

Section 12. Laboratory Considerations

This section contains information on the laboratory procedures performed in MTN-013/IPM 026.

12.1 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 will be performed at various locations in site clinics or laboratories, MTN Network Laboratories (NL), and specialty labs conducting ring PK analysis. Table 12-1 and table 12-2 highlight specimen and storage requirements. Table 12-3 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 NL 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 NL 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 12-1: Overview of Laboratory Testing Locations, Specimens, and Methods for MTN-013/ IPM 026

Test	Testing Location	Specimen Type	Tube or Container and tube size (recommended)	Kit or Method
Dipstick Urinalysis ¹	Clinic/Local lab	Urine	Plastic screw top cup	Siemens Multistix or Uristix
Urine pregnancy test	In clinic	Urine	Plastic screw top cup	Quidel Quick Vue One-Step or Combo hCG kit
CBC with Diff. and 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
Hepatitis testing for HBsAG & Anti-HCV	Local Lab	Serum	plain or serum separator, 4ml	Local methodology
Plasma Archive	Clinic/Local Lab	Plasma	EDTA 10mL tube	Network Lab procedure
Blood PK Dapivirine & Maraviroc levels	NL Pharmacology Core	Plasma	EDTA 10 mL tube	Network Lab procedure
HIV antibody screen and Western Blot	Clinic/Local Lab	Plasma, serum, or whole blood	EDTA or plain tube 4mL	FDA approved tests
Syphilis Serology	Local Lab	Serum or Plasma	EDTA tube, plain or serum separator, 4 ml	Local methodology
Trichomonas rapid test	Local lab or in clinic	Vaginal swab (supplied with kit)	Sterile tube with no additives	OSOM kit
Vaginal NAAT for Gonorrhea and Chlamydia	Local lab	Vaginal swab (supplied with kit)	Kit specific Transport tube	BD Probetec or Gen-Probe Aptima
Vaginal pH	In clinic	Vaginal swab	N/A	S/P pH Indicator Strips
Vaginal saline wet preparation (for BV and/or KOH wet mount) ²	In clinic	Vaginal swab	Slides	Network Lab procedure
Quantitative Vaginal Culture	MTN Network Lab	Vaginal swab	Port-a-Cul transport tubes by BD	Network Lab procedure
Vaginal smear Gram-stain	MTN Network Lab	Vaginal Swab	Slides	Network Lab procedure
Vaginal Biomarkers and PD	MTN Network Lab	Vaginal Swab	400uL PBS Cryovial	Network Lab procedure
Vaginal PK	NL Pharm. Core	Tear Test Strip	Glass 11 mL (16x100mm)	Network Lab procedure
Vag. Swab for Validation	NL Pharm. Core	Vaginal Swab	1.8 cryovial	Network Lab procedure
Used Vaginal Ring for PK residual assessment (Fenway & UAB only)	NL Pharmacology Core	Used VR	Autoclavable foil bags	Network Lab procedure
Used Vaginal Ring for biofilm culture, FISH, and SIM (Pitt only)	MTN Network Lab	Used VR	Petri dish	Network Lab procedure
Cervical Biopsy for PK	NL Pharmacology Core	Tissue	1.8 mL cryovial	Network Lab procedure
Cervical Biopsy for PD ex vivo challenge (Fenway & UAB)	MTN Network Lab	Tissue	90% FBS + 10% DMSO in a 1.8 mL cryovial	Network Lab procedure

Cervical Biopsy for PD ex vivo challenge (Pittsburgh only)	MTN Network Lab	Tissue	50 ml conical tube with 10 ml of biopsy transport medium	Network Lab procedure
Pap Smear ³	Local Lab	Ecto & Endo-cervical cells	Slides	Local methodology

1. Perform Urine Culture and Sensitivity as clinically indicated per local SOP.
2. Perform only if clinically indicated per local SOP.
3. Perform only if clinically indicated or if participant does not have a documented satisfactory Pap within the 12 months prior to Enrollment.

Table 12-2: 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	Spin 10 minutes at 3000 RPM	MTN Network Lab	Store frozen at site until notified by MTN
Plasma for blood PK	EDTA	10 mL	Spin within 2 hours after collection for 10 minutes at 3000 RPM (1500g)	John Hopkins Pharmacology Lab	Store frozen at site until conclusion of study
Vaginal smear for Gram-stain	None	2 slides	NON (No Additive)	MTN Network Lab	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 Network Lab	Ship on ice packs to NL on the day of collection.
Tear Test Strip for Vaginal PK	Tear Test Strips (TFS)	Glass tube(s)	Place absorbed strip in tube and cap.	John Hopkins Pharmacology Lab	Store frozen at site until conclusion of study
Vaginal Dacron swab for Validation	None	1 swab	Place swab into a cryovial & cap.	John Hopkins Pharmacology Lab	Store frozen at site until conclusion of study
Vaginal Dacron Swab for Vaginal Biomarkers and PD	400uL PBS	1.5ml Micro tube	Put swab in 400uL PBS, break off shaft	MTN Network Lab	Store frozen at site until notified by MTN
Used vaginal ring for PK residual assessment (Fenway & UAB only)	None	Autoclavable sterilizer bag	Place VR in autoclavable foil pouch & autoclave	(To be determined)	Room temp. storage at site until conclusion of study
Used vaginal ring for culture, FISH, and SIM (Pitt only)	None	Petri Dish	Place IVR into sterile petri dish	MTN Network Lab	Immediately deliver to Lorna Rabe's MTN NL upon collection
Cervical Tissue Biopsy for PK	None	1.8mL Cryovial	Cool cryovial on ice prior to biopsy transfer	John Hopkins Pharmacology Lab	Store frozen at site until conclusion of study
Cervical Tissue Biopsy for PD (ex vivo challenge) (Fenway & UAB only)	90% FBS + 10% DMSO (TFM)	1.8 mL Cryovial	90% FBS + 10% DMSO in cryovial (slow freeze)	MTN Network Lab	Store frozen at site until conclusion of study
Cervical Tissue Biopsy for PD (ex vivo challenge) (Pitt only)	Biopsy Transport Medium (BTM)	50 ml conical tube	Pre-cool conical tube with 10 ml of biopsy transport medium	MTN Network Lab	Immediately transport to Pamela Kunjara at MTN NL lab

Table 12-3: Overview of Laboratory Tests by visit for MTN-013/ IPM 026

		SCR	ENR D0	D1 D2	D3	D5	D7	D14	D21	D28	D29 D30	D31	D35 D42	D52	Interim	
		Up to and incl. 45 days prior to ENR								Ring removal		hCG, Vag PK, & Cervical biopsy are randomized for days 31, 35, and 42				
URINE	Qualitative hCG	X	X	*	*	*	*	X	*	X	*	†	†	X	*	
	Dipstick UA	X	*	*	*	*	*	*	*	*	*	*	*		*	
	Urine Culture	*	*	*	*	*	*	*	*	*	*	*	*		*	
BLOOD	HIV-1 Test	X	X							*				X	*	
	CBC w Diff & Plt	X	X							X				X	*	
	Chemistries	X	X							X				X	*	
	HBsAG & Anti-HCV	X ¹														
	Syphilis Serology	X													*	
	Plasma Archive		X													
	Blood Maraviroc & Dapivirine levels		x @ hr 0,1,2,4,6	X	X	X	X	X	X	X	x @ hr 0,1,2,4,6	X	X	X	X	
VAGINAL	Vaginal pH	*	X	*	X	*				X	*	X		X	*	
	Gram Stain		X		X					X		X		X		
	Quant vag. culture		X				X			X				X		
	KOH wet mount	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
	Wet Mount for BV	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
	Test for Trichomonas	X	*	*	*	*	*	*	*	*	*	*	*	*	*	
	NAAT for GC/CT	X	*	*	*	*	*	*	*	*	*	*	*	*	*	
	Tear Test Strip for Vag PK		X	X	X	X	X	X	X	X	X		†	†	X	
	Vaginal Swab for Validation				X						X					
	Vag Swab Biomarkers		X		X						X		X		X	
	Vaginal Swab for PD same swab as biomarkers		δ x								δ x		δ x			
	Collect Used VR										x ⊖				▲	
	CERVICAL	Cervical Biopsy for PK									x		†	†		
Cervical Biopsy for PD (ex vivo challenge)										x						
Pap Smear		*														
		SCR	ENR D0	D1 D2	D3	D5	D7	D14	D21	D28	D29 D30	D31	D35 D42	D52	Interim	

¹ if not previously completed at the Screening Visit prior to LoA #01 approval, then perform at the next study visit

X Required.

* If clinically indicated.

⊖ Designated site(s) only: a biofilm (Pitt) and PK residual assessment (UAB & Fenway) will be performed on the used ring that is removed at the Day 28 visit if the ring is removed by a study clinician. In the event that a VR is removed at an earlier visit, the used ring will have a biofilm assessment if assessment criteria are met, including VR removal by a study clinician/designee.

† Day 31, 35, or 42 are based on 1/3 group randomization assignment.

▲ Execute if an early termination visit or not already performed, collect VR.

δ On these days, the swab will be shared with biomarkers.

12.2 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. If the date is handwritten, it should be in indelible ink (such as a Sharpie pen).

Microscope slides used for evaluation of vaginal fluids also will be labeled with SCHARP provided PTID labels. 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 12.4 for tests that will be entered into LDMS and labeled with LDMS-generated labels.

12.3 Procedures for Specimens that can not be evaluated

Specimens will be redrawn or recollected if it is found that they can not be evaluated per site SOP's. Sites will monitor specimen management problems as part of ongoing Quality Assurance. In cases where additional specimens need to be recollected either due to a laboratory error (lost or broken specimen or clerical error) or clinic error (clerical error), a protocol event form may be required.

12.4 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 plasma, blood PK, vaginal smears for gram stain, vaginal cultures, vaginal PK, vaginal biomarkers and PD, used VR for PK residual assessment, VR cultures, VR for FISH & SIM, cervical biopsy for PK, and cervical biopsy for PD.

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).

Questions related to use of LDMS in MTN-013/IPM 026 may be directed to Wayne Hall or LDMS Technical (User) Support. Usual business hours for LDMS User Support are 7:30 am - 6:00 pm (ET) on Monday and Fridays and 7:30 am - 8:00 pm (ET) on Tuesdays, Wednesdays, and Thursdays. During business hours, please contact LDMS User Support as follows:

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

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 NL 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 NL 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 NL 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 Image 1)
- Enter the PK time point information in Time and Time Unit area (See Image 1)

IMAGE 1: LDMS Entry Screen

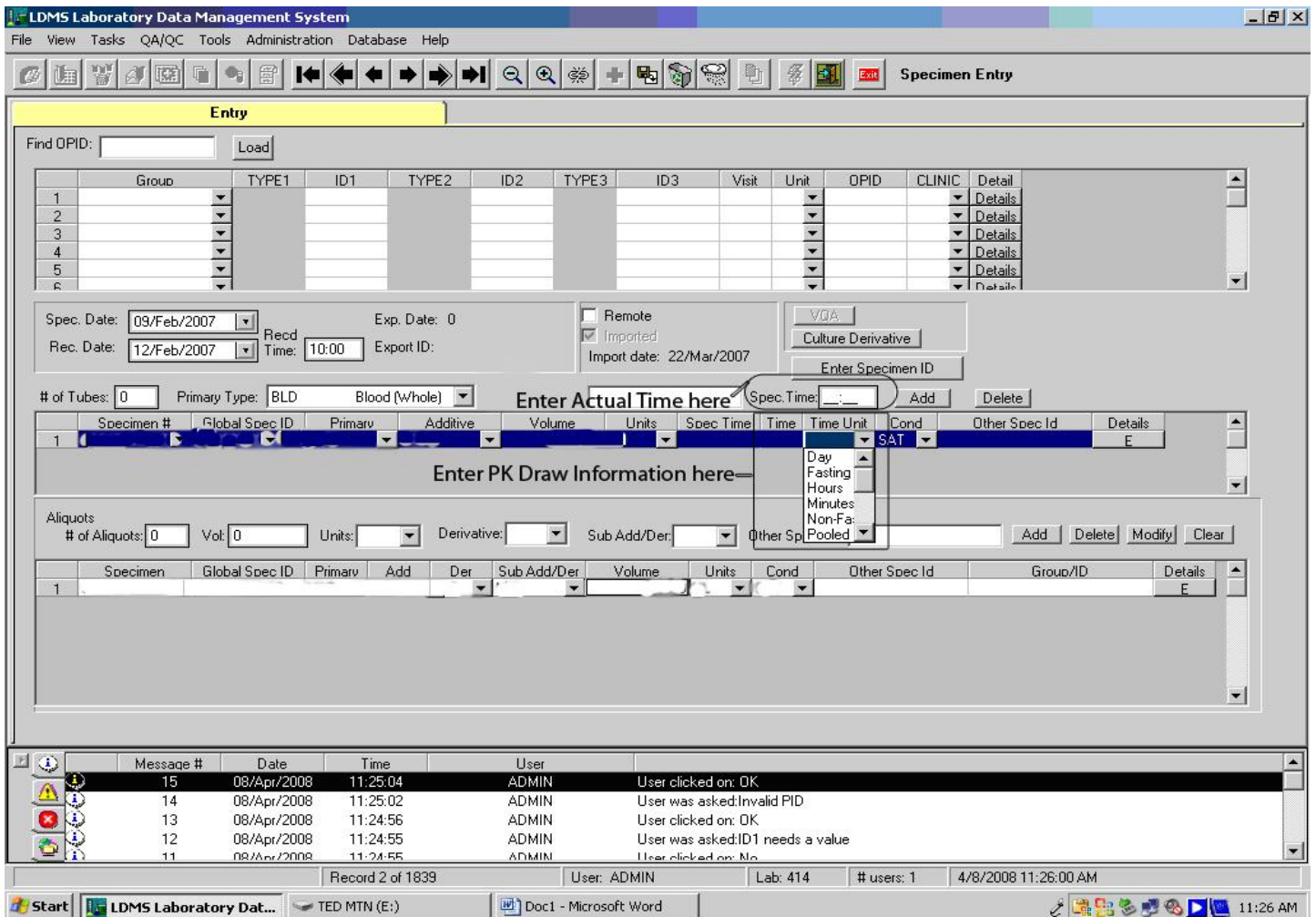


Table 12-4: LDMS Specimen Management Guide to Logging in MTN-013 Specimens

The table below should be used as a guide when logging in MTN-013/IPM 026 specimens. Please use the LDMS codes listed below when logging in specimens for each test listed. See Appendix 12-2 for a copy of the LDMS tracking sheets created for this study.

Test	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALIQOT DERIVATIVE	ALIQOT SUB ADDITIVE/ DERIVATIVE	Aliquot volume	Units	INSTRUCTIONS FOR PROCESSING LAB
Plasma for Storage	BLD	EDT (purple top)	PL1/2	N/A	1-2	mL	Prepare as many 1.5 mL aliquots as possible with a total volume of aliquots ≥ 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 and Maraviroc analysis)	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.
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 NL and store other slide on-site.
Vaginal Swab for Quantitative Culture	VAG	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 NL on the day of collection.
Vaginal PK Tear Flo Strip	VSC	NON	TFS	N/A	2 strips/ 2 tubes (1 strip in each tube)	Each	Pre & post weigh each tube+strip. Place in transport tube and store at ≤-20°C. Send to NL Pharmacology Core at end of study.
Vaginal Swab for Validation	VAG	NON	SWB	N/A	1 swab in cryovial	Each	Place Dacron swab in a labeled cryovial. Store sample tubes at ≤-20°C. NL will coordinate shipment at end of study.

Test	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALIQOT DERIVATIVE	ALIQOT SUB ADDITIVE/ DERIVATIVE	Aliquot volume	Units	INSTRUCTIONS FOR PROCESSING LAB
Vaginal Swab for PD & Biomarkers	VAG	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. NL will coordinate shipment at end of study.
Used Vaginal Ring for PK residual assessment <i>Fenway & UAB only</i>	IVR	NON	IVR	NA	1 auto-claved ring in bag	Each	Autoclave used vaginal ring in autoclavable foil bag and store at room temp. NL will coordinate shipment at end of study.
Used vaginal ring for Biofilm, FISH and SEM <i>Pittsburgh only</i>	IVR	NON	IVR	N/A	Petri dish	Each	Immediately place ring into a sterile container and transport to MTN NL for processing.
Cervical Biopsy for PK	CVB	NON	TIS	N/A	2 1.8mL cryovial	Each	Pre (without biopsy) and post (with biopsy) weigh cryovials. Place tissues (2 biopsies/PK time- point) in pre-chilled cryovials and store at ≤-70°C locally
Cervical Biopsy for PD (ex vivo challenge) <i>Fenway & UAB</i>	CVB	TFM	TIS	N/A	1 1.8mL cryovial	Each	Place biopsy for ex vivo challenge into a 2ml cryovial containing 1ml of sterile freezing mix (90% FBS + 10% DMSO), Freeze in a slow rate freezing container for 24 hrs, and store at ≤-70°C . Send to MTN NL at end of study.
Cervical Biopsy for PD (ex vivo challenge) <i>Pittsburgh only</i>	CVB	BTM	TIS	N/A	1 50 mL conical tube	Each	Place biopsy into a 50 ml conical tube containing 10 ml of chilled biopsy transport medium, take to MTN lab immediately for processing.

BLD: Whole Blood	NON: No Additive	TIS: Tissue
BTM: Biopsy Transport Medium	PAC: Port-a-Cul Vial	TFM: Tissue Freezing Medium (90% FBS + 10% DMSO)
CVB Cervical Biopsy	PBS: Phosphate buffered saline	TFS: Tear Flow Strip (same as Tear Test Strip)
EDT: EDTA	PL1/2: Single or double spun plasma	TIS: Tissue
GRS: Gram Stain	SLD: Slide	VAG: Vagina
IVR: Intravaginal ring	SWB: Swab	VSC: Vaginal Secretions

12.5 Urine Testing for Pregnancy and 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 aliquotted for each test when possible. When performing multiple tests from one specimen, the correct order is pregnancy testing first, then the urine dipstick.

12.5.1 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 20-30 ml of midstream urine in a sterile collection cup. Instruct the participant to screw the lid tightly onto the cup after collection.

12.5.2 Pregnancy Testing

The Quidel QuickVue One-Step hCG urine or Quidel QuickVue Combo hCG urine/serum 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-013/IPM 026. 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 can not 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.

Participants who become pregnant will be permanently discontinued from VR use and will be instructed to return the study VR. All protocol-specified study procedures will continue except the following: urine hCG testing, provision of study product; acceptability, protocol and product use adherence assessments; pelvic exams; PK specimen collection (blood and pelvic samples); and provision counseling for product adherence and contraception.

12.5.3 Dipstick Urinalysis

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

Only leukocytes and nitrites are required at the screening visit. Any of the Siemens urine reagent test strips can be used. Perform this test according to site SOPs and the package insert. Assess and record results for leukocytes and nitrites. If leukocytes or nitrites are

positive, perform a urine microscopy and a urine culture according to local SOP. To avoid overgrowth of bacteria, refrigerate specimen before and during transport to laboratory.

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

12.6 Blood Specimens for HIV testing, Hematology, Chemistries, Syphilis, Plasma Archive, Blood Dapivirine, Blood Maraviroc

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.

12.6.1 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 syphilis, chemistries, and/or HIV testing.
- Lithium Heparin tubes may also be drawn for chemistry 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, plasma archive, Maraviroc, and Dapivirine Level. If whole blood for hematology testing and plasma are to be taken from the same tube, the hematology 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.

12.6.2 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.

HIV infection status will be assessed using an FDA-approved HIV immunoassay per the HIV testing algorithm (see appendix 12-2 in this section or appendix II of the MTN-013/IPM-026 protocol). If the screening test is negative, the participant will be considered HIV-seronegative. If the screening test is positive or indeterminate, an FDA-approved Western Blot (WB) or Immunofluorescent Antibody (IFA) test will be performed on the original screening sample (Sample 1). If there is insufficient sample to perform WB or IFA, then additional blood must be recollected and must still be regarded as screening Sample 1 per the algorithm. If the WB or IFA is negative or indeterminate, contact the NL for guidance. If the WB or IFA is positive for the screening visit, patient is considered seropositive and will not be eligible for enrollment. If the WB or IFA is positive for any other

visit, a second specimen (Sample 2) will be drawn for confirmatory testing. If the WB or IFA is negative or indeterminate, the site should contact the NL for further instructions.

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

12.6.3 Hematology Testing

Complete blood counts will be performed at all sites according to protocol at the Screening and Final Clinic Visits.

Each of the following must be analyzed and reported:

- Hemoglobin
- Hematocrit
- Platelets
- White blood cell count with differential
- Red blood cell count

These tests will be performed on EDTA whole blood per local site SOP's.

12.6.4 Serum Chemistries

Chemistry tests will be performed on serum and/or plasma per local SOP's.

Liver Function

- Aspartate aminotransferase (AST)
- Alanine transaminase (ALT)

Renal Function

- At Screening, the participant's creatinine clearance rate will be calculated, per the Cockcroft-Gault formula listed in protocol Section 5.3. The Creatinine Clearance Calculator is located at the MTN website (<http://www.mtnstopshiv.org/node/3594>) in the Study Implementation Materials section of the MTN-013/IPM 026 protocol.

12.6.5 HBsAg test Anti-HCV

Hepatitis B Surface Antigen (HBsAg) and Hepatitis C Virus antibody (Anti-HCV) testing will be performed upon screening visit, however if not previously completed at the Screening Visit prior to LoA #01 approval, then perform at the next study visit. HBsAg and Anti-HCV will be performed on serum and/or plasma per local SOP's.

12.6.6 Syphilis Testing

Syphilis testing will be performed using an FDA approved 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 microhemagglutinin assay for *Treponema pallidum* (MHA-TP), *Treponema pallidum* hemagglutination assay (TPHA), *Treponema pallidum* particle agglutination (TP-PA), or fluorescent treponemal antibody (FTA-ABS). All positive RPR results must have a titer

obtained and reported. 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.

For reactive RPR tests observed during screening, a confirmatory test result must be received. If a confirmation test is positive, then the participant will not be eligible for enrollment. Appropriate clinical management should include repeat RPR tests at quarterly intervals following syphilis diagnosis to confirm treatment effectiveness. If the RPR titer does not decrease four-fold or revert to seronegative within three months after treatment, treatment should be repeated.

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

Questions related to result interpretation in relation to eligibility and enrollment in the study should be directed to the MTN-013/IPM 026 Protocol Safety Physicians (mtn013safetymd@mtnstpshiv.org).

12.6.7 Plasma Archive

For plasma archive, use collection tubes with EDTA anticoagulant. Aliquot plasma into 2 ml cryovials, store at $\leq -70^{\circ}\text{C}$, and batch onsite until the MTN NL 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 on ice after collection, freeze within 24 hours.
- Prepare as many 1.5 mL aliquots as possible with a total volume of aliquots greater than or equal (\geq) to 4ml
- If total volume is less than 2.0 mL, redraw as soon as possible.
- If less than 4 mL of plasma are available, store that plasma and inform the MTN NL for instruction.
- If samples are hemolyzed, store the aliquots as per normal and enter comments in LDMS.
- The MTN NL will send instructions to the site when shipping and/or testing is required.

12.6.8 Blood Dapivirine and Maraviroc

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 3000 rpm (1500 x g) for 10 minutes. **The centrifugation must be completed and sample placed in the freezer within 8 hours of blood collection.**
3. Use a pipette to aliquot approximately 1.5-2.0 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 $\leq -20^{\circ}\text{C}$ 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 Network Lab in Baltimore, MD and assayed for Dapivirine and Maraviroc at conclusion of study unless informed otherwise. The back-up samples will be retained at the site until advised by the MTN-013/IPM 026 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

12.7 Testing of Vaginal Specimens

Refer Section 7 of this manual for further information of the required sequence of specimen collection and diagnostic procedures to be performed during study pelvic exams.

12.7.1 Vaginal pH

Vaginal 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, as follows:

- During pelvic examination, collected vaginal fluid via swab (Dacron or cotton) and then swab onto the pH strip (Do not insert the pH strip into the vagina).
- Match the resulting color of the indicator strip to the color scale provided with the strips to determine the pH value.
- 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.

12.7.2 Gram Stains on Vaginal Fluid

Dried vaginal fluid smears will be prepared for Gram staining and assessment for bacterial vaginosis at the MTN NL. 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 NL 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:

- Use a pencil to write the PTID and specimen collection date on one side of the frosted end of one microscope slide. 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. Also write "V" for vaginal on each label.
- 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.
- Allow the specimens to air-dry on the slides. Do not heat-fix.
- Deliver the slides and an 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 slides with LDMS labels. Place the LDMS label on the frosted end of the slide, on the opposite side of the slide from the SCHARP-provided label, on top of the pencil markings.
- The primary slides will be positioned in a plastic slide holder and sent to the MTN NL 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 Womans Research Institute. (See shipping instructions below).
- 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).

12.7.3 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 NL. Shipping instructions follow.

- Collect the specimen for culture by rotating 2 Dacron swabs several times over the lateral wall of the vagina. Insert swabs into 1 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 NL.)
- 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.

12.7.4 Shipping instructions to MTN NL

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 NL 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.

12.7.5 Vaginal Fluid Wet Mount Testing for BV and yeast (Only if indicated)

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

- potassium hydroxide (KOH) prep
- Saline prep

These procedures are for diagnosis of bacterial vaginosis and candidiasis as summarized in Table 12-5 below.

If wet prep slides are read in-clinic by clinical staff, results may be recorded directly onto 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 onto 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 NL will administer a web-based proficiency test approximately every six months. The MTN NL 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 NL 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 NL for additional information and guidance on performing and documenting the proficiency

testing. Also contact the MTN NL when new laboratory staff is hired, so that appropriate training can take place prior to such staff performing wet mounts for study purposes.

Table 12-5: 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: 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.

12.7.6 Rapid Test for Trichomoniasis

This testing will be done using the Genzyme Rapid Trichomonas test with vaginal swabs per site SOPs approved by the MTN NL. 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.

12.7.7 NAAT Chlamydia and Gonorrhea Testing

Note: 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 NL for testing. Contact the NL prior to sending specimens for GC/CT testing.

Collect vaginal or cervical samples (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.

12.7.8 Tear Test Strip for Vaginal PK Assessment

The tear test strips are used to absorb Cervicovaginal secretions and the consequent analysis of Dapivirine and Maraviroc. The tear strips will be placed in two different locations in the vagina near the introitus (before the speculum is inserted) and at the surface of the cervix (after the speculum is in place). TearFlo strips have been supplied in glass tubes and are marked with the locations that sampling will occur at.

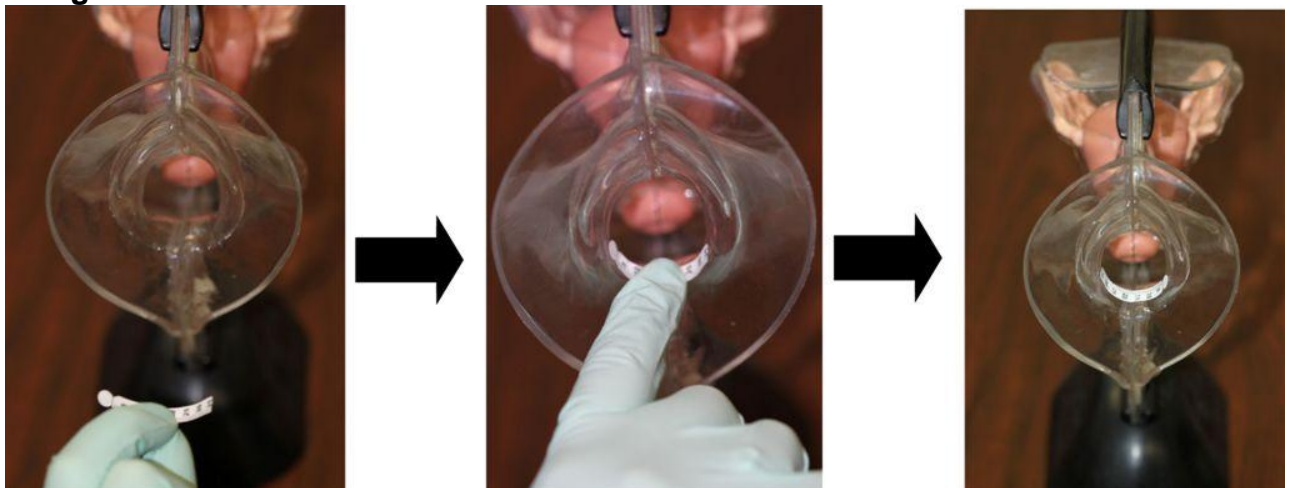
Procedure for Tear Test Strip Sampling:

1. Tear test strip specimen should be collected within one hour of PK blood draw.
2. Please ensure that a new Tear Test Strip and new disposables (gloves, tweezers, tubes, etc.) are used for each sample, as Dapivirine and Maraviroc are very sensitive to cross-contamination.
3. Test Tear Strips (called TearFlo Strips) have been supplied in 11 ml (16x100mm) glass tubes and are marked with the locations that sampling will occur at. Label the proper tube with participant study and sample identification information.
4. Perform any internal or external QC (using certified weights) that would be necessary for the analytical scale to accurately weigh samples to a weight of at least 0.1 milligrams.
5. Weigh the glass tube containing the TearFlo Strip (including the lid) on an analytical balance. Document this pre-weight of each labeled cryovial on the CRF.

6. Remove the TearFlo strip from the tube using forceps. Make sure that the vaginal location sampled is the same that is marked on the tube. Insert the strip into the vagina and apply the strip to the epithelial surface of each designated sampling location:

- Introitus: Before the speculum is in place and while using clean gloves, separate the vulva and use fingers to position the tear test strip into the introitus perpendicular and horizontal to the vaginal canal (e.g. circumferentially along the posterior hymenal ring). Curve the strip in a semi-circle shape and apply enough pressure so the entire surface of the strip adheres to the posterior part of the vaginal wall (see Figure 1). After tear strip is in place, remove fingers to allow vulva to remain closed for 2 minutes to allow strip to absorb. Once absorption is complete, remove strip with forceps.

Figure 1



- Cervix: Position the entire or at least part of the tear strip on the surface of the cervix. Either lateral or vertical positioning (in relationship with the vagina) on the face of the cervix will be acceptable.

Each strip should be left in place until the strip becomes partly moist for up to 2 minutes. If absorption is poor (less than 1/3rd of the strip becomes moist) after 2 minutes, reposition the strip to a slightly different location in close proximity to the target location. Lay the entire strip flat onto the tissue for an additional 3 minutes. Optimally, half or larger part of the strip is the desired amount of saturation. Adsorption usually takes approximately two minutes, but may take a little longer. The site clinician should routinely check the absorbance of the strip which varies. The total specimen collection time should not surpass 5 minutes.

7. Immediately place strip back into the glass tube after sampling is complete and reattach screw top lid.
8. Weigh the glass tube containing the absorbed TearFlo Strip (including the lid) on an analytical balance and record in the CRF.

9. Within 2 hours, place the sample tubes in the freezer at $\leq -20^{\circ}\text{C}$.
10. At the end of the study, ship in dry ice on Monday or Tuesday to the MTN Network Lab in Baltimore, MD (Johns Hopkins University).

Procedure notes:

- If excess mucus or menses clot has accumulated, a large cotton-tipped cotton swab may be used to gently remove this material before inserting the strip.
- First strip should be held in place on the vaginal surface (inside the vagina) near the vaginal opening (introitus)
- Second strip should be placed on the surface of the Cervix

The shipping address for PK Tear Strip 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**

12.7.9 Vaginal Swab for PD (Pharmacodynamics) and/or Biomarkers

The pharmacodynamics (PD) of Dapivirine and Maraviroc will be studied to determine the effectiveness of the drugs by evaluating the anti-HIV-1 activity present in the genital tract. Biomarkers will also 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 and PD analysis at the MTN NL. The swab will be used for biomarkers for visit days: 0, 3, 28, 31, and 52. The swab will be used for both biomarkers and PD (the swab will be shared) on visit days: 0, 28, and 31.

Procedure:

- 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 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 NL 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

12.7.10 Vaginal Swab for Validation

One vaginal swab will be used to absorb Cervicovaginal secretions for the analysis and validation method that will be performed at the Clinical Pharmacology Department in Johns Hopkins University School of Medicine. This swab will be collected on visit day 3 and 28.

- Collect vaginal fluid using a Dacron swab from the posterior fornix.
- Place swab in a 1.8 cryovial marked PK Swab, break off stick, and cap.
- Within 2 hours, place the sample tubes in the freezer at $\leq -20^{\circ}\text{C}$.
- At the end of the study, ship in dry ice on Monday or Tuesday to the MTN Network Lab in Baltimore, MD (Johns Hopkins University).

The shipping address for PK Swab validation 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**

12.8 Testing of Intravaginal Ring (VR)

VR's will be collected by the study clinician at all three sites. Rings collected at UAB and Fenway will be dedicated for residual drug analysis. Rings collected at the University of Pittsburgh will be dedicated for biofilm assessment.

12.8.1 Testing of Intravaginal Ring (VR) for Residual PK: UAB, Fenway and Pittsburgh Sites

Note: Used rings from the Pittsburgh site will be used for residual PK analysis testing on the additional 8 participants reassigned to the Pitt site and if biofilm testing cannot be performed i.e. if the ring was not removed by the study clinician.

Used rings will be collected at the end of the study to be analyzed for residual levels of Dapivirine and Maraviroc. The used rings may contain vaginal secretions and therefore all used rings must be treated as a biohazard and autoclaved in foil pouches. The autoclaved rings will remain in the foil pouch and stored at the site until further notice from the MTN NL.

Step 1: On visit day 28, the clinician will remove the used ring and rinse the ring with sterilized water and blot it dry. 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 Participant Follow-up Section 6.6.1).

Step 2: Site staff will place the ring into the 5"X8" FoilPAK pouch (see figure 1) and seal it with the autoclave tape. Using permanent ink pen, label the bag with the participant ID number and visit number. Add a Biohazard sticker (very important if the pouch is not immediately autoclaved) to the outer FoilPAK, making sure not to cover the identifier information.

Step 3: Rings must be autoclaved at 121°C for a minimum of 15 minutes with the pressure at 15 psi.

Step 4: All pouches must now have identifying information such as participant number and visit number for each ring.

Step 5: Place the FoilPAK pouches, into the 4 litre Biohazard bag (see figure 2). Use the returned ring shipping manifest to document the ring number information being placed into the bag. **Once the biohazard bag is sealed, it CANNOT be re-opened until received at the final destination.**

Step 6: Store used rings within the biohazard bag under room temp or refrigerated conditions until ready for shipment. All refrigerators where rings are stored must contain a biohazardous label on the outside indicating the storage of biohazardous material.

Step 7: The use of LDMS is required to log in all used rings.

Step 8: At the end of the study, NL will coordinate shipment to:

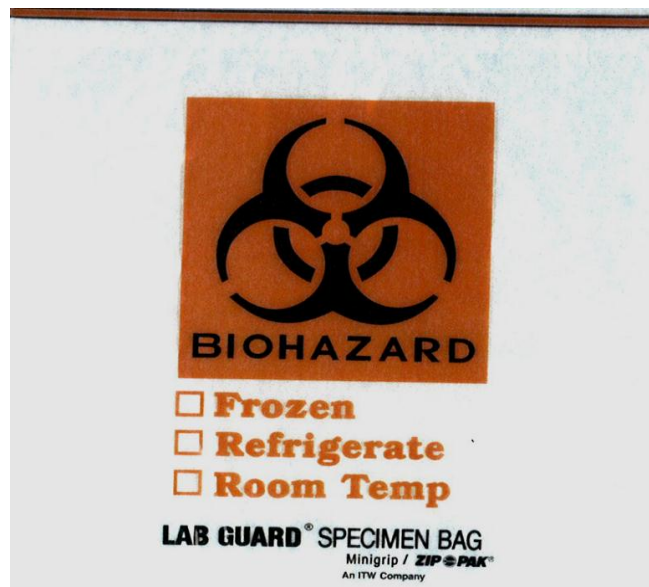
**Pharmavize
Attn Dave Seghers
Kleimoer 4
9030 Mariakerke
BELGIUM
Tel 0032 9 267 65 00
Fax 0032 9 267 65 10**



Figure 1

FoilPAK* Heat-Sealable Pouch. 4.5mil white tri-laminate foil barrier. With three side seal, pouch. Retortable pouch withstands autoclaving, retorting, boiling, and freezing. Seals at approximately 165[degree]C. Size: 12.7x20.3cm. For food and medical industries.

Figure 2



12.8.2 Testing of Intravaginal Ring (VR) for Biofilms (Pittsburgh site only)

Note: Used rings for which biofilm testing cannot be performed i.e. if the ring was removed by the study participant or has been contaminated, should be autoclaved and prepared for residual PK analysis testing.

The VR will be tested by culture to quantify and identify the bacteria adhering to the ring. Sections of the ring will also be evaluated using scanning electron microscopy (SEM) to view and photograph any biofilms present on the rings and to measure the size of the biofilms. Another section of the ring will be stained using fluorescence in situ hybridization (FISH) with a universal oligonucleotides probe that targets most bacteria to detect the bacteria present in the film. **Only rings that have been removed by the clinician will be tested.** This is to ensure the viability of the organism on the ring and eliminate the possibility of external organisms contaminating the ring.

The clinician will remove the ring at the exit visit and place into a sterile petri dish. The lid of the petri dish is to be taped closed and immediately delivered to the MTN Hillier lab for processing.

Notes: If the clinician removes the ring before the end of the study that will not be reinserted, then a biofilm assessment will be performed on the ring. Any ring removed by the participant and not reinserted will not be assessed for biofilms.

If the ring can not be delivered immediately, call the Hillier Laboratory at 412-641-6041 (or 412-641-6042).

12.9 Testing of Cervical Specimens

12.9.1 Cervical Biopsy

The cervical biopsy should be the last part of the exam. Three biopsy specimens (2 for PK and 1 for PD) are collected on Day 28 from different areas of the cervix. Biopsy specimens for PK will also be collected on 31, 35, or 42, depending on the participant's randomized assignment. The biopsy will be collected as described in the site SOP. Standard cervical biopsy instruments (Kevorkian, Tischler, etc) with a bite size measuring approximately 3 x 5 or 3 x 7 mm are used. 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 013 SSP Section 10.5.2.

12.9.2 Cervical Biopsies for PK analysis

1. Label 2 1.8 ml cryovials (Nunc or Nalgene) with the appropriate sample/study identification information.
2. Weigh the labeled cryovial using an analytical balance with a sensitivity rating of 0.1 milligrams or better. Document this pre weight of each labeled cryovial on the CRF.
3. Cool cryovials on ice prior to vaginal biopsy transfer.
4. Transfer each cervical biopsy to its designated cool pre-weighed cryovial.
5. Obtain the post weight for each cryovial containing a cervical biopsy using an analytical balance and document on the CRF. Note: Prior to weighing, condensation on the cryovial should be removed.
6. Immediately freeze the cryovial containing the cervical biopsy in dry ice or liquid nitrogen.
7. Store the labeled cryovials containing the biopsies at $\leq -70^{\circ}\text{C}$. Document the date and time the cryovial containing the cervical biopsy was frozen.
8. 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**

Note: All frozen PK samples (Blood PK, Tear Strip PK, and Biopsy PK) will be going to John Hopkins at the end of the study and may be shipped together on dry ice. Make sure all sample types are arranged in their own container with correct LDMS labeling. Shipping should be scheduled for a Monday or Tuesday pick up to ensure delivery before the weekend.

12.9.3 Cervical Biopsy for PD (HIV ex vivo challenge):

On day 28, one of the three biopsies collected will be used for the HIV ex vivo challenge. The Pittsburgh site will retain a fresh biopsy that will be transported immediately to NL in transport media. Fenway and UAB will follow the frozen biopsy procedure to ensure the correct method of freezing mucosal tissue for its viable recovery.

Fresh PD Biopsy procedure (Pitt only):

A fresh biopsy will be collected and inserted using forceps into a 50 ml conical tube with 10 ml of biopsy transport medium (kept at 4°C). Release tissue directly into transport media by gently shaking tube until biopsy is dislodged from forceps. Immediately transport to MTN Charlene Dezzutti's lab. If Pitt CRS staff can not take to MTN NL laboratory, call extension 1-6157 for biopsy pick-up. If there is no answer, call pager#: 412-917-9343.

Frozen PD Biopsy Procedure (Fenway & UAB):

1. Thaw 900 µL FBS (pre-aliquoted in a 1.8ml cryovial) in the refrigerator overnight prior to use.
2. Add 100 µL of DMSO to a 1.8 ml cryovial containing 900 µL FBS (previously frozen) and mix. This mix must be prepared at least 30 minutes in advance and kept refrigerated prior to introducing the biopsy.
3. Weigh the labeled cryovial using an analytical balance with a sensitivity rating of 0.1 milligrams or better. Document the pre-weight of the labeled cryovial on the CRF.
4. Transfer the cervical biopsy to its designated pre-weighed cryovial.
5. Obtain the post-weight for each cryovial (containing the cervical biopsy in transport media) using an analytical balance and document on the CRF. **Note:** Prior to weighing, condensation on the cryovial should be removed.
6. Place the cryovial containing the tissue/biopsy and freezing media into a pre-cooled* slow rate freezing container**, then freeze at $\leq -70^{\circ}\text{C}$ for 24 hours.
7. Cryovials can then be removed from the freezing container, placed in storage boxes and returned to $\leq -70^{\circ}\text{C}$. For prolonged storage (greater than 2 months), store in liquid nitrogen if available.
8. At the conclusion of the study, ship to NL. Place samples on dry ice, ensuring enough dry ice is included to maintain freezing conditions for the duration of shipment and any potential disruption in the delivery process.

9. At the end of the study, ship on Monday or Tuesday to ensure delivery before the weekend.

*Pre-cooled: pre-cool the slow rate freezing container to 4°C for 30 minutes prior to adding cryovial with tissue

** Slow Rate Freezing Container: Any can be used such as Mr. Frosty or CoolCell™

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**

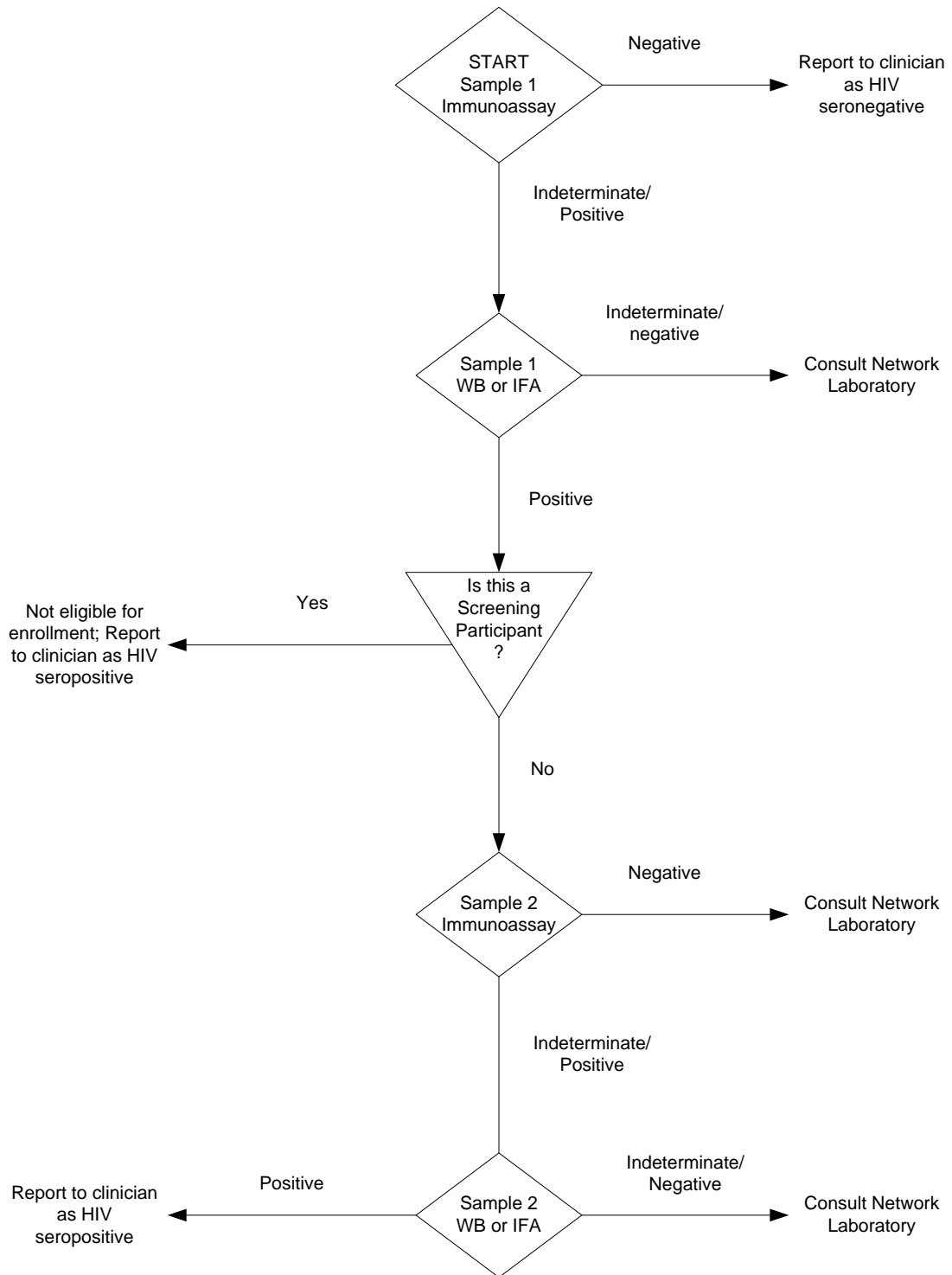
12.9.4 Papanicolaou (Pap) Test (* only if indicated)

Pap smears are only required if clinically indicated or if a participant has not had a documented normal test within 12 months. 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. 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 12-1: HIV ANTIBODY TESTING ALGORITHM



Appendix 12-2 MTN-013/IPM 026 LDMS Tracking Sheet

For login of stored specimens into LDMS

Participant ID			Visit Code		Specimen Collection Date		
<input type="text"/> <input type="text"/> <input type="text"/> -	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> -	<input type="text"/>	<input type="text"/> <input type="text"/> .	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/>
Site Number	Participant Number	Chk			MMM	yy	

# of TUBES or SPECIMENS	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALIQUOT DERIVATIVE	ALIQUOT SUB ADD/DER	INSTRUCTIONS FOR PROCESSING LAB
<input type="checkbox"/>	Blood (BLD) <i>Plasma Archive</i> Collection Time ____:____ <i>hour : min</i>	EDT (purple top)	PL1/2	N/A	Prepare as many 1.5 mL aliquots as possible with a total volume of aliquots ≥ to 4ml. If sample is collected and held at room temp, freeze within 4 hours. If refrigerated after collection, freeze within 24 hours.
<input type="checkbox"/>	Blood (BLD) <i>PK-Single time-point</i> Collection Time ____:____ <i>hour : min</i>	EDT (purple top)	PL1/2	N/A	Transport to lab ASAP. Centrifuge, split, and label two or more cryovials with a minimum of 1.5mL of plasma in each cryovial. Freeze within 8 hrs of blood collection.
<input type="checkbox"/>	Vaginal Tear Test Strip (VSC) Collection Time ____:____ <i>hour : min</i>	NON	TFS	N/A	Place in glass transport tube and store at ≤-20°C.
<input type="checkbox"/>	Vaginal Smear for Gram Stain (VAG)	NON	SLD	GRS	Re-label with LDMS label. Make 2 slides. Ship one slide to MTN NL and store other slide on-site.
<input type="checkbox"/>	Vaginal Swab for Culture (VAG)	PAC	SWB	N/A	Insert 2 vaginal Dacron swabs into PAC, and ship overnight on ice packs to MTN NL on the day of collection.
<input type="checkbox"/>	Vaginal swab for PD & Biomarkers (VAG)	PBS (400 µL PBS)	SWB	N/A	Place Dacron swab in a labeled 1.5 mL micro tube containing 400 µL PBS. Store sample tubes at ≤-70°C.
<input type="checkbox"/>	Vaginal Swab for Validation (VAG)	NON	SWB	N/A	Insert 1 vaginal Dacron swabs into cryovial and store at ≤-20°C.

Comments:

Initials: _____
Sending Staff Receiving Staff

LDMS Data Entry Date: / / _____
dd *MMM* *yy* LDMS Staff

Purpose: *This non-DataFax form is used to document collection and entry of study specimens into the Laboratory Data Management System (LDMS).*

General Information/Instructions: *A copy of this form accompanies specimens for storage (in their original specimen collection containers) to the LDMS entry laboratory. Once the specimens have been entered into LDMS, this form is kept on file at the LDMS entry laboratory. If the site chooses, a copy of this completed form may be made once the specimens have been entered into LDMS and the copy kept in the participant's study notebook. This is not required, however. Because this form is a non-DataFax form, this form should NOT be faxed to SCHARP DataFax.*

Item-specific Instructions:

- **Visit Code:** Record the visit code of the visit at which the specimens were collected.
- **TUBES or SPECIMENS COLLECTED:** In the box provided, record the total number of tubes or specimens collected for that primary specimen type. If no LDMS specimens of the primary specimen type were collected, record "0."
- **Primary Specimen, Primary Additive, and Aliquot Derivative Codes:** See table below for a listing of the codes.

BLD: Whole Blood	NON: No Additive	TIS: Tissue
BTM: Biopsy Transport Medium	PAC: Port-a-Cul	TFM: Tissue Freezing Medium (90% FBS + 10% DMSO)
CVB Cervical Biopsy	PBS: Phosphate buffered saline	TFS: Tear Flow Strip (same as Tear Test Strip)
EDT: EDTA	PL1/2: Single or double spun plasma	TIS: Tissue
GRS: Gram Stain	SLD: Slide	VAG: Vagina
IVR: Intravaginal ring	SWB: Swab	VSC: Vaginal Secretions

- **Initials – Sending Staff:** The clinic staff person who completed the form and/or who is sending the LDMS form and specimens to the LDMS entry lab, records his/her initials here.
- **Initials – Receiving Staff:** The laboratory staff person who received this form (and the LDMS specimens accompanying the form), records his/her initials here.
- **LDMS Data Entry Date:** Record the date the LDMS specimens listed on this form were entered into LDMS.
- **LDMS Data Entry Date – LDMS Staff:** The LDMS laboratory staff person who entered the specimens into LDMS, records his/her initials here.

MTN-013/IPM 026 LDMS Specimen Tracking Sheet

For login of stored specimens into LDMS

Participant ID			Visit Code		Specimen Collection Date		
[] [] []	[] [] [] [] [] []	[]	[] [] []	[] []	[] [] [] []	[] []	[] []
Site Number	Participant Number	Chk			dd	MMM	yy

# of TUBES or SPECIMENS	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALIQUOT DERIVATIVE	ALIQUOT SUB ADD/DER	INSTRUCTIONS FOR PROCESSING LAB
<input type="checkbox"/>	Cervical Biopsy for PK (CVB) Collection time: ____: ____ hour : min	NON	TIS	N/A	Place tissue in a pre-chilled cryovial and store at $\leq -70^{\circ}\text{C}$ locally.
<input type="checkbox"/>	Fenway & UAB only Cervical Biopsy for PD (ex vivo challenge) (CVB) Collection time: ____: ____ hour : min	TFM	TIS	N/A	Place biopsy for ex vivo challenge into a 1.8 ml cryovial containing 1ml of sterile freezing mix (90% FBS + 10% DMSO), Freeze in a slow rate freezing container for 24 hrs, and store at $\leq -70^{\circ}\text{C}$. Send to MTN NL at end of study.
<input type="checkbox"/>	Fenway & UAB only Used vaginal ring for PK residual assessment (IVR) Collection time-Day 28 ____: ____ hour : min	NON	IVR	N/A	Autoclave used ring in autoclavable bag and store at room temp.
<input type="checkbox"/>	Pittsburgh only Cervical Biopsy for PD (ex vivo challenge) (CVB) Collection time: ____: ____ hour : min	BTM	TIS	N/A	Place biopsy into a 50 ml conical tube containing 10 ml of chilled biopsy transport medium, take to MTN NL immediately for processing.
<input type="checkbox"/>	Pittsburgh only Used vaginal ring for Biofilm, FISH and SEM (IVR) Collection time-Day 28 ____: ____ hour : min	NON	IVR	N/A	Immediately place ring into a sterile container and transport to MTN NL for processing.

Comments:

Initials: _____
Sending Staff
Receiving Staff

LDMS Data Entry Date: [] [] [] [] [] [] / _____
dd
MMM
yy
LDMS Staff

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MTN-013/IPM 026 LDMS Specimen Tracking Sheet

Enrollment and Day 28 Blood PK *For login of stored specimens into LDMS*

Participant ID		Visit Code		Specimen Collection Date		
<input type="text"/> <input type="text"/> <input type="text"/> - <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> - <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/>		<input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/>			
<small>Site Number</small>	<small>Participant Number</small>		<small>Chk</small>	<small>dd</small>	<small>MMM</small>	<small>yy</small>
# of TUBES or SPECIMENS	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALIQUOT DERIVATIVE	ALIQUOT SUB ADD/DER	INSTRUCTIONS FOR PROCESSING LAB	
<input type="checkbox"/>	Blood PK – 0 Hour (BLD) Collection Time ____:____ <i>hour : min</i>	EDT (purple top)	PL1/2	N/A	Transport to lab ASAP. Centrifuge, split, and label two or more cryovials with a minimum of 1.5mL of plasma. Freeze within 8 hrs of blood collection.	
<input type="checkbox"/>	Blood PK – Hour 1 (BLD) Collection Time ____:____ <i>hour : min</i>	EDT (purple top)	PL1/2	N/A	Transport to lab ASAP. Centrifuge, split, and label two or more cryovials with a minimum of 1.5mL of plasma. Freeze within 8 hrs of blood collection.	
<input type="checkbox"/>	Blood PK – Hour 2 (BLD) Collection Time ____:____ <i>hour : min</i>	EDT (purple top)	PL1/2	N/A	Transport to lab ASAP. Centrifuge, split, and label two or more cryovials with a minimum of 1.5mL of plasma. Freeze within 8 hrs of blood collection.	
<input type="checkbox"/>	Blood PK – Hour 4 (BLD) Collection Time ____:____ <i>hour : min</i>	EDT (purple top)	PL1/2	N/A	Transport to lab ASAP. Centrifuge, split, and label two or more cryovials with a minimum of 1.5mL of plasma. Freeze within 8 hrs of blood collection.	
<input type="checkbox"/>	Blood PK – Hour 6 (BLD) Collection Time ____:____ <i>hour : min</i>	EDT (purple top)	PL1/2	N/A	Transport to lab ASAP. Centrifuge, split, and label two or more cryovials with a minimum of 1.5mL of plasma. Freeze within 8 hrs of blood collection.	

Comments:

Initials: _____
Sending Staff Receiving Staff

LDMS Data Entry Date: / / _____
dd MMM yy LDMS Staff

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