

# Section 10. Laboratory Considerations

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## Table of Contents

<b>10.1. Overview and General Guidance</b> .....	<b>2</b>
<b>10.2. Specimen Labeling</b> .....	<b>6</b>
<b>10.3. Procedures for Specimens that cannot be evaluated</b> .....	<b>6</b>
<b>10.4. Use of LDMS</b> .....	<b>7</b>
10.4.1. Logging in PK Samples: .....	8
10.4.2. Entering weight measurements of vaginal swabs for PK in LDMS: For UAB, Pittsburgh, and Fenway sites only .....	8-9
10.4.3. LDMS Codes for specimen log in .....	10
<b>10.5. Testing for GC/CT (Neisseria gonorrhea and Chlamydia trachomatis) by NAAT</b> .....	<b>11</b>
10.5.1. Urine .....	11
10.5.2. Vaginal Swab (option if using Gen-Probe Aptima for Trichomonas testing) .....	11
<b>10.6. Urine Testing for Pregnancy and Urinary Tract Infection</b> .....	<b>11</b>
10.6.1. Specimen Collection .....	11
10.6.2. Pregnancy Testing.....	12
10.6.3. Urinary Tract Infection .....	12
<b>10.7. Blood Specimens for Chemistry, Hematology, HIV testing, Syphilis, Plasma Archive, Blood Dapivirine</b> .....	<b>12</b>
10.7.1. Specimen Collection and Initial Processing .....	12
10.7.2. Chemistry (Alanine transaminase, Aspartate aminotransferase, and Creatinine), and Hematology (CBC with Platelets).....	12
10.7.3. HIV Testing.....	13
10.7.4. Syphilis Testing.....	14
10.7.5. Plasma Archive.....	14
10.7.6. Blood for PK (Dapivirine) .....	15
<b>10.8. Cervicovaginal Lavage (CVL) for Biomarkers, Aliquot storage, and Cell Pellet</b> .....	<b>15</b>
10.8.1. Processing of the CVL Cell Pellet and Supernatant for biomarker and storage .....	15
10.8.2. CVL Biomarkers and Cell Pellet .....	16
<b>10.9. Vaginal Specimens for Herpes Lesions, Gram Stain, Microbiology Culture, Vaginal Fluid pH, Vaginal Wet Mount, Trichomonas, Swab for Biomarkers, Vaginal Secretions for PK, and IVR for PK</b> .....	<b>16</b>
10.9.1. Herpes Lesion Testing.....	16
10.9.2. Gram Stains of Vaginal Fluid .....	16
10.9.3. Microbiology: Vaginal Swab for Quantitative Culture .....	17
10.9.4. Vaginal Fluid pH .....	18
10.9.5. Vaginal Fluid Wet Mount Testing if indicated for BV and Yeast (KOH).....	19
10.9.6. Test for Trichomonas vaginalis .....	20
10.9.7. Vaginal Swab for Biomarkers .....	21
10.9.8. Vaginal Swabs for PK.....	21
<b>10.10. Testing of Intravaginal Ring (IVR)</b> .....	<b>23</b>
<b>Appendix 10-1: HIV ANTIBODY TESTING ALGORITHM</b> .....	<b>24</b>

## 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/>

Laboratory procedures may be performed in the study site clinics or laboratories, approved commercial laboratories and in the MTN Laboratory Center (MTN LC), including the MTN Pharmacology Core at Johns Hopkins University Clinical Pharmacology Analytical Laboratory (JHU CPAL). Table 10-1 is an overview of the specimens that are collected. Table 10-2 highlights storage and shipment requirements of specimens (including derivatives of initial specimens) that are stored. Table 10-3 lists the tests to be performed at each visit. Regardless of whether tests are performed in clinic or laboratory settings, study staff that performs the tests must be trained in proper, associated QC procedures prior to performing the tests for study purposes; training documentation should be available for inspection at any time.

Sites are responsible to ensure that specimen volumes do not exceed what is described in the informed consent process. The MTN LC may request details of collection containers and volumes for this purpose.

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

Ideally, one method, type of test kit, and/or combination of test kits will be used for each protocol specified test throughout the duration of the study. If for any reason a new or alternative method or kit must be used after study initiation, site laboratory staff must perform a validation study of the new method or test prior to changing methods. The MTN LC must be notified before the change and can provide further guidance on validation requirements.

Provided in the remainder of this section is information intended to standardize laboratory procedures across sites. Adherence to the specifications of this section is essential to ensure that primary and secondary endpoint data derived from laboratory testing will be considered acceptable to all regulatory authorities across study sites.

**Table 10-1  
Overview of Laboratory Testing Locations, Specimen Collection, and Methods for MTN-023/IPM 030**

<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>
Urine pregnancy test (hCG)	In clinic	Urine	Plastic screw top cup	Quidel Quick Vue, Fisher HealthCare Sure-Vue Urine hCG, Cardinal Health hcG Cassette Rapid Test, or OSOM Card Pregnancy Test
Urine Culture or Dipstick <sup>1</sup>	Local lab	Urine	Plastic screw top cup	Local methodology
Urine or vaginal NAAT for GC/CT <sup>2</sup>	Local lab	Urine or vaginal swab	Kit specific Transport tube	BD Probetec or Gen-Probe Aptima
CBC with Platelet	Local Lab	Whole Blood	EDTA 4-mL tube	Local methodology
Chemistries (AST, ALT, Creatinine)	Local Lab	Serum or Heparinized plasma	Plain or serum separator 4-mL	Local methodology
HIV antibody screen and confirmatory test	Clinic/Local Lab	Plasma, serum, or whole blood	EDTA or plain tube 4-mL or greater	FDA approved tests
Blood PK (Dapivirine)	JHU CPAL	Plasma	EDTA 5-mL or greater tube	JHU CPAL collection procedure
Syphilis Serology	Local Lab	Serum or Plasma	EDTA tube, plain or serum separator, 4-mL or greater	Local methodology
Plasma Archive or HIV Confirmation & Resistance Testing	Clinic/Local Lab/MTN Virology LC	Plasma	EDTA 4-mL or greater tube	MTN LC procedure
Cervicovaginal Lavage (CVL) for biomarkers & Cell Pellet	MTN LC	Fluid & pellet recovered from CVL	15-mL Conical Tube	MTN LC Procedure
Vaginal Swab(s) for biomarkers	MTN LC	Vaginal Swab	2.0-mL cryovial with 400-uL PBS	MTN LC procedure
Vaginal Swab for PK	JHU CPAL	Swab	2.0-mL Cryovial	JHU CPAL collection procedure
Trichomonas Rapid Test	Local lab or in clinic	Vaginal swab (supplied with kit)	OSOM: Sterile tube with no additives Aptima: kit swab and transport tube	OSOM kit or Aptima <sup>2</sup>
Vaginal pH	In clinic	Vaginal swab	N/A	S/P pH Indicator Strips
Quantitative Vaginal Culture	MTN LC	Vaginal swab	BD Port-a-Cul transport tubes	MTN LC procedure
Vaginal smear for Gram stain	MTN LC	Vaginal Swab	Slides	MTN LC procedure
Used Vaginal Ring (VR) for PK residual assessment	Parexel, South Africa	Used VR	Biohazard labeled amber bag	Parexel, South Africa lab procedure
Vaginal saline wet preparation (for BV and/or KOH wet mount) <sup>1</sup>	In clinic	Vaginal swab	tube with 6 drops of saline	MTN LC procedure
Herpes Lesion Testing <sup>1</sup>	Local lab	Local method	Local method	Local methodology

1. Perform only if clinically indicated per local SOP.

2. If Trichomonas is being tested with Gen-Probe Aptima, GC/CT Gen-Probe Aptima NAAT can also be performed using the same vaginal swab.

**Table 10-2: Overview of Specimens for Storage and Shipment**

Stored Specimens	Storage Tube Type or Size (recommended)	Processing	Ship to:	Shipping schedule
Plasma for archive or Confirmation of HIV Resistance Testing	2-mL cryovial	Pipet two 1-mL aliquots of plasma into tubes. Freeze at $\leq -70^{\circ}\text{C}$ within 24 hours after blood draw, if blood refrigerated, or within 4 hours if blood left at room temp	MTN LC	Store frozen at site until notified by MTN LC
Plasma for blood PK (Dapivirine)	2-mL cryovial	Pipet two $\geq 1$ -mL aliquots of plasma into tubes. Freeze at $\leq -70^{\circ}\text{C}$ within 8 hours after collection	JHU CPAL	Store frozen at site until conclusion of study
Cervicovaginal Lavage (CVL) supernatant: Biomarker	2-mL cryovial	Refrigerate CVL until processing. Aliquot 1-mL supernatant into one tube for CVL biomarkers and $\geq 5$ tubes labeled 'extra CVL'. Freeze at $\leq -70^{\circ}\text{C}$ within 8 hours of collection.	MTN LC	Store frozen at site until notified by MTN LC
CVL Cell Pellet	2-mL cryovial	Refrigerate CVL until processing. Add 0.5-mL normal saline to cell pellet. Freeze at $\leq -70^{\circ}\text{C}$ within 8 hours of collection	MTN LC	Store frozen at site until notified by MTN LC
Used Vaginal Ring for PK residual assessment	3"x5" amber Zippit pouch	Place VR in pouch	MTN LC	Room temp. storage at site until conclusion of study
Vaginal Swab(s) for biomarkers	2-mL cryovial	Keep refrigerated until frozen at $\leq -70^{\circ}\text{C}$ within 8 hours of collection	MTN LC	Store frozen at site until notified by MTN LC
Vaginal Swab for PK	2-mL cryovial	May be kept on ice until frozen. Freeze at $\leq -70^{\circ}\text{C}$ within 2 hours of collection	JHU CPAL	Store frozen at site until conclusion of study
Vaginal smear for Gram-stain	2 slides	Allow to air dry. Store at room temp,	MTN LC	Ship one slide with Port-a-cul to MTN LC. Store 2nd slide at site until conclusion of study
Vaginal Swabs for Quantitative Vaginal Culture	2 swabs in transport tube	Place swab in BD Max V or Port-a-Cul & break off shaft. Refrigerate until shipped.	MTN LC	Ship on ice packs to MTN LC for next day delivery. <sup>1</sup>

<sup>1</sup> If specimens are collected after the last FedEx pickup, store the Port-a-cul tube at  $4^{\circ}\text{C}$  and ship the following day. Check with your local FedEx office for last pickup times and the options for Saturday pickup or drop off locations.

**Table 10-3: Overview of Laboratory Tests by Visit for MTN-023/ IPM 030**

	SCR	ENR	2-Wk Visit	4-Wk Visit	8-Wk Visit	12-Wk Visit	16 Wk Visit	20 Wk Visit	24-Wk Final Clinic Visit/Early Termination Visit	1-Wk and 25- Wk Termination Phone Call
<b>LABORATORY</b>										
Urine hCG	X	X	X	X	X	X	X	X	X	
Urine or vaginal NAAT for GC/CT	X	*	*	*	*	*	*	*	X	
Dipstick UA and/or urine culture, per local standard of care	*	*	*	*	*	*	*	*	*	
Serum chemistries (4 mL) <sup>2</sup>	X	*	*	*	*	*	*	*	X	
CBC with platelets (4 mL) <sup>2</sup>	X	*	*	*	*	*	*	*	X	
HIV-1 serology (4 mL) <sup>2</sup>	X	X	*	*	*	X	*	*	X	
PK- Blood (Plasma) (5 mL) <sup>2</sup>			X	X	*	X	*	*	X	
PK-Vaginal fluid			X	X	*	X	*	*	X	
Syphilis serology (4 mL) <sup>2</sup>	X	*	*	*	*	*	*	*	*	
Plasma archive (4 mL) <sup>2</sup>		X	*+	*+	*+	*+	*+	*+	*+	
Gram stain		X		X		X			X	
Vaginal pH	*	X	*	X	*	X	*	*	X	
Vaginal swab for quantitative vaginal culture		X		X		X			X	
Vaginal swab for biomarkers		X		X		X			X	
CVL for biomarkers		X				X			X	
Vaginal swab for Rapid Trichomonas	X	*	*	*	*	*	*	*	X	
Vaginal swab for Trichomonas/GC/CT	X	*	*	*	*	*	*	*	X	
Saline wet mount for BV	*	*	*	*	*	*	*	*	*	
KOH wet mount for candidiasis	*	*	*	*	*	*	*	*	*	
Herpes lesion testing	*	*	*	*	*	*	*	*	*	
<b>STUDY PRODUCT</b>										
Collect study product			*	X	X	X	X	X	X	
Approximate Total volume of blood needed-may vary depending on local laboratory requirements. <sup>2</sup>	16 mL	20 mL	21 mL	21 mL	16 mL	21mL	16mL	16mL	21 mL	

X = required, \* = if indicated/needed, X- = sites to reference SOPs, +=Plasma for confirmation of HIV viral load and resistance testing.

<sup>2</sup> Maximum volume needed for study requirement, if all specimens are collected including “if clinically indicated”.

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

Microscope slides used for evaluation of vaginal fluids also will be labeled with PTID labels provided by SCHARP. PTIDs are pre-printed on these labels; however, study staff must write the specimen collection date on each label. The visit code also may be written on the label.

When specimens are tested at the local lab, any additional labeling required for on-site specimen management and chain of custody will be performed in accordance with site SOPs. Refer to Table 10-4 for specimens that will be entered into LDMS and labeled with LDMS-generated labels.

## 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.

In cases where additional specimens need to be recollected either due to a laboratory error (lost, broken tube, clerical, etc.) or clinic error, a protocol deviation form may be required.

The 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 compromised specimen integrity
- Any situation that may indicate a protocol deviation

If site staff has any question 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 used for the storage and shipping of laboratory specimens. LDMS must be used at all sites to track the collection, storage, and shipment of the sample types described in Table 10-4. An LDMS tracking sheet listing the sample types in Table 10-4 is initiated by the clinical staff at time of sample collections and is transported with the primary samples to the LDMS laboratory. The LDMS laboratory logs the specimens into LDMS, which formats appropriate labels for sample derivatives. It is crucial to be aware of proper label formats to ensure that specimens are correctly labeled.

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).

Each site will be required to:

- Maintain the current version of LDMS
- Monitor updates relating to the use of LDMS
- Back up their LDMS data (frequency determined by site) locally
- Export their data to FSTRF at least on a weekly basis.

Exported data are used by the MTN Statistical Data Management Center (SDMC) to:

- Generate a monthly specimen repository report
- Reconcile data entered in LDMS with data entered on study case report forms (CRFs)
- Create 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 time frame and for following up with sites that do not resolve discrepancies within two weeks.

The MTN SDMC reviews the discrepancy reports for critical samples (e.g., plasma needed for confirmatory HIV testing) that appear to be missing, and works with the 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.

Questions related to use of LDMS in MTN-023/IPM 030 may be directed to Lorna Rabe or LDMS Technical (User) Support. Usual business hours for LDMS User Support are 7:00 am - 6:00 pm (ET) from Monday through Friday. All other hours and weekends, an on-call LDMS User Support specialist will be available:

Email: [ldmshelp@fstrf.org](mailto:ldmshelp@fstrf.org)  
Phone: +716-834-0900, ext 7311  
Fax: +716-898-7711

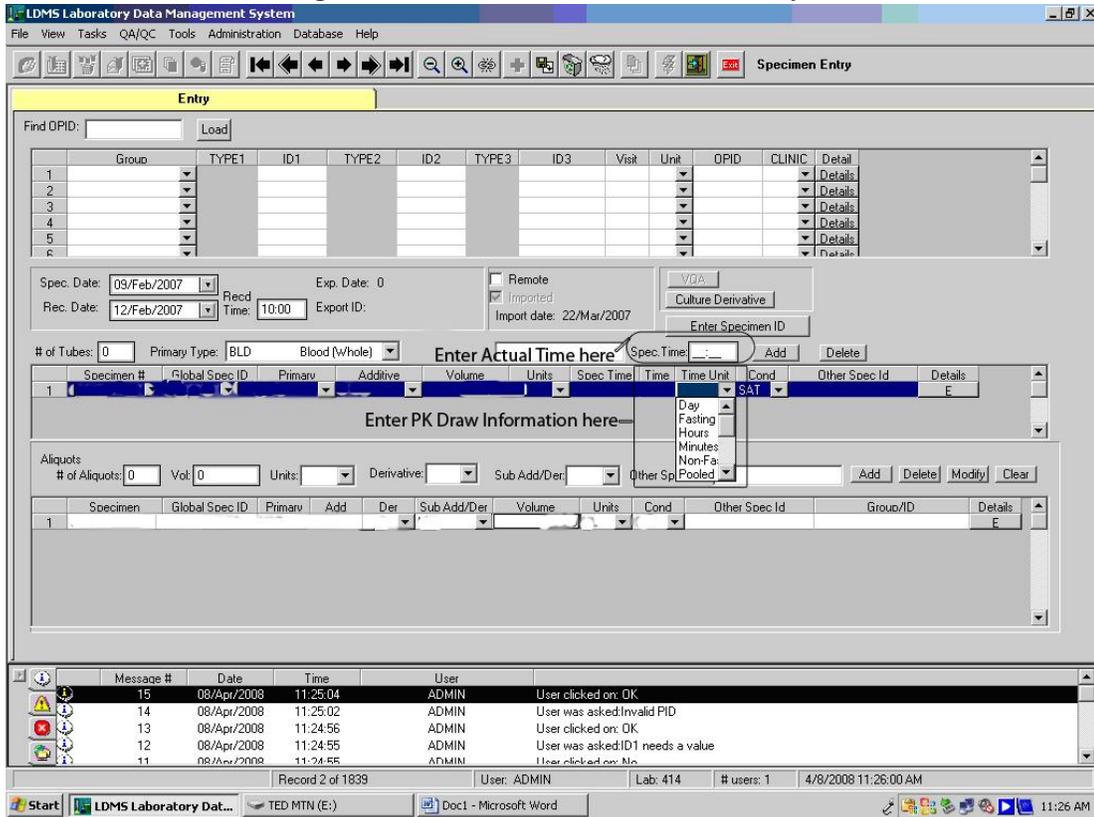
##### Off-Hours Contact Information:

If you are locked out of your LDMS or are experiencing errors that prevent you from completing your LDMS lab work during off-hours, page LDMS User Support using the LDMS Web Pager utility. Alternatively, you may e-mail the paging system directly: [ldmspager1@fstrf.org](mailto:ldmspager1@fstrf.org). Please allow at least 15 minutes to get a response before sending another e-mail to the paging system.

### 10.4.1. Logging in PK Samples:

- Enter the actual time in the Specimen Time area (See Figure 10-1).
- Enter the PK time point information in Time and Time Unit area (See Figure 10-1).

Figure 10-1: LDMS Screen for Time Entry



### 10.4.2. Entering weight measurements of vaginal swabs for PK in LDMS: For UAB, Pittsburgh, and Fenway sites only

The volume field in LDMS can be used for displaying weight measurements with proper units. Once the net-weight is attained by subtracting the pre-weight from the post-weight, the result can be entered into LDMS as shown in figures 10-2 and 10-3.

- In the primary sample field (section A), enter the sample(s) information. Make sure to place the correct draw 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 as in Section B for the primary sample. In the "# of Aliquots" field, enter 1. For "Volume", enter the net-weight and change the "Units" to MG (milligrams). Enter the correct derivative and Sub-Add/Der, and click the add button (See Figure 10-2).

Example: Pre-weight Swab: 1972.1 mg, Post weight Swab: 2097.4 mg, Net weight of Swab is 125.3 mg (2097.4 - 1972.1 = 125.3). Place the 125.3 under "Volume" and change "Units" to MG, the "Derivative" to SWB, change the Sub-Add/Der to N/A, and press add. The finished aliquot should look like Figure 10-3.

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

**A**

Group	TYPE1	ID1	TYPE2	ID2	TYPE3	ID3	Visit	Unit	OPID	CLINIC	Detail
1 Practice Group	PD	1234567	PROTOCOL		ID3						Details
2											Details
3											Details
4											Details
5											Details
6											Details

Spec. Date: 23/Dec/2013 Exp. Date:  Remote  
 Rec. Date: 23/Dec/2013 Recd. Time: 08:00 Export ID:  Imported  
 VQA Culture Derivative Enter Specimen ID  
 # of Tubes:  Primary Type: VGL Other Spec ID:  Spec. Time: 07:31 Add Delete

Specimen #	Global Spec ID	Primary	Additive	Volume	Units	Spec Time	Time	Time Unit	Cond	Other Spec Id	Details
1 414/1305682		VGL	NON	1.00	EA	07:31			SAT		DB

**B**

# of Aliquots:  Vol: 125.3 Units: MG Derivative: SWB Sub Add/Der: N/A Other Spec ID:  Add Delete Modify Clear

Specimen	Global Spec ID	Primary	Add	Der	Sub Add/Der	Volume	Units	Cond	Other Spec Id	Group ID	Details
1 414/1305684		VGL	NON	SWB	N/A	125.30	MG	SAT		Practice Group/1234567	DB

**Figure 10-3: LDMS Screen for Weight Entry Example in 10.4.2**

**A**

Group	TYPE1	ID1	TYPE2	ID2	TYPE3	ID3	Visit	Unit	OPID	CLINIC	Detail
1 Practice Group	PD	1234567	PROTOCOL		ID3						Details
2											Details
3											Details
4											Details
5											Details
6											Details

Spec. Date: 23/Dec/2013 Exp. Date:  Remote  
 Rec. Date: 23/Dec/2013 Recd. Time: 08:00 Export ID:  Imported  
 VQA Culture Derivative Enter Specimen ID  
 # of Tubes:  Primary Type: VGL Other Spec ID:  Spec. Time: 07:31 Add Delete

Specimen #	Global Spec ID	Primary	Additive	Volume	Units	Spec Time	Time	Time Unit	Cond	Other Spec Id	Details
1 414/1305682		VGL	NON	1.00	EA	07:31			SAT		DB

**B**

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

Specimen	Global Spec ID	Primary	Add	Der	Sub Add/Der	Volume	Units	Cond	Other Spec Id	Group ID	Details
1 414/1305684		VGL	NON	SWB	N/A	125.30	MG	SAT		Practice Group/1234567	DB

### 10.4.3. LDMS Codes for specimen log in

The table 10-4 should be used as a guide when logging in MTN-023/IPM 030 specimens into LDMS. When logging in specimens for each sample type listed, please use the LDMS codes listed below:

BLD: Whole Blood	IVR: Used Intravaginal Ring	PBS: Phosphate buffered saline
CEN: Cell Pellet	N/A: Not Applicable	PL1/2: Single or double spun plasma
GRS: Gram stain slide	NON: No Additive	SWB: Swab
EDT: EDTA	NSL: Normal Saline	VGL: Vagina
FLD: Fluid Supernatant	PAC: Port-a-Cul	CVL: Cervicovaginal lavage

Tracking sheets can be found in the Study Implementation Materials section on the MTN-023/IPM 030 webpage.

**Table 10-4 LDMS Specimen Management Guide to Logging in MTN-023/ IPM 030 Specimens**

<b>SAMPLE TYPE</b>	<b>PRIMARY SPECIMEN</b>	<b>PRIMARY ADDITIVE</b>	<b>ALIQUOT DERIVATIVE</b>	<b>ALIQUOT SUB ADDITIVE/ DERIVATIVE</b>	<b>Aliquot volume</b>	<b>Units</b>
Plasma for Storage (archive) or HIV confirmation	<b>BLD</b>	<b>EDT</b>	<b>PL1 or PL 2</b>	<b>N/A</b>	1.0-mL	mL
Plasma for PK (Dapivirine)	<b>BLD</b>	<b>EDT</b>	<b>PL 1 or PL 2</b>	<b>N/A</b>	>1.0-mL in 2-mL cryovial	mL
Cervicovaginal Lavage (CVL) supernatant	<b>CVL</b>	<b>NSL</b>	<b>FLD</b>	<b>N/A</b>	1-mL in 2-mL cryovial	mL
Cervicovaginal Lavage (CVL)Cell Pellet	<b>CVL</b>	<b>NSL</b>	<b>CEN</b>	<b>NSL</b>	1-mL in 2-mL cryovial	mL
Vaginal Swab(s) for biomarkers	<b>VGL</b>	<b>PBS (400-uL PBS)</b>	<b>SWB</b>	<b>N/A</b>	1 swab in 2-mL cryovial	Each
Vaginal Smear for Gram Stain	<b>VGL</b>	<b>NON</b>	<b>SLD</b>	<b>GRS</b>	2 smears	Each
Vaginal Swab for Quantitative Culture	<b>VGL</b>	<b>PAC</b>	<b>SWB</b>	<b>N/A</b>	2 swabs in 1 Max V transporter	Each
Vaginal PK Swab	<b>VGL</b>	<b>NON</b>	<b>SWB</b>	<b>N/A</b>	1 swab	Each or MG
Used Vaginal Ring for PK residual assessment	<b>IVR</b>	<b>NON</b>	<b>IVR</b>	<b>NA</b>	1 pouch	Each

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

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

### **10.5.1. Urine**

#### **10.5.1.1 Specimen Collection**

- The participant should not have urinated within one hour prior to urine collection.
- Provide the participant with a sterile, plastic, preservative-free screw-top urine collection cup labeled with a SCHARP-provided PTID label.
- Instruct the participant not to clean the labia prior to specimen collection.
- Collect the first 20-30 mL of voided urine (not midstream urine) in a sterile collection cup if testing for GC/CT, pregnancy, or urinalysis dipstick.
  - Note: If a culture is required, then also collect midstream urine.
- Instruct the participant to screw the lid tightly onto the cup after collection.

#### **10.5.1.2 Urine Processing for Testing**

The laboratory that is performing the test must provide the clinic with the appropriate transport tube for the test being performed: ProbeTec or Gen-Probe.

- Open the kit and label the transport tube with the participants PTID number and date.
- Hold the transport tube upright and firmly tap the bottom of the tube on a flat surface to dislodge any large drops from inside the cap.
- Use the transfer pipet provided in the kit to fill the transport tube with urine to the level indicated by the black lines on the tube. Do not under fill or overfill the tube.
- Transport to the local laboratory according to the specific manufacturers recommendations.
- Testing will be done at the local laboratories according to the site SOP.

### **10.5.2 Vaginal Swab (option if using Gen-Probe Aptima for Trichomonas testing)**

#### **10.5.2.1 Specimen Collection**

- Affix SCHARP-provided label on tube from GenProbe Aptima vaginal sample collection kit. Collect the specimen for GC/CT testing by rotating the swab from the Aptima collection kit several times over the lateral wall of the vagina. Insert swab into foil top GenProbe Aptima tube. Store at room temperature until testing.
- Transport to the local laboratory according to the specific manufacturers recommendations.
- Testing will be done at the local laboratories according to the site SOP.

## **10.6 Urine Testing for Pregnancy and Urinary Tract Infection**

### **10.6.1 Specimen Collection**

- The participant should not have urinated within one hour prior to urine collection.
- Provide the participant with a sterile, plastic, preservative-free screw-top urine collection cup labeled with a SCHARP-provided PTID label.
- Instruct the participant not to clean the labia prior to specimen collection.
- Collect the first 20-30 mL of voided urine (not midstream urine) in a sterile collection cup if testing for GC/CT, pregnancy, or urinalysis dipstick.

- Note: If a culture is required, then also collect midstream urine.
- Instruct the participant to screw the lid tightly onto the cup after collection.

## 10.6.2 Pregnancy Testing

The Quidel QuickVue One-Step hCG urine, Quidel QuickVue Combo hCG urine/serum, Fisher HealthCare Sure-Vue Urine hCG, Cardinal Health hCG Cassette Rapid test, or OSOM Card Pregnancy test must be used at all sites. Perform the test according to site SOPs and the package insert. Do not perform any other urine pregnancy tests for confirmatory purposes.

Pregnancy status is a critical participant safety consideration in MTN-023/IPM 030. 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.

If the urine pregnancy test cannot adequately be interpreted because of interfering factors, for example excess blood or extreme cloudiness due to amorphous material, the sample can be spun down and the urine supernatant can be used. If the test continues to have interferences such as gross hemolysis making the test difficult to read, then another urine sample will need to be collected.

In the rare event a participant becomes pregnant, refer to section 7.5.2 of the SSP.

## 10.6.3 Urinary Tract Infection

Urine Dipstick and/or Culture: Perform only if indicated and by local standard of care. Instruct participant to collect midstream urine. However, the first 20-30 mL of voided urine must also be collected if GC/CT testing is needed.

## 10.7 Blood Specimens for Chemistry, Hematology, HIV testing, Syphilis, Plasma Archive, Blood Dapivirine

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.

### 10.7.1 Specimen Collection and Initial Processing

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

- Allow plain tubes (no additive or serum separator tubes) to clot, then centrifuge per site SOPs to yield serum for syphilis and/or HIV testing.
- EDTA tubes should be gently inverted at least eight times after specimen collection to prevent clotting. EDTA tubes are used for hematology, HIV testing, Dapivirine quantification, and plasma archive. If whole blood for hematology testing and plasma is to be taken from the same tube, hematological tests must be completed before the tube is centrifuged and aliquoted. If whole blood is to be used for multiple tests, ensure that the tube is well mixed before removing any specimen.

*Note: If locally available tube top colors do not correspond with the tube additives specified above, use appropriate tubes based on the additives, not the listed tube top colors.*

### 10.7.2 Chemistry (Alanine transaminase, Aspartate aminotransferase, and Creatinine), and Hematology (CBC with Platelets)

Testing will be performed per the local standard of care. Tests performed for Chemistry are: Alanine transaminase (ALT), Aspartate aminotransferase (AST), and Creatinine. Hematology tests are: Hemoglobin, Hematocrit, Platelets, MCV, and White blood cell count (WBC).

### 10.7.3 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-023/IPM 030 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 is considered Sample 1. If Sample 1 is HIV positive by the confirmatory test a second specimen (Sample 2) is drawn and sent to the MTN Virology LC for confirmation.

HIV test result interpretation is as follows:

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

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

- If the confirmatory test is negative, indeterminate or invalid, contact the virology LC for guidance at [mtnvirology@mtnstopshiv.org](mailto:mtnvirology@mtnstopshiv.org). It is not recommended for participants with discrepant HIV testing results to continue enrollment into MTN-023/ IPM 030.
- If the confirmatory test is positive for the screening visit, the participant is considered seropositive and is not eligible for enrollment.

#### **Follow-Up Participants**

- If the confirmatory test on sample 1 is negative, indeterminate or invalid, contact the virology LC for guidance at [mtnvirology@mtnstopshiv.org](mailto:mtnvirology@mtnstopshiv.org).
- If the confirmatory test is positive at a follow-up visit, a second specimen (Sample 2) will be drawn for additional confirmatory testing (HIV RNA and resistance testing) at the MTN Virology LC.
  - Draw enough whole blood to store a total of 5 mL of plasma to send to the virology core. The virology core can work with less, but 5 mL is the desired amount to complete all testing.
  - **NOTE: Draw extra blood with Sample 2, if required for local standard of care or at discretion of clinician. This blood is sent directly to a local lab following their procedures.**
- Processing of SAMPLE 2 is similar to Plasma for Archive:
  - Log into LDMS, but with special ID = CON.
  - Centrifuge at 1500xg and aliquot 1- 1.5-mL plasma into 2-mL cryovials and freeze at  $\leq -70^{\circ}\text{C}$ .
- Alert the MTN Virology Core, 412-383-8138, about shipment.
- Package and ship 3 aliquots immediately on dry ice to:

Dr. Urvi Parikh  
University of Pittsburgh  
3550 Terrace St.  
Scaife Hall S804  
Pittsburgh, PA 15261
- MTN Virology Core will provide test results to the site.
  - If positive, the participant is HIV positive.

- If negative, indeterminate or invalid, the MTN Virology Core will supply guidance.

#### 10.7.4 Syphilis Testing

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

1. Rapid plasma reagin (RPR) screening test followed by a confirmatory test for *Treponema pallidum*. Any FDA approved *Treponema pallidum* confirmatory test can be used, such as the Enzyme Immunoassay (EIA), microhemagglutinin assay for *Treponema pallidum* (MHA-TP), *Treponema pallidum* hemagglutination assay (TPHA), *Treponema pallidum* particle agglutination (TPPA), or fluorescent treponemal antibody (FTA-ABS). All positive RPR results must have a titer reported. For reactive RPR tests observed during screening, a confirmatory test is performed and appropriate clinical management action must be taken prior to enrollment in the study. Enrolled participants considered positive should include repeat non-treponemal assay tests at quarterly intervals following syphilis diagnosis to evaluate treatment effectiveness. If the RPR or VDRL titer does not decrease four-fold or revert to seronegative within three months after treatment, further investigation and/or treatment may be warranted.
2. Perform syphilis assessment using a specific FDA approved treponemal test (such as EIA, MHA-TP, TPHA, TPPA, or FTA-ABS) and confirming positive test results with a non-treponemal assay (RPR or VDRL). If the confirmatory non-treponemal assay is reactive at screening visit, appropriate clinical management action must be taken prior to enrollment in the study. If the RPR or VDRL is negative, this may indicate prior treatment, late latent disease, or a false positive test. MTN LC recommends additional testing using an alternative treponemal test other than the original treponemal test used for the original assessment so the participant can be correctly evaluated. (Of note, the FTA-ABS should not be used as the alternative confirmatory test due to performance issues). If the second confirmatory test is negative, the participant is not considered infected with syphilis. If the second confirmatory test is positive, the participant has had prior exposure to syphilis and depending on clinical scenario may or may not require treatment.

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

Questions related to result interpretation concerning eligibility and enrollment in the study should be directed to the MTN-023 Protocol Safety Physicians ([mtn023safetymd@mtnstopshiv.org](mailto:mtn023safetymd@mtnstopshiv.org)).

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

#### 10.7.5 Plasma Archive

For plasma archive, affix SCHARP label on the 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 1.0-mL aliquots.
- 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 1500xg (relative centrifugal force in 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 two 1.0-mL aliquots that will be stored in consecutive locations in 'Plasma Archive' storage box.
  - If total volume is less than 0.5-mL, redraw as soon as possible.
  - If less than 1-mL of plasma is available, store the plasma and contact the MTN LC for instruction.
  - If samples are hemolyzed, store the aliquots as per normal and enter comments in LDMS.
  - If plasma is for HIV confirmation and resistance testing store at least three vials with at least 1.0mL each.
- The MTN LC will send instructions to the site when shipping and/or testing is required.

### 10.7.6 Blood for PK (Dapivirine)

Affix SCHARP label to a 5-mL or greater EDTA Vacutainer tube. Collect blood 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. Pipette ≥1.0-mL aliquots of the resulting plasma into two or more cryovials. One of these will serve as the primary sample; the others will serve as a back-up in case the primary samples are accidentally destroyed during shipment.
4. Prepare two storage boxes for Plasma PK and label one as "primary samples" and the other as "back-up samples". Transfer the tubes from each participant in chronological order into the storage boxes. All aliquots are labeled and tracked using LDMS.
5. Store the samples at ≤-70°C until shipped to MTN LC Pharmacology Core JHU CPAL.
6. Prior to shipping, prepare a shipment box (a foam chest) filled with dry ice sufficient for a 48 hour period with an appropriate shipping label.
7. Primary samples will be shipped to the MTN LC Pharmacology Core JHU CPAL and assayed for Dapivirine at conclusion of study unless informed otherwise. The back-up samples will be retained at the site until advised by the MTN-023/IPM 030 leadership group.

#### The shipping address for PK samples:

James Johnson  
 Johns Hopkins University  
 Division of Clinical Pharmacology  
 600 N. Wolfe Street, Osler 523  
 Baltimore, MD 21287

Lab Phone#: (410) 955-9710 or (410) 614-9978  
 Email: [jjohnso6@jhmi.edu](mailto:jjohnso6@jhmi.edu)

### 10.8. Cervicovaginal Lavage (CVL) for Biomarkers, Aliquot storage, and Cell Pellet

CVL aliquots will be collected, processed, and used for testing of biomarkers, supernatant storage, and preservation of the cell pellet. See SSP Section 7.6.3 for CVL collection procedures.

#### 10.8.1 Processing of the CVL Cell Pellet and Supernatant for biomarker and storage

The CVL aliquots and the cell pellet are labeled and tracked using LDMS.

1. CVL specimens are kept on wet ice or refrigerated and should be processed within 8 hours

of collection.

2. All the CVL liquid will be spun at 800×g for 10 minutes in the 15-mL conical collection tube.
3. Remove supernatant from the cell pellet in as many 1-mL aliquots as possible into 2-mL cryovials, assuring there are at least 1 aliquot for biomarker testing and a minimum of 5 back-up aliquots marked as 'Extra CVL'.
4. Re-spin the 15-mL conical tube containing cells for 10 minutes at 800×g.
5. Pull off and save any additional supernatant making sure not to remove any cells or debris.
6. Re-suspend the cell pellet in 0.5-mL normal saline in a cryovial.
7. Freeze all CVL aliquots and cell pellet at  $\leq -70^{\circ}\text{C}$  within 8 hours of collection and track in LDMS.
8. Prepare three sets of CVL storage boxes for shipment.
  - The one aliquot for biomarker testing is placed in a 'CVL for Biomarker' box. This set of boxes will be the first shipment of CVL's that will be requested.
  - The remaining supernatant aliquots are stored in consecutive locations, (i.e. all participant's 'Extra CVL' aliquots stored together) in another set of boxes for 'Extra CVL'.
  - The third set of boxes stores the CVL cell pellet.
9. The MTN LC will send instructions to the site when shipping is required.

#### 10.8.2 Shipping of CVL Biomarkers and Cell Pellet

CVL supernatant aliquots for biomarkers and CVL cell pellet samples will be batched and shipped on dry ice to MTN LC at end of study to:

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

#### 10.9 Vaginal Specimens for Herpes Lesions, Gram Stain, Microbiology Culture, Vaginal Fluid pH, Vaginal Wet Mount, Trichomonas, Swab for Biomarkers, Vaginal Secretions for PK, and IVR for PK

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

##### 10.9.1 Herpes Lesion Testing

Testing will be performed per the local standard of care.

##### 10.9.2 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 labeled and logged using LDMS. The primary slide will be shipped to the MTN LC with the Max V or Port-a-Cul. The secondary slide will be archived on site until written notification that the slide may be discarded is received from the Statistical Center for HIV/AIDS Research & Prevention (SCHARP).

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 = VGL) and label the slides with LDMS labels. Place the LDMS label on the frosted end of the slide on top of the pencil markings (same side as sample).



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

### 10.9.3 Microbiology: Vaginal Swab for Quantitative Culture

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

- Collect the specimen for culture by rotating two Dacron swabs several times over the lateral wall of the vagina. Do not collect culture swabs in the exact same area 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). If using the BD Max V transporter, insert the two swabs attached to the cap into the tube. If using the Port-a-cul transporter, insert swabs into one Port-A-Cul transport tube (labeled with a SCHARP label), submerging the swabs into the gel and breaking off the shafts of the swabs, and capping. (The BD Max V or Port-A-Cul transport tubes will be provided by MTN LC.)

- The specimen may be kept at controlled room temperature for up to 4 hours. It must be refrigerated after that and shipped with ice packs.
- Deliver the Max V or Port-A-Cul and the LDMS specimen tracking sheet to the local LDMS laboratory.
- Using the LDMS Tracking Sheet, log the slides into LDMS (specimen type = VGL) and label the Max V or Port-A-Cul tube with LDMS labels.
- Use LDMS to generate a shipping manifest for the cultures to be shipped.
- Shipping schedules:
  - Monday through Thursday, on the day of collection, for all samples collected prior to the FedEx pickup deadline, prepare shipment of the Max V or Port-A-Cul tube and the vaginal smear for gram stain for standard overnight next day delivery. If the samples are collected after the FedEx pickup deadline, refrigerate the Max V or Port-a-cul tube and include in the shipment for the following day.
  - All samples collected on Friday should be in a package marked for Saturday delivery.
    - If a site plans to have weekend clinic hours they should contact their FedEx office for drop off locations and hours on Saturday for Monday delivery.
    - Specimens collected on a Sunday can be refrigerated until shipped on Monday for Tuesday delivery.
- Place the Max V or Port-A-Cul in a biohazard bag and secure in the leak-proof container with absorbent material. Place the container, ice packs, slides, and a copy of the manifest in a cardboard box lined with Styrofoam.
- Use diagnostics packing code 650, UN3373.
- Confirm the address is correct (see below). Because the Research Institute is not open for delivery on the weekend, the specimens shipped on Friday must be sent to the *hospital* address for delivery on Saturday.

#### Shipping instructions to MTN LC:

If sending Monday through Thursday, send to the Institute:

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

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

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

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

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

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

#### 10.9.4 Vaginal Fluid pH

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

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

1. Obtained by the clinician during the pelvic examination
2. Collected by the clinician in a non-speculum exam

Note: a speculum is not required for pH sample collection.

Vaginal Fluid pH Procedure:

1. Swab onto the pH strip (Do not insert the pH strip into the vagina).
2. Match the resulting color of the indicator strip to the color scale provided with the strips to determine the pH value.
3. Record the pH value directly onto the appropriate case report form. It is not necessary to record pH values onto laboratory log sheets or other source documents prior to recording values onto case report forms.

### 10.9.5 Vaginal Fluid Wet Mount Testing, if indicated for bacterial vaginosis (BV) and KOH preparation for Yeast

Wet mount procedures for this study are only performed if indicated, and consists of two different preparations:

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

These procedures are for diagnosis of BV and candidiasis as summarized in Table 10-5 below.

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

The MTN LC requires all wet mount readers are assessed by the LC for competency of the wet mount tests; therefore, the MTN LC will administer a web-based proficiency test approximately every six months. The MTN LC will post wet mount slides on the MTN web pages for this purpose every 6 months; results will be entered directly on the website (contact: Lorna Rabe: lrabe@mwri.magee.edu). The MTN LC will report results back to the Laboratory Manager and also specify any corrective action that may be needed based on the results. Contact the MTN LC for additional information and guidance on performing and documenting the proficiency testing. Also contact the MTN LC when new laboratory staff is hired, so that appropriate training can take place prior to such staff performing wet mounts for study purposes.

**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)

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

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

- Use a pencil to write the PTID and specimen collection date on one side of the frosted end of two microscope slides. Affix a SCHARP-provided PTID label to the other side of the slides (on the frosted end, under the pencil markings) and write the specimen collection date in indelible ink (e.g. Sharpie pen) on each label.
- Immediately following collection from the lateral vaginal wall via swab (Dacron or cotton), smear vaginal fluid specimens onto each slide. Alternatively, the swab may be placed in a glass or plastic tube with approximately six drops (100- $\mu$ L) sterile physiologic saline to allow for non-immediate slide preparation. In this case, vaginal fluid specimens should be smeared onto the two slides upon receipt from the collecting clinician.
- Apply one drop of 10% KOH to one slide and immediately perform whiff test for a "fishy" amine odor. Then apply cover slip. Examine the KOH slide at both 100X and 400X magnification for yeast and pseudohyphae.
- 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.

#### **10.9.6 Test for *Trichomonas vaginalis***

Testing for *Trichomonas* can be done with either the OSOM Rapid *Trichomonas* test (manufactured by Sekisui Diagnostics) or the Gen-Probe Aptima. If using the Aptima test, GC/CT can be tested from the same swab, thus eliminating the need for a urine specimen for GC/CT.

##### **10.9.6.1 OSOM Rapid *Trichomonas* test**

- Use the rayon swab provided with the kit for collection
- Affix a SCHARP-provided PTID label to a clean glass or plastic tube with a cap.
- Collect specimen using kit-provided swab from the lateral vaginal wall (fluids also may be collected from the posterior fornix; avoid collecting specimens from the cervix).
- Immediately place the swab in the labeled tube, break off the shaft of the swab, and cap the tube.
- Testing is expected to be performed during the participant visit. However, specimens may be stored at room temperature for 24 hours or refrigerated for 36 hours before testing.

##### **10.9.6.2 Gen-Probe Aptima NAAT (This same sample can be used for GC/CT testing)**

- Use the Gen-Probe 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

### 10.9.7 Vaginal Swab for Biomarkers

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

Procedure:

- Affix a SCHARP-provided PTID label to the provided 2-mL cryovial containing 400- $\mu$ L PBS (1X Concentration)
  - Label should indicate vaginal biomarker or VGL PBS to distinguish this specimen from other vaginal specimens.
- Collect vaginal fluid using a Dacron swab from the posterior fornix.
- Place the swab into the 2-mL cryovial containing 400- $\mu$ L PBS, break off swab shaft, and cap the vial.
- Immediately refrigerate or place vial on ice and freeze at  $\leq -70^{\circ}\text{C}$  within 8 hours of collecting the sample collection.
- Mark this sample on the LDMS tracking sheet and transport to the LDMS laboratory.
- The sample will be labeled and tracked using LDMS.
- Batch ship on dry ice to MTN LC at end of study:

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

### 10.9.8 Vaginal Swabs for PK

Vaginal fluid for PK will be collected at all sites; however, Pittsburgh, UAB and Fenway sites will weigh the swabs before and after collection. The Memphis, Colorado and Bronx sites will not weigh the swabs.

#### 10.9.8.1 Procedure for Vaginal Fluid Sampling for PK assessment and weighing swab (UAB, Pittsburgh, and Fenway sites only):

Materials:

2-mL Nalgene cryovials containing pre-cut Polyester-Tipped (Dacron) Swab  
 Hemostat or Ring Forceps (recommend 8 inches or longer)  
 Analytical scale (accurate to 0.1 milligrams)

1. PK swabs must be collected within one hour of PK blood draw.
2. Ensure that new, clean, or sterilized supplies (gloves, hemostat, swabs, tubes, etc.) are used for each sample, as Dapivirine is very sensitive to cross-contamination.
3. Before starting procedures, write participant identification information on a SCHARP label and affix the label to the cryovial containing the pre-cut swab.
4. Perform QC that would be required for the analytical scale to accurately weigh samples to a weight of at least 0.1 milligrams.
5. Perform Pre-Weight measurement by weighing the capped cryovial with pre-cut swab.
6. Record this pre-weight on the LDMS Tracking Sheet.
7. Uncap the pre-weighed cryovial. Put on new gloves and use a clean hemostat to clamp on to the exposed shaft of the swab to collect vaginal fluid. Use a clean gloved hand

to assist, if needed.

8. Insert the hemostat holding the swab into the upper vagina near the cervix to the location nearest to where the ring was residing. For 10-20 seconds, rotate in a circular motion touching all walls to absorb as much fluid as possible.
9. Immediately place swab tip into the cryovial after sampling and recap.
10. Perform Post Weight:
  - Weigh the capped cryovial containing the absorbed swab tip
  - Record on the LDMS Tracking sheet
11. Transport sample to the LDMS laboratory where the sample will be labeled and tracked using LDMS.
12. Record the pre and post weights into the "PK swab excel worksheet". This will automatically calculate the weight of the fluid collected. This worksheet will be sent to the PK lab along with the specimens at the end of the study.
13. Within 2 hours, place the sample tubes in the freezer at  $\leq -70^{\circ}\text{C}$ .

#### **10.9.8.2 Procedure for Vaginal Fluid Sampling for PK assessment (No weighing of swabs, Colorado, Bronx and Memphis sites):**

Materials:

2-mL Nalgene cryovials with affixed SCHARP label  
Polyester-Tipped (Dacron) Swab  
(The MTN LC will provide pre-cut Dacron swabs inserted into the cryovial. This is to assure consistent size of swabs for analysis)

PK swabs must be collected within one hour of PK blood draw.

1. Ensure that new, clean, or sterilized supplies (gloves, hemostat or ring forcep, swabs, tubes, etc.) are used for each sample, as Dapivirine is very sensitive to cross-contamination.
2. Before starting procedures, label the cryovial containing the pre-cut swab with participant study and sample identification information.

Collection of vaginal fluid:

1. Label the cryovial with SCHARP label with PTID, date, and visit number.
2. Use a clean gloved hand to handle the swab.
3. Open the cryovial and attach the pre-cut swab to hemostat or ring forcep.
4. Insert the swab into the vagina to the location nearest to where the ring was residing. For 10-20 seconds, rotate in a circular motion touching all walls to absorb as much fluid as possible.
5. Immediately place swab tip into the labeled cryovial after sampling and recap.
6. Record this sample on the LDMS tracking sheet.
7. Transport sample to the LDMS laboratory where the sample will be labeled and tracked using LDMS.
8. Within 2 hours, place the sample tubes in the freezer at  $\leq -70^{\circ}\text{C}$ .

#### **10.9.8.3 Shipping of PK swab samples to MTN LC JHU CPAL**

At the end of the study, ship PK swab samples on dry ice on Monday or Tuesday to the MTN LC JHU CPAL:

**James Johnson  
Johns Hopkins University  
Division of Clinical Pharmacology  
600 N. Wolfe Street, Osler 523  
Baltimore, MD 21287**

#### 10.10 Testing of Intravaginal Ring (IVR)

Used rings will be analyzed for residual levels of Dapivirine, and will be collected at visit weeks 4, 8, 12, 16, 20, and 24. The used rings may contain vaginal secretions and therefore treated as a biohazard. The rings will remain in the amber pouch and stored at room temperature until further notice from the MTN LC. Rings that are defective or inserted briefly and removed for various reasons may be destroyed at the site via biohazard procedures.

##### Removal of ring by clinician<sup>¥</sup>:

1. Wear lab coat, gloves, and protective face guards when performing this step.
2. The clinician will remove the used ring and place in a clean container\* with tap water.  
\*Use a disposable container or a reusable container that was cleaned using 10% bleach solution for 20 minutes or sterilized.
3. Move the ring around in the water or swirl the container to remove vaginal material.
4. Take the ring out of the water and blot dry with paper towels or gauze.
5. The ring should be dry before storing in pouch.
6. Dispose of blotting materials and contaminated water according to your institution biohazard policy.

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

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

1. Site staff will place the ring into a new 3"X5" amber Zippit pouch (see figure 10-4) that was provided by LC to store the rings.
2. Label the pouch with a SCHARP label consisting of the participant ID number, visit number, and collection date.
3. Add a biohazard sticker if one is not already attached to the pouch, making sure not to cover the identifier information.
4. The use of LDMS is required to label and log in all used rings.
5. Store the used ring within the biohazard labeled amber pouch at room temperature.
6. At the end of the study, the MTN LC will contact site to coordinate shipment.

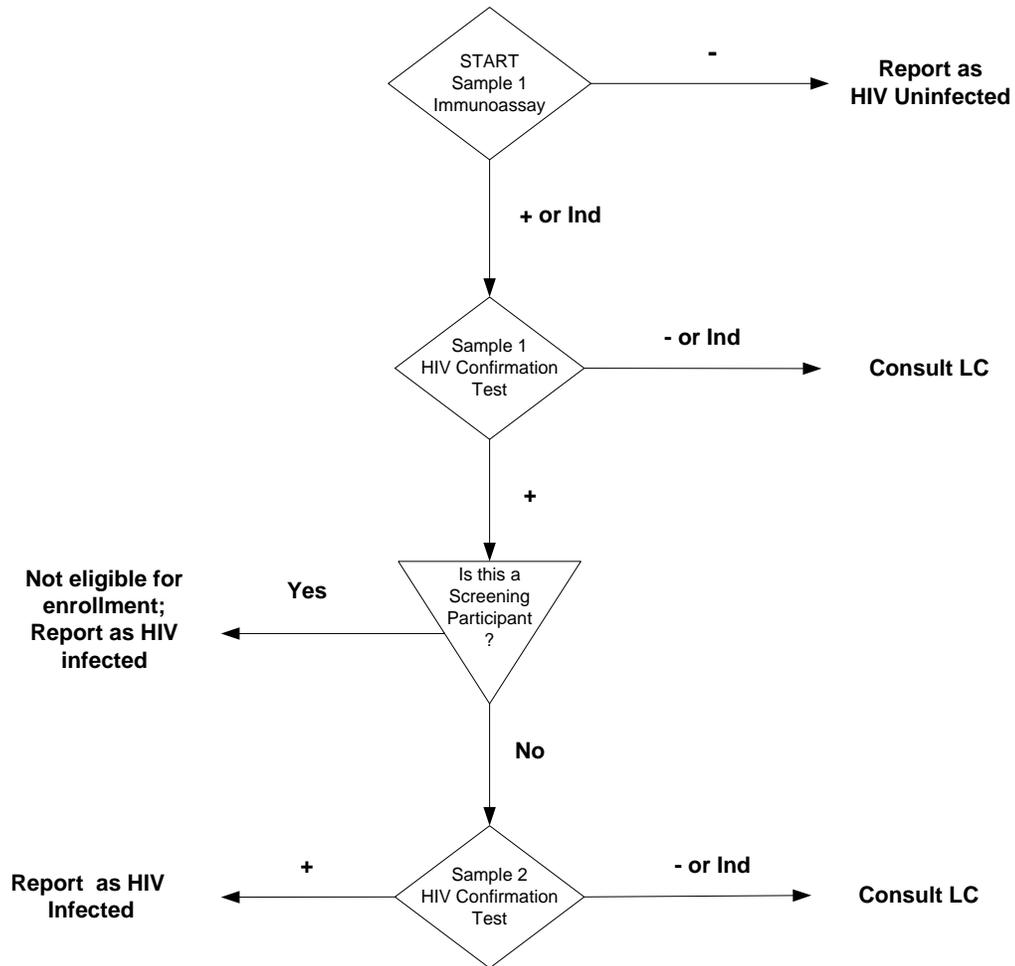
¥ If the ring is removed by the participant prior to the clinic visit and will not be reinserted, then the used ring is still prepared for residual drug analysis. After the used ring is taken out of the participant's bag (bag or pouch they returned the ring in), follow directions starting with step 1.

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



# Algorithm For HIV Testing

## Appendix 10-1: HIV ANTIBODY TESTING ALGORITHM



Ind: Indeterminate test results  
LC: Laboratory Center