

Section 10. Laboratory Considerations

This section contains information on the laboratory procedures performed in MTN-024/ IPM 031.

Table of Contents

The page numbers are hyperlinked to the contents below

10.1 Overview and General Guidance	10-2
Table 10-1 :Overview of Laboratory Testing Locations, Specimens, and Methods	10-3
Table 10-2: Overview of Specimens for Storage and Shipment	10-4
Table 10-3: Overview of Laboratory Tests by visit	10-6
10.2 Specimen Labeling	10-6
10.3 Procedures for Specimens that cannot be evaluated	10-6
10.4 Use of LDMS	10-6
Figure 10-1: LDMS Entry Screen	10-7
10.4.1: Weight measurements in LDMS	10-8
Figure 10-2: LDMS Weight Entry Screen	10-8
Table 10-4 LDMS Specimen Management Guide	10-9
Table 10-5 LDMS Codes	10-10
10.4.2 Off-Hours Contact Information:	10-11
10.5 Urine Testing for CT/GC (Chlamydia Trachomatis and Neisseria Gonorrhoea), Pregnancy, Urinary Tract Infection.	10-11
10.5.1 Specimen Collection	10-11
10.5.2 Testing for Chlamydia Trachomatis and Neisseria Gonorrhoeae by NAAT	10-11
10.5.3 Pregnancy Testing	10-12
10.5.4 Urinary Tract Infection	10-12
10.6 Blood Specimens for Chemistry, FSH, Hematology, HIV testing, Syphilis, Plasma Archive, Blood Dapivirine	10-12
10.6.1 Specimen Collection and Initial Processing	10-12
10.6.2 Chemistry (Alanine transaminase, Aspartate aminotransferase, and Creatinine), FSH (Follicle Stimulating Hormone), and Hematology (CBC with Platelets)	10-13
10.6.3 HIV Testing	10-13
10.6.4 Syphilis Testing	10-13
10.6.5 Plasma Archive	10-14
10.6.6 Blood for PK (Dapivirine)	10-15
10.7 Cervicovaginal Lavage (CVL) for Biomarkers, Aliquot storage, and Cell Pellet.	10-15
10.7.1 Collection procedure for CVL	10-16
10.7.2 Processing of the CVL Cell Pellet and Supernatant for biomarker and storage	10-17
10.7.3 CVL Biomarkers	10-17
10.7.4 CVL Cell Pellet	10-18
10.8 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.	10-18
10.8.1 Herpes Lesion Testing	10-18

10.8.2 Gram Stains on Vaginal Fluid	10-18
10.8.3 Microbiology: Vaginal Swab for Quantitative Culture	10-19
10.8.4 Vaginal Fluid pH	10-20
10.8.5 Vaginal Fluid Wet Mount Testing if indicated for BV and Yeast (KOH)	10-21
Table 10-6 Summary of Wet Prep Assessments and Diagnostic Criteria	10-21
10.8.6 Rapid Test for Trichomonas	10-22
10.8.7 NAAT Chlamydia and Gonorrhea Testing	10-22
10.8.7 Vaginal Swab for Biomarkers	10-23
10.8.8 Vaginal Swab for PK	10-23
10.8.9 Testing of Intravaginal Ring (IVR)	10-24
Figure 10-3: 3”X5” amber Zippit pouch	10-25
10.9 Cervical Specimens for Biopsy PK, Cytobrush for Flow Cytometry, and Pap Test.	10-25
10.9.1 Cervical Biopsy	10-25
10.9.2 Cytobrush for Flow Cytometry at Pittsburgh & Case Western sites	10-26
10.9.3 Papanicolaou (Pap) Test (*only if indicated)	10-28
APPENDIX 10-1: HIV ANTIBODY TESTING ALGORITHM	10-29

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 (LC [formally known as Network Lab or NL]), including the MTN Pharmacology Core at Johns Hopkins University (Clinical Pharmacology Analytical Laboratory [CPAL]). Table 10-1 and table 10-2 highlight specimen, storage and shipment requirements. Table 10-3 lists the tests to be performed at each visit for each group.

Regardless of whether tests are performed in clinic or laboratory settings, study staff that performs the tests must be trained in proper associated QC procedures prior to performing the tests for study purposes; training documentation should be available for inspection at any time.

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

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

Ideally, one method, type of test kit, and/or combination of test kits will be used for each protocol specified test throughout the duration of the study. If for any reason a new or alternative method

or kit must be used after study initiation, site laboratory staff must perform a validation study of the new method or test prior to changing methods. The MTN LC must be notified before the change and can provide further guidance on validation requirements.

Provided in the remainder of this section is information intended to standardize laboratory procedures across sites. Adherence to the specifications of this section is essential to ensure that 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, Specimens, and Methods for MTN-024/ IPM 031**

Test	Testing Location	Specimen Type	Tube or Container and tube size (recommended)	Kit or Method
Urine pregnancy test (hCG)	In clinic	Urine	Plastic screw top cup	One-Step or Combo hCG Quidel Quick Vue, or Fisher HealthCare Sure-Vue Urine hCG kit
Urine Culture or Dipstick ¹	Local lab	Urine	Plastic screw top cup	Local methodology
Urine NAAT for GC/CT ¹	Local lab	Urine	Kit specific Transport tube	BD Probetec or Gen-Probe Aptima
CBC with Platelet	Local Lab	Whole Blood	EDTA 4mL tube	Local methodology
Chemistries (AST, ALT, Creatinine)	Local Lab	Serum or Heparinized plasma	Plain or serum separator 4 mL	Local methodology
HIV Antibody Screen	Clinic/Local Lab	Plasma, serum, or whole blood	EDTA or plain tube 4 mL	Bio-Rad HIV-1,2+O EIA or other FDA approved test
HIV Confirmation	Local Lab	Plasma or serum	EDTA or plain tube 4 mL	Multispot or other FDA-approved test
Blood PK (Dapivirine)	LC JHU CPAL	Plasma	EDTA 10 mL tube	JHU CPAL collection procedure
Syphilis Serology	Local Lab	Serum or Plasma	EDTA tube, plain or serum separator, 4 ml	Local methodology
FSH (Follicle Stimulating Hormone)	Local Lab	Serum	Plain or serum separator, 4 ml	Local methodology
Plasma Archive	Clinic/Local Lab	Plasma	EDTA 10mL tube	LC procedure
Cervicovaginal Lavage (CVL) for biomarkers & Cell Pellet	LC & LC JHU CPAL	Fluid & pellet recovered from CVL (saline used)	15 mL Conical Vial	LC Procedure
Vaginal Swab(s) for biomarkers	LC	Vaginal Swab	1.8 cryovial	LC procedure
Vaginal Swab for PK (subset only)	LC JHU CPAL	Swab	2.0 Cryovial	LC procedure

Vaginal NAAT for Gonorrhea and Chlamydia	Local lab	Vaginal swab (supplied with kit)	Kit specific Transport tube	BD Probetec or Gen-Probe Aptima
Trichomonas Rapid Test	Local lab or in clinic	Vaginal swab (supplied with kit)	Sterile tube with no additives	OSOM kit
Vaginal pH	In clinic	Vaginal swab	N/A	S/P pH Indicator Strips
Quantitative Vaginal Culture	LC	Vaginal swab	Port-a-Cul transport tubes by BD	LC procedure
Vaginal smear Gram-stain	LC	Vaginal Swab	Slides	LC procedure
Used Vaginal Ring for PK residual assessment	IPM Designated lab	Used VR	Biohazard labeled amber bag	LC procedure
Vaginal saline wet preparation (for BV and/or KOH wet mount) ¹	In clinic	Vaginal swab	tube with 6 drops of saline	LC procedure
Herpes Lesion Testing ¹	Local lab	Local method	Local method	Local methodology
Cervical Biopsy for PK (intensive PK subset only)	LC JHU CPAL	Tissue	1.8 mL cryovial	JHU CPAL collection procedure
Cervical Cytobrush Flow Cytometry ² (Case & Pitt Only)	Local Lab	Cytobrush	50 ml Conical vial	LC collection procedure
Pap Smear ³	Local Lab	Ecto & Endo-cervical cells	Slides	Local methodology

1. Perform only if clinically indicated per local SOP.
2. To be collected on participants with a cervix at specified site(s).
3. Perform only if clinically indicated or if participant does not have a documented satisfactory Pap within the 12 months prior to Enrollment.

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

Specimen and subsequent testing	Additive	Tube type or size recommended	Processing	Ship to:	Shipping schedule
Plasma for archive	EDTA	10 mL	Freeze plasma at $\leq -70^{\circ}\text{C}$ within 4 hours of draw	MTN LC	Store frozen at site until notified by MTN
Plasma for blood PK (Dapivirine)	EDTA	10 mL	Freeze plasma within 8 hours after collection	LC JHU CPAL	Store frozen at site until conclusion of study
Cervicovaginal Lavage (CVL) supernatant: Biomarker	Normal Saline (as a Cervicovaginal rinse)	2ml cryovial	Freeze supernatant within 8 hours of collection	MTN LC	Store frozen at site until notified by MTN
Cervicovaginal Lavage Cell Pellet (CVL pellet)	NSL	2ml cryovial	Freeze cell pellet within 8 hours of collection	MTN LC	Store frozen at site until notified by MTN

Cervical Cytobrush for Flow Cytometry Case Western & Pitt	tRPMI	2 ml cryovial	Process within 2 hours of collection.	LC JHU CPAL	Store frozen at site until notified by MTN
Used Vaginal Ring for PK residual assessment	None	3"X5" amber Zippit pouch	Place used VR in pouch	MTN LC	Store room temp at site until notified by MTN
Vaginal smear for Gram-stain	None	2 slides	NON (No Additive)	MTN LC	Ship one slide the day of collection. Store 2 nd slide at site until conclusion of study
Vaginal Swabs for Quantitative Vaginal Culture	Port-a-Cul (PAC)	2 swabs in transport tube	Place swab in Port-a Cul & break off shaft	MTN LC	Ship on ice packs to LC on the day of collection.

Table 10-3: Overview of Laboratory Tests by visit for MTN-024/ IPM 031

	SCR	ENR	4-Wk Visit	8-Wk Visit	12-Wk Final Clinic Visit/Early Termination Visit
CLINICAL					
Pelvic examination	X	X	X	X	X
LABORATORY (vaginal and cervical swabs as required)					
Urine hCG	X	*			
Urine NAAT for GC/CT			*	*	*
Dipstick UA and/or urine culture, per local standard of care	*	*	*	*	*
Serum chemistries (AST, ALT, Creat.)	X	*	*	*	X
CBC with platelets	X	*	*	*	X
HIV-1 serology	X	*	*	*	X
FSH	X				
PK- Blood			X	X	X
Syphilis serology	X				
Plasma archive		X			
Gram stain		X	X	X	X
Vaginal pH		X	X	X	X
Quantitative vaginal culture		X	X	X	X
Cytobrush for flow cytometry (only at Case & Pitt)		∅			∅
CVL for biomarkers, cell pellet, & supernatant storage		X	X		X
Vaginal swab(s) for biomarkers		X	X		X
Vaginal Swab for PK (subset Only ¹)			X	X	X
Cervical biopsies for PK (Intensive PK subset only ²)					X
Rapid Trichomonas	X	*	*	*	*
Vaginal NAAT for GC/CT	X	*			
Pap smear interpretation	*				
Saline wet mount for BV	*	*	*	*	*
KOH wet mount for candidiasis	*	*	*	*	*
Herpes lesion testing	*	*	*	*	*
STUDY PRODUCT					
Participants will receive study VR (Vaginal Ring)		X	X	X	
Collect study product			X	X	X

1: The vaginal fluid for PK will be performed on a subset of 45 participants

2 The tissue biopsy will be performed on a subset of 15 women (from the set of 45 participants having vaginal fluid PK)

X = required, * = if indicated, ∅ = To be collected on participants with a cervix at selected site(s)

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 tests 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 LC must be notified in the following cases

- Any time a participant must return to the clinic for specimen collection
- When PK specimens are missed
- Insufficient blood volume is collected for the plasma archive
- Any time specimens have been mishandled, possibly compromised specimen integrity
- Any situation that may indicate a protocol deviation

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

10.4 Use of LDMS

The Laboratory Data and Management System (LDMS) is a program used for the storage and shipping of laboratory specimens. It is supported by the Frontier Science Foundation (FSTRF). LDMS must be used at all sites to track the collection, storage, and shipment of the sample types described in Table 10-4

Detailed instructions for use of LDMS are provided at: <https://www.fstrf.org/ldms> (may require a password).

All sites will be required to maintain the current version of LDMS and monitor updates relating to use of the LDMS. It is crucial to be aware of proper label formats to ensure that specimens are

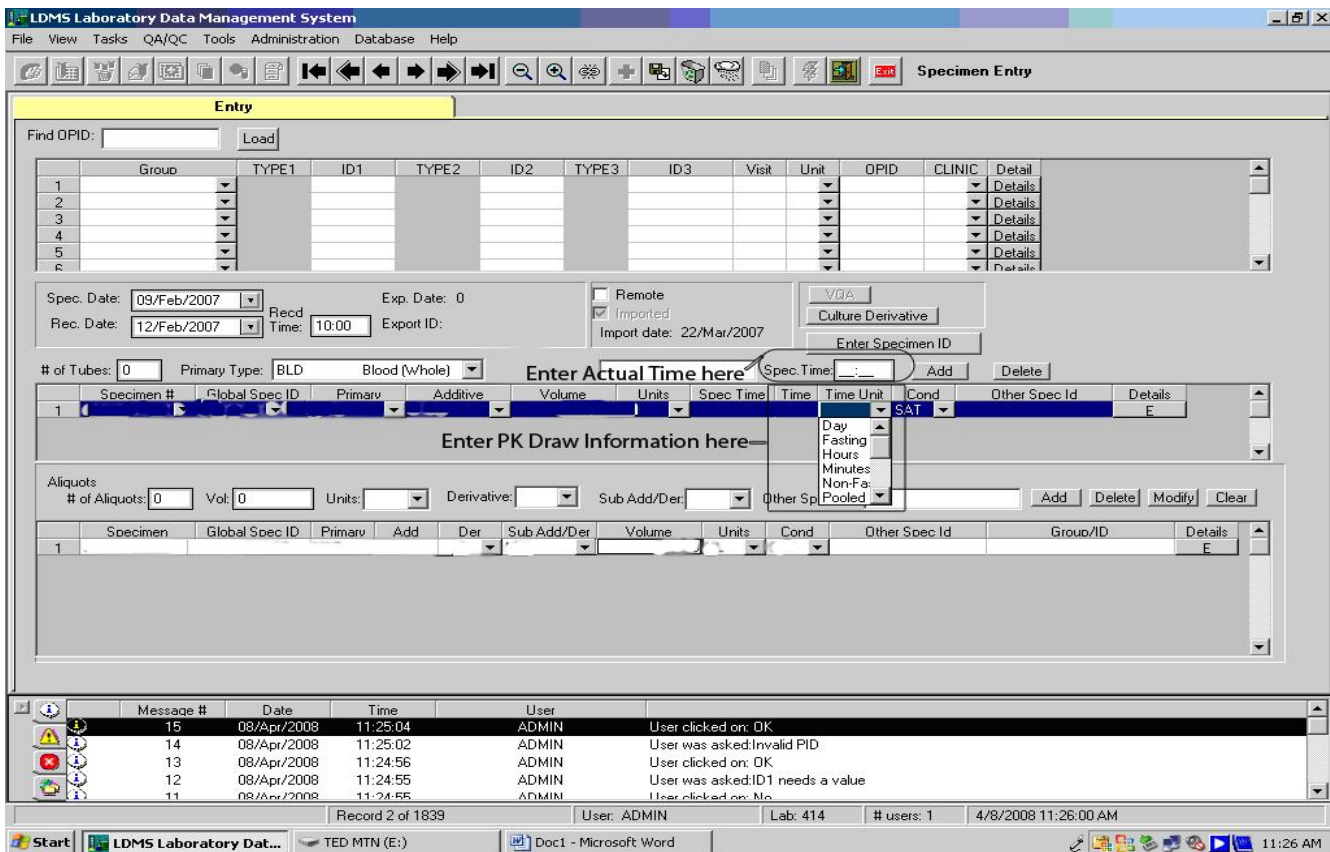
correctly labeled. Sites will be responsible to back up their LDMS data (frequency determined by site) locally and to export their data to FSTRF (at least weekly).

Each site must export its LDMS data to Frontier Science (FSTRF) on a weekly basis. Exported data are used by the MTN SDMC to generate a monthly specimen repository report and to reconcile data entered in LDMS with data entered on study case report forms. Any discrepancies identified during the reconciliation are included in a monthly discrepancy report for each site. Sites are expected to resolve all discrepancies within two weeks of receipt of the report. The MTN LC is responsible for reminding sites to adhere to the two week timeframe and for following up with sites that do not resolve discrepancies within two weeks. The MTN SDMC reviews the discrepancy reports for critical samples (e.g., plasma needed for confirmatory HIV testing) that appear to be missing, and works with the LC and site staff to undertake appropriate corrective action. All corrective action should be documented in paper-based clinic and/or laboratory records as appropriate, and entered in the details section of LDMS. The LC and SDMC will discuss and document any items that, although resolved, appear 'irresolvable' in LDMS.

Logging in 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 Entry Screen



10.4.1: Weight measurements in LDMS

The volume field in LDMS can be used for displaying weight measurements with proper units. Once the *net-weight* is attained by subtracting the *pre-weight* from the *post-weight*, the result can be entered into LDMS as shown in Figure 10-2. In the primary sample field (section **A**), enter the sample(s) that are weighted. Under volume *Units*, enter *each* (EA) in this field and enter '1' for *Volume*. Enter the exact time drawn in the *Spec Time* field. Also, to help identify two of the same type of samples (Ex: biopsies), enter additional identifying information under *Other Spec ID* (see example in Figure 10-2). To put in the weights, make an aliquot (in section **B**) for each of the primary samples that were entered from section **A**. Enter the net-weight and change the units to milligrams. Here is an example of a biopsy weight: Pre-weight=3583.5 mg, Post weight= 3621.1 mg; therefore, the net weight= 37.6 (3621.1-3583.5=37.6). Enter the 37.6 under *Volume*, change the *Unit* to MG.

Figure 10-2: LDMS Weight Entry Screen

The screenshot shows the LDMS Weight Entry Screen. Section A is a form for entering specimen details, and Section B is a table for entering aliquot details.

Section A:

- Spec. Date: 22/Apr/2013, Exp. Date: (empty)
- Rec. Date: 22/Apr/2013, Recd. Time: 08:30, Export ID: (empty)
- Primary Type: CVB, Other Spec ID: (empty), Spec Time: 08:00

Specimen #	Global Spec ID	Primary	Additive	Volume	Units	Spec Time	Time	Time Unit	Cond	Other Spec Id	Details
1	414/13001307	CVB	NON	1.00	EA	08:00			SAT	BIOPSY 1	DB
2	414/13001307	CVB	NON	1.00	EA	08:05			SAT	BIOPSY 2	DB

Section B:

- # of Aliquots: 0, Vol: 0, Units: (empty), Derivative: (empty), Sub Add/Der: (empty), Other Spec ID: (empty)

Specimen	Global Spec ID	Primary	Add	Der	Sub Add/Der	Volume	Units	Cond	Other Spec Id	Group/ID	Details
1	414/13001308	CVB	NON	TIS	N/A	37.60	MG	SAT	BIOPSY 1	Practice Group/123456	RDB
2	414/13001308	CVB	NON	TIS	N/A	22.50	MG	SAT	BIOPSY 2	Practice Group/123456	RDB

Table 10-4 should be used as a guide when logging in MTN-024/ IPM 031 specimens. Please use the LDMS codes listed below when logging in specimens for each test listed. Tracking sheets can be found in the Study Implementation Materials section on the MTN-024/ IPM 031 webpage.

Table 10-4 LDMS Specimen Management Guide for MTN-024/ IPM 031 Specimens

Test	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALIQOT DERIVATIVE	ALIQOT SUB ADDITIVE/ DERIVATIVE	Aliquot volume	Units	INSTRUCTIONS FOR PROCESSING LAB
Plasma for Storage (archive)	BLD	EDT	PL1/2	N/A	1.5	mL	Prepare as many 1.5 mL aliquots as possible with a total volume of aliquots \geq to 4ml. If sample is collected and held at room temp, freeze within 4 hours. If refrigerated after collection, freeze within 24 hours.
Plasma for PK (Dapivirine)	BLD	EDT	PL1/2	N/A	1.5-2 in 2ml cryovial	mL	Transport to lab ASAP. Centrifuge, split, and label two or more cryovials with a minimum of 1.5mL in each. Freeze within 8 hrs of blood collection.
Cervicovaginal Lavage (CVL) supernatant	CVL	NSL	FLD	N/A	1 ml in 2ml cryovial	mL	CVL supernatant for biomarkers. Freeze at $\leq -70^{\circ}\text{C}$ within 8 hours of collection.
							CVL supernatant: 3 or more additional aliquots (used for backup or future testing marked 'extra CVL') & freeze at $\leq -70^{\circ}\text{C}$ within 8 hours of collection.
Cervicovaginal Lavage (CVL) Cell Pellet	CVL	NSL	CEN	NSL	1 ml in 2ml cryovial	mL	CVL cell pellet: suspended in 0.5 mL of normal saline & freeze at $\leq -70^{\circ}\text{C}$ within 8 hours of collection.
Cervical Cytobrush for Flow Cytometry (Case Western & Pittsburgh)	CER	RPM	CTB	N/A	Brush in 20 ml tRPMI	Each	Keep on ice and deliver to Flow Cytometry ASAP to process within 2 hours from collection.

Test	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALIQOT DERIVATIVE	ALIQOT SUB ADDITIVE/ DERIVATIVE	Aliquot volume	Units	INSTRUCTIONS FOR PROCESSING LAB
Cervical Biopsies for PK	CVB	NON	TIS	N/A	1 biopsy in each 1.8mL cryovial	mg	Collect 2 biopsies. Perform Pre (without biopsy) and Post (with biopsy) weights. Mark vials Biopsy 1 & Biopsy 2. and store at <-70°C. Enter weights on LDMS tracking sheet.
Vaginal Swab(s) for biomarkers	VGL	PBS (400 uL PBS)	SWB	N/A	1 swab in 1.5 ml micro tube	Each	Place Dacron swab in a labeled cryovial containing 400 µL PBS. Store sample tubes at ≤ -70°C.
Vaginal Smear for Gram Stain	VAG	NON	SLD	GRS	2 smears	Each	Re-label with LDMS label. Make 2 slides. Ship one slide to MTN LC and store other slide on-site.
Vaginal Swab for Quantitative Culture	VGL	PAC	SWB	N/A	2 swabs in 1 Port-a-cul tube	Each	Insert 2 vaginal Dacron swabs into PAC, and ship overnight on ice packs to MTN LC on the day of collection.
Vaginal PK Swab	VGL	NON	SWB	N/A	1 swab	Each	Pre & post weigh each tube+pre-cut swab. Store at <-70°C. Enter weights on LDMS tracking sheet.
Used Vaginal Ring for residual PK	IVR	NON	IVR	NA	1 pouch	Each	Store at room temp.

Table 10-5 LDMS Codes

BLD: Whole Blood	FLD: Fluid Supernatant	PL1/2: Single or double spun
CVB Cervical Biopsy	IVR: Used Intravaginal Ring	SWB: Swab
CVL: Cervical Vaginal Lavage	N/A: Not Applicable	RPM: RPMI Transport Media
CEN: Cell Pellet	NON: No Additive	TIS: Tissue
CER: Cervix	NSL: Normal Saline	VAG: Vaginal Swab
CTB: Cytobrush	PAC: Port-a-Cul	VGL: Vagina
EDT: EDTA	PBS: Phosphate buffered saline	

Questions related to use of LDMS in MTN-024/ IPM 031 may be directed to Wayne Hall or LDMS Technical (User) Support. Usual business hours for LDMS User Support are 7:00 am - 6:00 pm (ET) from Monday through Friday. All other hours and weekends, an on-call user support specialist will be available. Contact LDMS User Support at:

Email: ldmshelp@fstrf.org
Phone: +716-834-0900, ext 7311
Fax: +716-898-7711

10.4.2 Off-Hours Contact Information:

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

10.5 Urine Testing for CT/GC (Chlamydia Trachomatis and Neisseria Gonorrhoea), Pregnancy, Urinary Tract Infection.

The urine tests performed at each study visit will depend on the time point of the visit and the clinical presentation of the participant. At study visits when urine testing is required, a single specimen will be collected and then aliquoted for each test when possible. When performing multiple tests from one specimen, the correct order is pregnancy testing first, and then the urine dipstick (if clinically indicated).

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 not to clean the labia prior to specimen collection.
- Collect the first 20-30 mL of voided urine (not midstream urine) in a sterile collection cup if testing for CT/GC.
 - Note: If testing using urine dipstick, culture, and/or pregnancy test, then collect midstream urine.
- Instruct the participant to screw the lid tightly onto the cup after collection.

10.5.2 Testing for Chlamydia Trachomatis and Neisseria Gonorrhoeae by NAAT

Testing for Chlamydia and Gonorrhoea is performed 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 LC for testing. Contact the LC prior to sending specimens for GC/CT testing.

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

- Open the UPT kit and remove the UPT and transfer pipette. Label the UPT with the participants PTID number and date.

- Hold the UPT upright and firmly tap the bottom of the tube on a flat surface to dislodge any large drops from inside the cap.
- Fill the transport tube with urine to the level indicated by the black line on the tube. Do not under fill or overfill the tube.
- Transport to the local laboratory according to the specific manufacturers recommendations.
- Testing will be done at the local laboratories according to the site SOP.

10.5.3 Pregnancy Testing

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

Pregnancy status is a critical participant safety consideration in MTN-024/IPM 031. All sites must maintain an adequate inventory of the QuickVue One-Step test kits at all times. Inventory should be monitored closely and re-supply orders placed at least 8-12 weeks in advance of actual need (or longer if needed per site procurement policies and procedures). The date and time of pregnancy testing must be documented.

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

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

10.5.4 Urinary Tract Infection

Urine Dipstick and/or Culture: Perform only if indicated and by local standard of care. Instruct participant to collect midstream urine, unless the sample is to be used for GC/CT testing which requires the collection of the first 20-30 mL of voided urine.

10.6 Blood Specimens for Chemistry, FSH, 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.

10.6.1 Specimen Collection and Initial Processing

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

- Allow plain tubes (no additive or serum separator tubes) to clot, then centrifuge per site SOPs to yield serum for chemistry, FSH, syphilis, and 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, 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.

10.6.2 Chemistry (Alanine transaminase, Aspartate aminotransferase, and Creatinine), FSH (Follicle Stimulating Hormone), 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), Creatinine and FSH. Hematology tests are: Hemoglobin, Hematocrit, Platelets, White blood cell count (WBC), and Red blood cell count (RBC).

10.6.3 HIV Testing

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

HIV infection status will be assessed according to the HIV testing algorithm as presented in Appendix 10-1 in this section (or appendix II of the MTN-024/ IPM 031 protocol). The first specimen drawn is considered Sample 1, and the confirmatory specimen drawn is considered Sample 2.

Test result interpretation is as follows:

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

Screening Participants

- If the confirmatory test is negative, indeterminate or invalid, contact the LC for guidance at mtnvirology@mtnstopshiv.org. It is not recommended for participants with discrepant HIV testing results to continue enrollment into MTN-024/ IPM 031.
- 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 is negative, indeterminate or invalid, contact the LC for guidance at 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.
 - If the confirmatory test on Sample 2 is positive, the participant is HIV positive.
 - If the confirmatory test on Sample 2 is negative, indeterminate or invalid, contact the LC for guidance at mtnvirology@mtnstopshiv.org.

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

10.6.4 Syphilis Testing

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

1. Rapid plasma reagin (RPR) screening test followed by a confirmatory test for *Treponema pallidum*. Any FDA approved *Treponema pallidum* confirmatory test can be used such as the Enzyme Immunoassay (EIA), microhemagglutinin assay for *Treponema pallidum* (MHA-TP), *Treponema pallidum* hemagglutination assay (TPHA), *Treponema pallidum* particle agglutination (TPPA), or fluorescent treponemal antibody (FTA-ABS). All positive RPR results must have a titer reported. For reactive RPR tests observed during screening, a confirmatory test is performed and appropriate clinical management action must be taken 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-024 Protocol Safety Physicians (mtn024safety@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.

10.6.5 Plasma Archive

For plasma archive, use collection tubes with EDTA anticoagulant. Aliquot plasma into 2 ml cryovials, store at $<-70^{\circ}\text{C}$, and batch onsite until the MTN 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 1500xg (relative centrifugal force in g) for 10 minutes, remove plasma.
 - Double spun: Spin blood at 800xg for 10 minutes, place plasma in a tube to spin again at 800xg for 10 minutes, remove plasma.
- Prepare as many 1.5 mL aliquots as possible with a total volume of aliquots greater than 4ml
- If total volume is less than 2.0 mL, redraw as soon as possible.
- If less than 4ml of plasma are available, store that plasma and inform the MTN 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 (Dapivirine)

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 1500xg for 10 minutes. The centrifugation must be completed and sample placed in the freezer within 8 hours of blood collection.
3. Use a pipette to aliquot approximately 1.5-2.0 mL of the resulting plasma into 2mL cryovials. One of these will serve as the primary sample; the others will serve as a back-up in case the primary samples are accidentally destroyed during shipment.
4. Prepare two storage boxes and label one as "primary samples" and the other as "back-up samples". Transfer the tubes from each participant in chronological order into the storage boxes. All samples will be tracked in LDMS.
5. Store the boxes with samples at $\leq -70^{\circ}\text{C}$ until shipped to MTN Pharmacology Core.
6. Prior to shipping, prepare a shipment box (a foam chest) filled with dry ice sufficient for a 24 hour period with an appropriate shipping label.
7. Primary samples will be shipped to the MTN Pharmacology Core at Johns Hopkins University 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-024/IPM 031 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

10.7 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.

10.7.1 Collection procedure for CVL

Suggested Materials

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

Preparation Notes

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

Preparation:

1. Explain procedure to study participant.
2. Position patient for pelvic examination.
3. Wash hands thoroughly prior to procedure and put on gloves.
4. Examine external genitalia. Document and report findings on CRF.

5. Carefully insert the speculum about halfway into the vagina.
6. Open speculum gently to visualize anatomy/positioning. Close speculum and gently advance it. Repeat opening the speculum to guide insertion until part of cervix, or upper vagina for women without a cervix, is visible.
7. Carefully open the speculum, without hitting the cervix (if still intact), and to position the upper vagina and cervix (if accessible) into view.
8. Visually inspect vagina and cervix, if applicable.

Sample Collection and Transport:

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

10.7.2 Processing of the CVL Cell Pellet and Supernatant for biomarker and storage

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

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 and save fluid in cryovials.
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. Store all supernatant in as many 1 mL aliquots as possible in 2mL cryovials, assuring there are at least 1 aliquot for biomarker testing. A minimum of 5 back-up aliquots is also required to be stored (mark as 'Extra CVL').
7. Freeze all aliquots at ≤-70°C within 8 hours of collection and track in LDMS.
8. If less than a total of 6 mL's (or less than 6 cryovials) of supernatant are recovered, contact the MTN LC.

9. Cell pellets will be suspended in 0.5 ml normal saline in a plastic cryovial and frozen at $\leq -70^{\circ}\text{C}$ within 8 hours of collection.
10. The MTN LC will send instructions to the site when shipping is required.

10.7.3 CVL Biomarkers

Biomarkers will be evaluated to determine the impact of the vaginal ring and drug may have on innate immune mediators, cytokines, or soluble factors.

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

Ship to:

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

10.7.4 CVL Cell Pellet

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

Ship to:

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

10.8 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.

10.8.1 Herpes Lesion Testing

Testing will be performed per the local standard of care.

10.8.2 Gram Stains on Vaginal Fluid

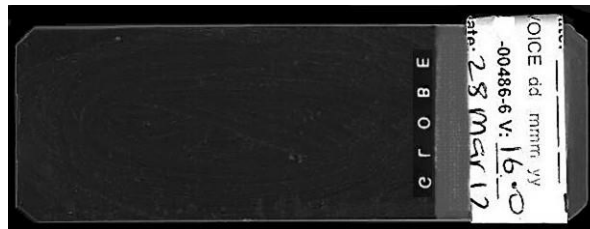
Dried vaginal fluid smears will be prepared for Gram staining and assessment for bacterial vaginosis at the MTN LC. Two slides (one designated as primary and the other as secondary) will be prepared at each required time point and both will be entered into LDMS. The primary slide will be shipped to the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

Instructions for slide preparation and shipping are provided below:

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



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



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



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

10.8.3 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 where another sample was collected (i.e: If the PK swab was collected first, then collect in a different location in the vagina preferably closer to the introitus). Insert swabs into one Port-A-Cul transport tube (labeled with a SCHARP label), submerging the swabs into the gel and breaking off the shafts of the swabs, and capping. (The 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 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 = VAG) and label the Port-A-Cul tube with LDMS labels.
- Use LDMS to generate a shipping manifest for the cultures to be shipped.
- Ship the Port-A-Cul tube and the vaginal smear for gram stain the same day of collection by overnight courier.
- Place the 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 taken 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 and 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.

10.8.4 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.

10.8.5 Vaginal Fluid Wet Mount testing if indicated for BV and Yeast (KOH)

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

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

These procedures are for diagnosis of bacterial vaginosis and candidiasis as summarized in Table 10-6 below.

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

CLIA regulations require semi-annual wet mount proficiency testing; therefore the MTN 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 10-6 Summary of Wet Prep Assessments and Diagnostic Criteria

Assessment	Saline Prep	KOH Prep
Whiff Test	Not applicable	Positive if fishy amine odor detected
Yeast	Positive if pseudohyphae and/or budding yeast are observed. Pseudohyphae and budding yeast may be obscured by epithelial cells. These cells will be lysed by KOH, thus pseudohyphae and budding yeast not observed in saline prep may be observed in KOH prep.	Positive if pseudohyphae or budding yeast are observed.
Clue Cells	Individual cells rather than clusters of cells should be examined. Positive if at least 20% clue cells observed. Cells must be completely covered with bacteria (<i>Gardnerella vaginalis</i> and/or anaerobic GNR) to be counted as clue cells.	Not applicable (clue cells are lysed by KOH)

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

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

- Use a pencil to write the PTID and specimen collection date on one side of the frosted end of two microscope slides. Affix a SCHARP-provided PTID label to the other side of the slides (on the frosted end, under the pencil markings) and write the specimen collection date in indelible ink (e.g. Sharpie pen) on each label.
- Immediately following collection from the lateral vaginal wall via swab (Dacron or cotton), smear vaginal fluid specimens onto each slide. Alternatively, the swab may be placed in a glass or plastic tube with approximately six drops (100 µL) sterile physiologic saline to allow for non-immediate slide preparation. In this case, vaginal fluid specimens should be smeared onto the two slides upon receipt from the collecting clinician.
- Apply one drop of 10% KOH to one slide and immediately perform whiff test for a “fishy” amine odor. Then apply cover slip.
- Apply one drop of sterile physiologic saline to the second slide, emulsify with the vaginal fluid specimen, and then apply cover-slip. Examine immediately at 10X magnification for epithelial cells, budding yeast, and pseudohyphae. Examine at 40X magnification to determine whether observed epithelial cells are clue cells and quantitate the cells. Clue cells are irregularly bordered squamous epithelial cells that are completely covered with bacteria (*Gardnerella vaginalis*). Clue cells must comprise at least 20 percent of the

observed epithelial cells in order for the saline prep to be considered positive for clue cells.

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

10.8.6 Rapid Test for Trichomonas

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

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

10.8.7 NAAT Chlamydia and Gonorrhea Testing

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

Collect vaginal sample (1 manufacturer's recommended swab) and transport to the local laboratory according to the specific manufacturer's recommendations.

Testing will be done at the local laboratories according to the site SOP.

10.8.8 Vaginal Swab for Biomarkers

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

Procedure for Biomarker Vaginal Swab:

- Collect vaginal fluid using a Dacron swab from the posterior fornix.
- Place the swab in a labeled 1.5 ml micro tube containing 400 μ L PBS (1X Concentration), break off and discard swab shaft, and cap the vial.
- Immediately refrigerate or place vial on ice and freeze at $\leq -70^{\circ}\text{C}$ within 8 hours of collecting the sample collection.
- Batch ship on dry ice to MTN LC at end of study.

Ship to:

Pamela Kunjara
Magee-Womens Research Institute
204 Craft Ave, Room A540
Pittsburgh, Pa. 15213

Phone# 412-641-6157
Email: pkunjara@mwri.magee.edu

10.8.9 Vaginal Swab for PK

Vaginal fluid for PK assessment will be performed on a subset of 45 women.

Procedure for PK Vaginal Swab:

Materials:

2 mL Nalgene cryovials containing pre-cut Polyester-Tipped (Dacron) Swab
Ring Forceps or Hemostat (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, label the cryovial containing the pre-cut swab with participant study and sample identification information.
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:
 - Weigh the capped cryovial with pre-cut swab.
 - Record this pre-weight on the LDMS Tracking Sheet.
6. Collection of vaginal fluid:
 - With new gloves and a clean ring forceps, uncap the pre-weighed cryovial and clamp on to the exposed shaft of the swab. Use a clean gloved hand to assist if needed.
Note: if shaft splinters and parts break off, then a **NEW PRE-WEIGHED CRYOVIAL WITH PRE-CUT SWAB WILL BE REQUIRED!** This occasionally happens when the very end of the plastic shaft is grasped with a thin hemostat.
 - Insert the hemostat holding 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.
7. Immediately place swab tip into the cryovial after sampling and recap.
8. Perform Post Weight:
 - Weigh the capped cryovial containing the absorbed swab tip
 - Record on the LDMS Tracking sheet.
9. Within 2 hours, place the sample tubes in the freezer at $\leq -70^{\circ}\text{C}$.
10. At the end of the study, ship in dry ice on Monday or Tuesday to the MTN LC in Baltimore, MD (Johns Hopkins University).

The shipping address for PK swab 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

10.8.10 Testing of Intravaginal Ring (IVR)

Used rings will be analyzed for residual levels of Dapivirine, and will be collected at visit weeks 4, 8, and 12. 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.

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

Important notes:

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

If the ring is removed by the participant prior to the clinic visit and will not be reinserted, then the used ring is still prepared for residual drug analysis (see Counseling Considerations Section 9.3.1). 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.

Step 2: Site staff will place the ring into a new 3"X5" amber Zippit pouch (see figure 10-3) that was provided by LC to store the rings. Label the pouch with the participant ID number and visit number. Add a biohazard sticker if one is not already attached to the pouch, making sure not to cover the identifier information.

Step 3: Store the used ring within the biohazard labeled amber pouch at room temperature.

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

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

Figure 10-3: 3”X5” amber Zippit pouch



10.9 Cervical Specimens for Biopsy PK, Cytobrush for Flow Cytometry, and Pap Test.

10.9.1 Cervical Biopsy

The Cervical biopsy will be performed on a subset of 15 women from the participants having vaginal fluid PK. Two biopsy specimens, each from different areas in the cervix, will be retrieved. The biopsy will be collected as described in the site SOP, and will be using standard cervical biopsy instruments (Kevorkian, Tischler, etc) with a bite size measuring approximately 3 x 5 mm. Topical anesthetic will be not be used. Bleeding may be controlled through a combination of applied pressure, silver nitrate and/ monsel's solution. More information can be found in the MTN 024/IPM 031 SSP Section 7.4.4.2

Cervical Biopsies for PK analysis process procedure:

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

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

10.9.2 Cytobrush for Flow Cytometry at Pittsburgh & Case Western sites

Supplies:

- Medscand Cytobrush Plus: LC will supply
The Cytobrush Plus can be ordered from Cooper Surgical at 800-243-2974, order # C0104. They come in a box of 100 (10 bags of 10 brushes/bag). If sites have trouble obtaining this item, contact the MTN LC.
- 50mL conical tube
- Trypan blue (0.4%): Cellgro Media tech #MT 25-900-CI 6 x 100mL
- D-PBS (without Ca⁺⁺/Mg⁺⁺): GIBCO Invitrogen Catalog # 14190-250 (10 x 500mL) or equivalent
- Transport Media (tRPMI):
 - Prepare tRPMI by making a 7.5% FBS solution of RPMI-1640: Prepare quantity that best fits laboratory needs. Here are 2 examples:
 1. Make 100mL: Adding 7.5mL of FBS into 92.5mL of RPMI-1640
 2. Make 500mL: Adding 37.5 mL of FBS into 462.5 mL RPMI-1640
 - Once tRPMI is prepared, store at 4°C and has a 30 day shelf life.
RPMI 1640: Invitrogen Catalog# 22400-105 10 x 500mL or 22400-089 1 x 500mL
Fetal Bovine Serum (FBS): Heat inactivated, Invitrogen Catalog #10082-147 500mL
- Hemocytometer or automated cell counter
- Refrigerated Centrifuge
- Petri dish

Specimen Collection Procedure:

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

Laboratory Processing Procedure:

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

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

*Squamous cells are expected to be rare on this specimen and will appear similar to squamous cells in urine. They will be larger than cervical mononuclear cells and will have a “fried egg” appearance. These should be counted in the same fashion as cervical mononuclear cells.

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

The Flow Cytometry procedure will begin here with antibody staining.

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

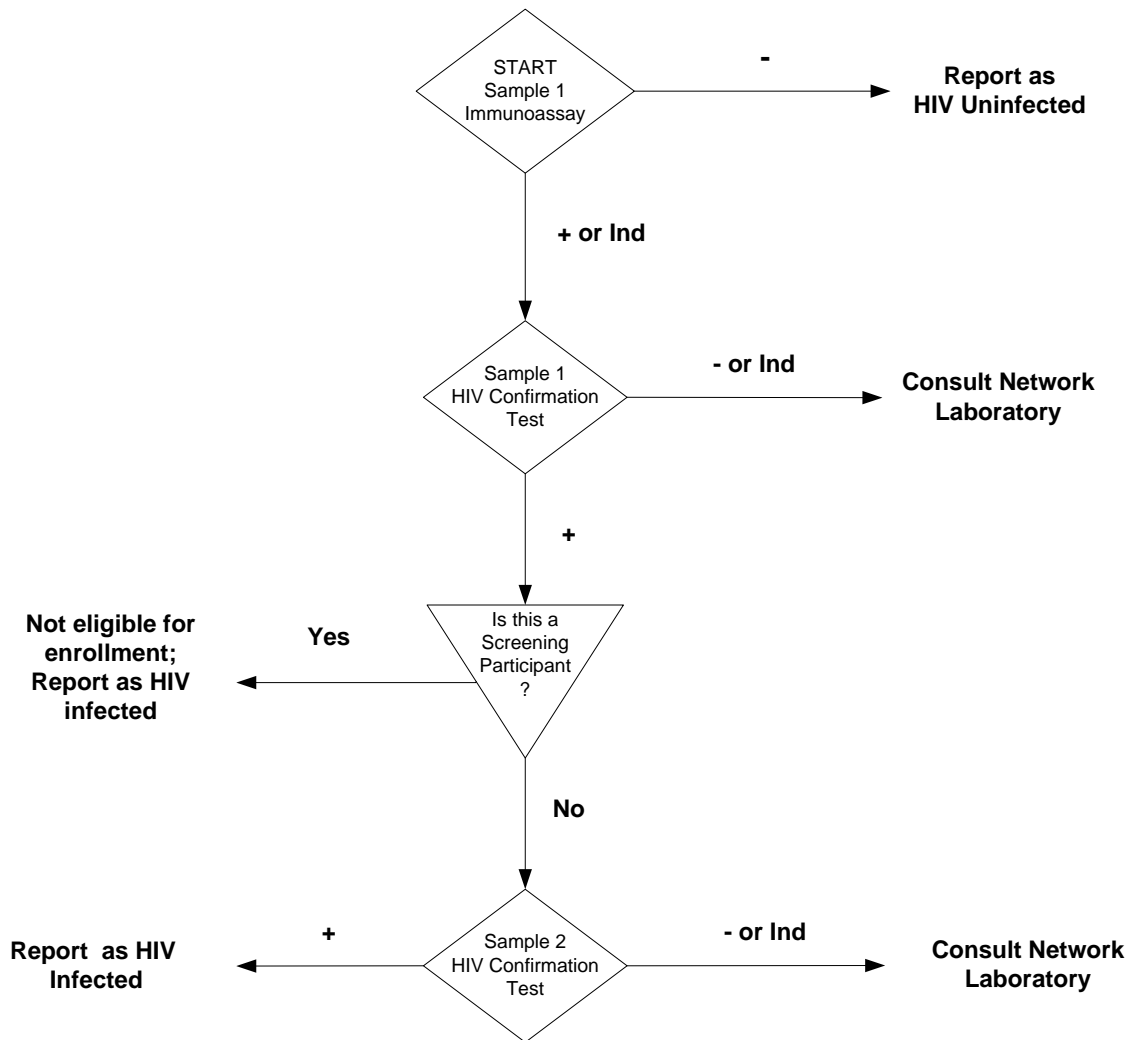
Pap smears are only required if clinically indicated or if a participant has not had a documented normal test within 12 months prior to Enrollment. If a Pap smears is required, ecto- and endocervical cells will be collected after all tissues have been visually inspected and all other required specimens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

At some study sites, Pap smear results may include notations of findings associated with certain STIs (e.g., trichomoniasis). Because Pap smear methods are not adequately sensitive and

specific for STIs, Pap smear findings associated with STIs should not be considered diagnostic of any infections. Rather, such findings should be handled as follows:

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

**APPENDIX 10-1:
HIV ANTIBODY TESTING ALGORITHM**



Ind: Indeterminate test results
Network Laboratory now
referred to as Lab Center