MTN-036/IPM 047 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.

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

1. 10mL 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. At this point you may note the estimated volume of the CVL for logging in the number of supernatant aliquots and the cell pellet into LDMS to create labels for the cryovials. For the final aliquot, the cryovial will most likely have volume  $< 1\text{-mL}$ ; therefore, please store as a backup.
4. Centrifuge the 15-mL collection tube of CVL at  $800\times g$  for 10 minutes.
5. Add LDMS label to each cryovial. Without disturbing the cell pellet, pipet as many 1-mL aliquots of supernatant as possible into cryovials.
6. Re-spin the 15 mL conical tube containing cells for 10 minutes at  $800\times g$ .
7. Without disturbing the cell pellet, pull off additional supernatant and add to a supernatant vial.
8. Resuspend cell pellet in 0.5 mL normal saline and store in a cryovial with an already affixed LDMS label.
9. Freeze all supernatants and cell pellet at  $\leq -70^{\circ}\text{C}$  within 2 hours of collection.
10. If less than a total of 6 mL's (or less than 6 cryovials) of supernatant are recovered, contact the MTN LC.
11. Store one aliquot each into boxes labeled CVL for PK and CVL for PD, and the remaining aliquots in CVL for biomarker.
12. Store the pellet into a box labeled CVL pellet.
13. 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 10.6.6.

### 10.7.8. Testing of Vaginal Ring (VR) for Remnant Content Analysis

Used VRs will be analyzed for residual levels of Dapivirine and will be collected at day 28 and 56 (participants receiving the 25 mg VR), PUEV or early termination visit. The used VRs may contain vaginal secretions and therefore treated as a biohazard. The VRs will remain in the amber pouch and stored at -20°C until further notice from the MTN LC. VRs that are defective or inserted briefly and removed for various reasons may be destroyed at the site via biohazard procedures.

#### Important notes:

- Immediately before VR removal, 0-HR time point blood, rectal fluid and CVF swabs, CVL and cervical biopsies, when applicable, for PK testing 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 rinse and dry the VR and place it in a container that is stored at room temperature. At the clinic, the used VR is still prepared for residual drug analysis. After the used VR is taken out of the container that the participant used to return it, follow directions starting with step 1 of "Removal of VR by clinician".

#### Materials:

- A disposable container or a reusable container that was cleaned using 10% bleach solution for 20 minutes or sterilized.
- Tap water
- PPE: lab coat, gloves, face guard
- Paper towel or gauze
- 3"x5" amber Zippit pouch with affixed biohazard label
- SCHARP label for amber pouch

#### 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 in a clean container with tap water.
3. Move the VR around in the water or swirl the container to remove vaginal material.
4. Take the VR out of the water and blot dry with paper towels or gauze.
5. The VR should be dry before storing in pouch.
6. Dispose of blotting materials and contaminated water according to your institution biohazard policy.

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

1. Site staff will place the VR into a new 3"x5" amber Zippit pouch (see figure 10-4) that was provided by MTN LC to store the VRs.
2. Label the pouch with the participant ID number and visit number.
3. Add a biohazard sticker if one is not already attached to the pouch, making sure not to cover the identifier information.
4. Store the used VR within the biohazard labeled amber pouch at -20°C.
5. The use of LDMS is required to log in all used VRs.
6. At the end of the study, MTN LC will contact the sites to coordinate shipment to the company that will perform remnant VR analysis. Used vaginal VRs will be shipped at room temperature (per IPM) to the company.

**Figure 10-4: 3"x5" amber Zippit pouch**



## 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

Two biopsy samples will be collected for a tissue PK level at Visit 8 (day 28) and visit 10 (day 91).

1. Label two 2-mL cryovials (Nunc or Nalgene) with a SCHARP label and write appropriate sample and study identification information. Label cryovials Biopsy 1 & 2, with 1 always being the first biopsy extracted.
2. Weigh each labeled cryovial using an analytical scale with a sensitivity rating of 0.1 milligrams or better. Document pre-weight on the LDMS tracking sheet.
3. Directly transfer the biopsy to the designated pre-weighed cryovial.
4. Obtain the post-weight for each cryovial containing a biopsy using an analytical scale and document on the LDMS tracking sheet.
5. Calculate the net weight, which should be greater than zero.
6. 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.
7. Document the time when the cryovial containing the biopsy is frozen on the LDMS tracking sheet.
8. Store the labeled cryovials containing the frozen biopsies at  $\leq -70^{\circ}\text{C}$ .
9. On the LDMS tracking sheet, calculate the net weight by subtracting the pre-weight from the post-weight.
10. 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.

## 10.9. Rectal Fluid Swab for PK

Rectal swabs will be collected at Enrollment (4-hours post insertion), Days 3, 7, 14, 28, 91 (prior to VR removal) and Final Contact visits. 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
- Polyester-Tipped (Dacron) Swab
- Zip-lock biohazard sample bag
- Plastic cup (without lid) or similar lightweight container, placed on middle of scale, to contain items to be weighed. (Some balances have an optional basket.)
- Scissors to cut swab shaft

### Instructions

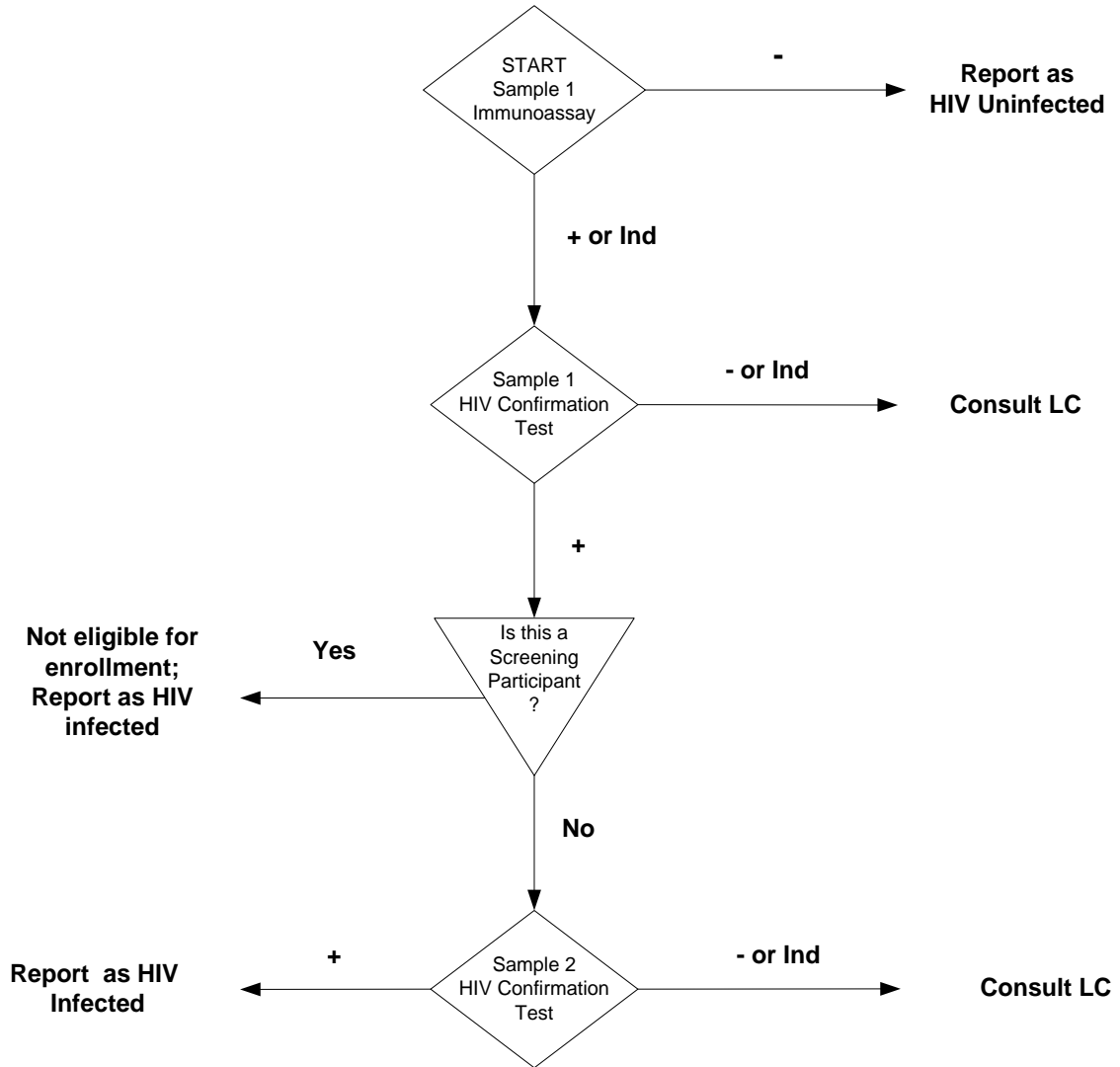
1. Place identically-labeled SCHARP labels on the cryovial and a biohazard sample bag.
2. Perform pre-weight. Handle items to be weighted with gloves.
  - a. Zero the cup or similar container on the scale.
  - b. Place the labeled 2-mL cryovial and packaged sterile Dacron swab upright in the cup. (Make 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. Place the swab in the cryovial and cut swab shaft using scissors at the pivot point. Be sure to hold onto the shaft to avoid losing it. Do **NOT** discard the shaft.
  - d. Place the cut shaft in the specimen bag.
  - e. Screw the lid back on the cryovial and place sample in the bag with the swab packaging and the swab shaft.
  - f. Document the collection time (the time the swab was removed from the rectum) on to the LDMS tracking sheet.
6. Perform Post Weight:
  - a. Zero the cup or similar lightweight container on the scale.
  - b. Weigh the capped cryovial containing the absorbed swab tip, the swab packaging and the remainder of the swab shaft (Suggestion: Place the swab shaft into the packaging and have it upright during weighing.)
  - c. Record post-weight on the LDMS Tracking sheet then calculate and record the NET weight.
8. Within 2 hours, place the sample tubes in the freezer at  $\leq -70^{\circ}\text{C}$ .
9. Log into LDMS (Table 10-3) and batch ship to JHU CPAL (LDMS Lab 194) upon request – see address listed in section 10.6.6.



Appendix 10-1: HIV ANTIBODY TESTING ALGORITHM



Ind: Indeterminate test results  
LC: Laboratory Center