

Section 9. Laboratory Considerations

9.1	Overview and General Guidance.....	2
9.2	Specimen Labeling	5
9.3	Procedures for Specimens that cannot be Evaluated.....	5
9.4	Use of LDMS	5
9.4.1	Logging in PK Samples.....	7
9.5	Urine Testing	8
9.5.1	Specimen Collection	8
9.5.2	Pregnancy Testing	8
9.5.3	Urine Chlamydia and Gonorrhea Testing	8
9.5.3.1	Instructions for transferring urine into the Gen-Probe UPT	8
9.5.3.2	Instructions for transferring urine into the GeneXpert transport reagent tube	9
9.5.4	Dipstick Urinalysis	9
9.5.5	Urine Culture	9
9.6	Blood Testing.....	9
9.6.1	Specimen Collection and Initial Processing	9
9.6.2	HIV Testing	9
9.6.3	Hematology Testing	10
9.6.4	Liver and Renal Function Testing	10
9.6.5	Syphilis Testing.....	10
9.6.6	Hepatitis B Surface Antigen and Hepatitis C Antibody	11
9.6.7	HSV Serology	11
9.6.8	INR/PT	11
9.6.9	Plasma Archive (baseline) and Plasma Storage.....	11
9.6.10	Blood for Dapivirine PK.....	12
9.7	Testing of Vaginal and Cervical Specimens	12
9.7.1	Papanicolaou (Pap) Test	12
9.7.2	Cervicovaginal Testing for GC/CT (Neisseria gonorrhea and Chlamydia trachomatis) by NAAT	12
9.7.3	Cervicovaginal Fluid for PK.....	12
9.7.4	Cervicovaginal Lavage (CVL) for PD and PK	14
9.7.5	Cervical Biopsies for PK	14
9.8	Testing of Rectal Specimens	14
9.8.1	Anal HSV-1/2	15
9.8.2	Rectal NAAT for Gonorrhea and Chlamydia	15
9.8.3	Rectal Microflora	15
9.8.4	Rectal Swab for PK.....	15
9.8.5	Rectal Sponge for Mucosal Safety.....	17
9.8.6	Rectal Enema for PD and PK	17
9.8.7	Rectal Biopsies for PK	17
9.8.8	Rectal Biopsy for Mucosal Gene Expression Array	18
9.8.9	Rectal Biopsy for Histology	18
9.8.10	Rectal Biopsies for PD	18
9.8.11	Rectal Biopsies for Mucosal T Cell Phenotyping	18
9.8.12	Rectal Biopsy for Proteomics.....	19
9.9	Appendices 9-1	20
	Procedure for preparing reagents for MTN-026	20

9.1 Overview and General Guidance

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

As transmission of HIV and other infectious agents can occur through contact with contaminated needles, blood, blood products, rectal, and vaginal secretions, 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 and Prevention 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), including the MTN Pharmacology Core (Johns Hopkins University Clinical Pharmacology Analytical Lab or JHU CPAL). Table 9-1 lists for each test, the testing location, specimen type, specimen container and kit/method (if specified). Table 9-2 specifies specimen collection for storage and shipment.

Regardless of whether tests are performed in clinic or laboratory settings, study staff that performs the tests must be trained in proper quality control (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.

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

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.

This section of the MTN-026 SSP Manual gives basic guidance to the sites, but is not an exhaustive procedure manual for all laboratory testing. This section must be supplemented with site Standard Operating Procedures (SOP). The MTN LC is available to assist in the creation of any SOPs upon request. Essential SOPs include but are not limited to:

- SOPs created by the site
- Specimen Collection and Transport*
- Chain of Custody *

*Must be approved by the MTN LC for study activation

**Table 9-1
Overview of Laboratory Testing Locations, Specimens,
And Methods for MTN-026**

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.

Test	Testing Location	Specimen Type	Tube or Container and tube size (recommended)	Kit/Method
Qualitative Urine hCG	Local Lab/Clinic	Urine	Plastic screw top cup	LC approved local methodology
Dipstick Urinalysis	Clinic	Urine	Plastic screw top cup	LC approved local methodology
Urine Culture	Local Lab	Urine	Plastic screw top cup	Not Specified
Urine NAAT for Gonorrhea and Chlamydia	Local Lab	Urine	Kit Specific Transport Tube	GenProbe Aptima or GeneXpert
Complete blood count w/diff and platelets	Local Lab	Whole Blood	EDTA tube 4mL	Local Methodology
Chemistries (Creatinine, ALT, AST)	Local Lab	Serum, plasma, or whole blood	Consult local lab requirements	Local Methodology
Syphilis Serology	Local Lab	Serum or Plasma	EDTA, plain or serum separator tube 4mL	Local Methodology
HIV-1/2 Testing	Local Lab/Clinic	Plasma, serum or whole blood	EDTA or plain tube 4mL	FDA approved tests
HSV-1 and HSV-2 IgG Serology	Local Lab	Serum	plain or serum separator tube 4mL	Local Methodology
Hepatitis B (HBsAg)	Local Lab	Serum or plasma	EDTA, plain or serum separator 4mL	Local Methodology
HCV	Local Lab	Serum or plasma	EDTA, plain or serum separator 4mL	Local Methodology
INR/PT	Local Lab	Whole Blood	Light Blue (Na Citrate) 4mL	Local Methodology
Plasma archive/storage	MTN LC	Plasma	EDTA tube 10mL	MTN LC Protocol
Plasma for PK	JHU CPAL	Plasma	EDTA tube 10mL	JHU CPAL Protocol
Vaginal NAAT for Gonorrhea and Chlamydia	Local Lab	Vaginal swab	Kit Specific Transport tube	GenProbe Aptima or GeneXpert
PAP test	Local Lab	Cervical Cells	Slides	Local Methodology
CVF for PK	JHU CPAL	Cervicovaginal Swab	Cryovial	JHU CPAL Protocol
CVL for PD and PK	MTN LC	Cervicovaginal Lavage	15mL conical tube	MTN LC Protocol
Cervical Biopsy for PK	JHU CPAL	2 cervical biopsies	Cryovial	JHU CPAL Protocol
Anal HSV 1 and 2	Local Lab	Anal Swab	Consult local lab requirements	Local Methodology
Rectal Microflora	MTN LC	Rectal Swab	Cryovial	MTN LC Protocol
Rectal NAAT for Gonorrhea and Chlamydia	Local Lab	Rectal Swab	Kit Specific Transport tube	GenProbe Aptima or GeneXpert
Rectal Swab for PK	JHU CPAL	Rectal Swab	Cryovial	JHU CPAL Protocol

Test	Testing Location	Specimen Type	Tube or Container and tube size (recommended)	Kit/Method
Rectal Sponge for Mucosal Safety	MTN LC	Rectal Sponge	5ml Cryotube	MTN LC Protocol
Rectal enema effluent for PD and PK	MTN LC	Rectal Enema	50mL Conical Tube	MTN LC Protocol
Rectal Biopsies for PK	JHU CPAL	2-5 Rectal Biopsies	1.8mL Cryovial	MTN LC Protocol
Rectal Biopsies for Mucosal gene expression array	MTN LC	2 Rectal biopsies	1.8mL Cryovial with RNAlater	MTN LC Protocol
Rectal Biopsy for Histology	MTN LC	1 Rectal biopsy	2.0mL tube	MTN LC Protocol
Rectal Biopsies for PD	Local Lab validated by MTN LC	2-4 Rectal Biopsies	Biopsy Transport Media	MTN LC Protocol
Rectal Biopsies for Mucosal T cell phenotyping*	Local Lab validated by MTN LC	7 Rectal biopsies	Biopsy Transport Media	MTN LC Protocol
Rectal Biopsies for Proteomics	MTN LC	1 Rectal biopsy	1.8mL Cryovial	MTN LC Protocol

Volumes may vary depending upon each site's testing platforms. Please confirm with the testing lab to determine minimum volume requirements.

Notes: Additional blood may be collected for any clinically indicated testing.
Red top tubes contain no additive.
Purple top tubes contain EDTA.
Light Blue top tubes contain Na Citrate.

Table 9-2
Overview of Specimens for Storage and Shipment

Specimen and Subsequent Testing	Additive	Tube type or size recommendation	Processing and Storage	Ship to:
Plasma Archive / Storage	EDTA	1x10mL	Spin 10 minutes at 1500xg (or double spin at 800xg). Aliquot and freeze.	Batch to MTN LC
Plasma for PK	EDTA	1x10mL	Spin 10 minutes at 1500xg. Aliquot and freeze within 8 hours of collection.	Batch to JHU CPAL
CVF for PK	None	Swab in Cryovial	Record net weight of swab and freeze within 2 hours of collection.	Batch to JHU CPAL
CVL for PD and PK	Normal Saline	15mL Conical Tube	Spin 10 minutes at 800xg. Aliquot supernatant and suspend cell pellet. Freeze supernatants and cell pellet within 8 hours of collection.	Batch to MTN LC
Cervical Biopsies for PK	None	Cryovial	Record net weight of biopsies then flash freeze and store at $\leq -70^{\circ}\text{C}$ within 2 hours of collection	Batch to JHU CPAL
Rectal Microflora	None	Swab in Cryovial	Freeze at $\leq -70^{\circ}\text{C}$ within 2 hours of collection.	Batch to MTN LC
Rectal Swab for PK	None	Swab in Cryovial	Record net weight of swab and freeze at $\leq -70^{\circ}\text{C}$ within 2 hours of collection	Batch to JHU CPAL
Rectal Sponge for Mucosal Safety	None	Merocel Sponge in 5mL Cryovial	Record net weight of sponge and freeze at $\leq -70^{\circ}\text{C}$ within 2 hours of collection	Batch to MTN LC

Specimen and Subsequent Testing	Additive	Tube type or size recommendation	Processing and Storage	Ship to:
Rectal Enema for PD and PK	None	50mL conical tube	Spin 10 minutes at 400xg. Aliquot supernatant and suspend pellet. Freeze supernatants and pellet within 8 hours of collection.	Batch to MTN LC
Rectal Biopsies for PK	None	1.8mL Cryovial	Record net weight of biopsies then flash freeze and store at $\leq -70^{\circ}\text{C}$ within 2 hours of collection	Batch to JHU CPAL
Rectal Biopsies for Mucosal gene expression array	RNA $later$	1.8mL Cryovial	Store at 4°C overnight (16-24 hours) then transfer to $\leq -70^{\circ}\text{C}$.	Batch to MTN LC
Rectal Biopsy for Histology	10% Formalin	2.0mL tube	Store at room temperature	Scheduled shipment to MTN LC
Rectal Biopsies for PD	Transport Media	50mL conical tube with 10mL media	Transport to local testing lab within 30 minutes of collection.	Supernatants batched to MTN LC
Rectal Biopsies for Mucosal T cell phenotyping	Transport Media	50mL conical tube with 10mL media	Transport to local testing lab on ice.	Local Testing Lab
Rectal Biopsy for Proteomics	None	1.8mL Cryovial	Flash freeze and store at $\leq -70^{\circ}\text{C}$ within 2 hours of collection	Batch to MTN LC

9.2 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 of specimen collection should also be included on the label. If the date is handwritten, it should be in indelible ink (such as a black Sharpie pen).

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. Specimens that are sent to the LC or are archived at the site will be entered into LDMS (Table 9-3) and labeled with LDMS-generated labels.

9.3 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 site is responsible for notifying the LC in the following cases

- Any time a participant must return to the clinic for specimen collection
- When PK specimens are missed or not collected within the allowable time frames
- 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 (Pam Kunjara at +1-412-641-6393 or PKunjara@mwri.magee.edu) as soon as possible for guidance.

9.4 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 to track the collection, storage, and shipment of specimens in Table 9-3.

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 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 the 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., blood 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.

Questions related to use of LDMS in MTN-026 may be directed to Pam Kunjara (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: +1-716-834-0900, ext. 7311

Fax: +1-716-898-7711

Table 9-3
LDMS Specimen Management Guide to Logging in MTN-026 Specimens

The table below should be used as a guide when logging in MTN-026 specimens for each test listed. Tests that are listed as "local lab" and specimens are not stored, are not required to be logged into the LDMS. The LDMS Tracking Sheet can be found on the MTN website (www.mtnstopshiv.org) under the MTN-026 study implementation materials.

Test	Primary	Additive	Primary Volume	No. of Aliquots	Aliquot Volume	Units	Derv	Sub Add/ Derv	Other Spec ID
Plasma Archive or Storage	BLD	EDT	10.0 ML	4-5	1.0	ML	PL1/2	N/A	
Plasma for PK	BLD	EDT	10.0 ML	4-5	1.0	ML	PL1	N/A	PK
CVF for PK	CVF	NON	1	1	Net Weight	MG	SWB	N/A	
CVL for PD and PK	CVL	NSL	10–15 ML	6+	1.0	ML	FLD	N/A	PD/PK/ EXT
				1	0.5	ML	CEN	NSL	
Cervical Biopsies for PK	CVB	NON	2	2	Net Weight	MG	BPS	N/A	
Rectal Swab for Microflora	REC	NON	1 EA	1	1.0	EA	SWB	N/A	MF
Rectal Swab for PK	REC	NON	1 EA	1	Net Weight	MG	SWB	N/A	PK
Rectal Sponge for Mucosal Safety	REC	NON	1 EA	1	Net Weight	MG	SPG	N/A	MS
Rectal Enema for PD and PK	REC	NSL	Varies	6+	1.0	ML	FLD	N/A	PD/PK/ EXT
				1	1.0	ML	PEN	NSL	
Rectal Biopsies for PK	FSR	NON	5 EA	5	Net Weight	MG	BPS	N/A	PK
Rectal Biopsy for Gene Expression	FSR	RNL	2 EA	2	1	EA	BPS	N/A	
Rectal Biopsy for Histology	FSR	FOR	1 EA	1	1	EA	BPS	N/A	

Test	Primary	Additive	Primary Volume	No. of Aliquots	Aliquot Volume	Units	Derv	Sub Add/ Derv	Other Spec ID
Rectal Biopsies for PD (Log each in separately)	FSR	BTM	1 EA	1	Net Weight	MG	BPS	N/A	PD
Rectal Biopsy Supernatant (Culture Derivative)	All from Primary Sample			1	500	UL	SUP	RPM	
Rectal Biopsy for PCR (Culture Derivative)	All from Primary Sample			1	1	ML	TIS	RNL	
Rectal Biopsies for T cell Phenotyping	FSR	BTM	7 EA	1	7	EA	BPS	N/A	PHENO
Rectal Biopsy for Proteomics	FSR	NON	1 EA	1	Net Weight	MG	BPS	N/A	PRO

BLD: Whole Blood

BPS: Biopsy

BTM: Biopsy Transport Media

CEN: Fresh Cells from a non-blood specimen

CVB: Cervical Biopsy

CVL: Cervicovaginal Lavage

CVF: Cervicovaginal Fluid

EDT: EDTA

FLD: Fluid

FOR: Formalin

FSR: Rectal biopsy by flexible sigmoidoscopy

NON: None

NSL: Normal Saline

PEN: Non-viable cells from non-blood specimen

PL1: Single spun Plasma

PL2: Double spun Plasma

REC: Rectal

RNL: RNAlater

SPG: Sponge

SWB: Swab

NOTE: The Other Spec ID field is a free text field. Please be sure to enter codes carefully.

EXT: Extra

MF: Microflora

MS: Mucosal Safety

PD: Pharmacodynamics

PHENO: Phenotyping

PK: Pharmacokinetics

PRO: Proteomics

9.4.1 Logging in PK Samples

- Enter the actual specimen collection time in the Specimen Time area (See Image 1)
- Time and Time Unit area (See Image 1) are used to enter the PK time point information (0 pre-dose, 60 min, 120 min) when applicable, otherwise leave blank.

IMAGE 1: LDMS Entry Screen

9.5 Urine Testing

The urine tests performed at the study visit will depend on the time point of the visit and the clinical presentation of the participant. In general at study visits when urine testing is required, a single specimen will be collected and aliquots will be made for each test when possible. When doing multiple tests from one specimen, an aliquot of urine should first be obtained for pregnancy testing (female participants only) and the remaining specimen should be reserved for chlamydia and gonorrhea testing. Collect urine specimens before collecting any pelvic specimens. Heavy menses may interfere with dipstick and pregnancy tests – sites should use discretion and contact the MTN LC if there are questions.

9.5.1 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 female participant not to clean the labia prior to specimen collection. Male participants should withdraw foreskin if present.
- Collect the first 15-60 mL of voided urine in a sterile collection cup. (Not mid-stream).
- Instruct the participant to screw the lid tightly onto the cup after collection.
- At visits when dipstick urinalyses and/or pregnancy testing is indicated, aliquot 5 to 10 mL for this test and store the remaining urine at 2-8°C or introduce the urine immediately into the UPT for subsequent Chlamydia and Gonorrhea testing.
- Note: only in situations where there is no NAAT testing and a clinician suspects a urinary tract infection, specimens may be collected per local specifications such as mid-stream clean catch.

9.5.2 Pregnancy Testing

At visits when pregnancy testing is required, aliquot approximately 5 to 10 mL of urine from the specimen collection cup and pipette from this aliquot for pregnancy testing. 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.

Either the Quidel QuickVue One-Step urine hCG or Quidel Quick Vue Combo urine and serum hCG 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.

The urine only kit and the combo kit are different kits and have different CAP method codes for EQA panels. If sites are running both kits, they must run CAP EQA panels on both kits. In most cases, the CAP results forms will only allow for entry of one kit. Sites can generally submit results to CAP for one kit and do a self-evaluation for the other kit. Consult SMILE, MTN LC or your PNL in case of questions regarding your EQA panels.

9.5.3 Urine Chlamydia and Gonorrhea Testing

This testing will be done using the Gen-Probe Aptima NAAT Method or the Cepheid GeneXpert NAAT method by the local laboratory.

The laboratory that is performing the test must provide the clinic with the appropriate transport tube for the test being performed.

9.5.3.1 Instructions for transferring urine into the Gen-Probe UPT

1. Collect urine as noted above.
2. Open the UPT kit and remove the UPT and transfer pipette. Label the UPT with the participants PTID number and date.
3. 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.
4. Uncap the UPT and use the transfer pipette to transfer enough urine to fill the tube to the level indicated on the tube between the black lines. Do not under fill or overfill the tube.

5. Cap tightly and invert the tube 3-4 times to ensure that the specimen and reagent are mixed.
6. The specimen can now remain at 2-30°C for 30 days.
7. Place the transport tube in a biohazard zip-lock bag and transport to the local laboratory for testing.
8. The results are sent to the clinic and are reported on a STI Tests CRF.

9.5.3.2 Instructions for transferring urine into the GeneXpert transport reagent tube

1. Collect urine as noted above.
2. Open the packaging of a disposable transfer pipette provided in the kit. Label the tube with the participants PTID number and date.
3. Remove the cap from the Xpert CT/NG Urine Transport reagent tube. Insert the transfer pipette into the urine cup so that the tip is near the bottom of the cup. Transfer approximately 7 mL of urine into the Xpert CT/NG Urine Transport reagent tube. The correct volume of urine has been added when the level reaches the black dashed line on the label.
4. Cap tightly and invert the tube 3-4 times to ensure that the specimen and reagent are mixed.
5. The specimen can remain at 2-30°C for 30 days.
6. Place the transport tube in a biohazard zip-lock bag and transport to the local laboratory for testing.
7. The results are sent to the clinic and are reported on a STI Tests CRF.

9.5.4 Dipstick Urinalysis

Dip the urinalysis test strip into an aliquot of urine. Perform this test according to site SOPs and the package insert. Assess and record results for glucose, protein, 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.

9.5.5 Urine Culture

Perform urine culture per local standard of care if ordered by clinician for clinical indications.

9.6 Blood Testing

The blood tests performed depend 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.

9.6.1 Specimen Collection and Initial Processing

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

After collection:

- Allow plain tubes (no additive or serum separator) to clot, then centrifuge per site SOPs.
- Lavender top tubes (additive = EDTA) should be gently inverted at least eight times after specimen collection to prevent clotting. If whole blood for hematology testing and plasma 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.
- Light blue top tubes (additive = Na Citrate) are used for coagulation determinations. These tubes should be gently inverted at least 4 times after specimen collection to prevent clotting.

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

9.6.2 HIV Testing

Although the HIV algorithm (Appendix II of the MTN-026 protocol) allows for EIA testing, rapid testing is recommended to obtain immediate results confirming participant eligibility throughout the study.

HIV testing must be validated at the study site per the CLIA standards, if applicable. All tests, and associated QC procedures, must be documented on local laboratory log sheets or other laboratory source documents.

HIV infection status at screening will be assessed using an FDA-approved HIV test per the HIV testing algorithm (see Appendix II in the current version of the MTN-026 protocol). If the test is negative, the participant will be considered HIV-seronegative. If the test is positive or indeterminate and this participant has already been enrolled into the study, an FDA-approved confirmatory test approved by the MTN LC will be performed on the original sample. If there is insufficient sample to perform confirmatory testing, then additional blood must be collected. If the confirmatory test is negative or indeterminate, contact the MTN LC for guidance.

Please notify the MTN Virology Core (mtnvirology@mtnstopshiv.org) via e-mail of all possible seroconverters identified during a follow up visit by submitting a MTN LC HIV Query Form which can be found on the MTN website. Once the MTN Virology Core has had an opportunity to review the form, a request for plasma storage to be shipped on dry ice to the MTN Virology Core may be issued. Be sure to provide the lab with the tracking number and details of each specimen prior to shipping.

Ship samples to MTN Virology Core (LDMS Lab 470)

Urvi Parikh
University of Pittsburgh
3550 Terrace Street
S804 Scaife Hall
Pittsburgh, PA 15261
Phone # 412-648-3103
Fax # 412-648-8521

Plasma storage (Section 9.6.9) is required for further MTN LC HIV testing (CD4, HIV RNA, and HIV drug resistance) of enrolled participants in the event of a positive HIV rapid or positive HIV EIA test result, and when additional samples are collected as part of algorithm testing at the site local lab to confirm a participant's HIV infection status.

All test results must be documented on local laboratory log sheets or other laboratory source documents. For non-CLIA sites, in addition to initialing or signing the testing logs to document review and verification of the results, the second lab staff member must also record the time at which the results were reviewed and verified.

9.6.3 Hematology Testing

Complete blood counts (CBC) with five-part differentials will be performed at all sites. 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 SOPs.

9.6.4 Liver and Renal Function Testing

The following tests will be performed to evaluate liver and renal function:

Liver Function

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

Renal Function

- Creatinine

These chemistry tests will be collected and performed according to local laboratory SOPs.

9.6.5 Syphilis Testing

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

- Rapid Plasma Reagin (RPR) or Venereal Disease Research Laboratory (VDRL) 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 or VDRL results must have a titer reported. For reactive RPR or VDRL tests observed during screening, a confirmatory test is performed and appropriate clinical management action must be taken prior to enrollment in the study. MTN LC recommends for enrolled participants considered positive, 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.
- 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. If the RPR or VDRL is negative, this may indicate prior treatment, late latent disease, or a false positive. 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 confirmation test is negative, the participant is not considered infected with syphilis. If the second confirmation 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-026 Protocol Safety Physicians (mtn026safetymd@mtnstopshiv.org).

9.6.6 Hepatitis B Surface Antigen and Hepatitis C Antibody

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

9.6.7 HSV Serology

Testing will be done for both HSV-1 and HSV-2 IgG antibody on serum per local SOPs

9.6.8 INR/PT

Testing will be performed on whole blood collected in light blue tubes (Na Citrate) per local SOP

9.6.9 Plasma Archive (baseline) and Plasma Storage

Plasma archive/storage is required at Enrollment and visits 7 and 16. Additionally, it is required for further MTN LC HIV testing (CD4, HIV RNA, and HIV drug resistance) of enrolled participants in the event of a positive HIV rapid or positive HIV EIA test result, and when additional samples are collected as part of algorithm testing at the site local lab to confirm a participant's HIV infection status.

For plasma archive and plasma storage, use collection tubes with EDTA anticoagulant. Aliquot plasma into 2 ml cryovials, store at $\leq -70^{\circ}\text{C}$, and batch onsite until the MTN LC requests shipping and/or testing.

1. If sample is collected and held at room temp, freeze plasma within 4 hours. If refrigerated or on ice after collection, freeze plasma within 24 hours.
2. If total whole blood volume is less than 2.0 mL, redraw as soon as possible.
3. Spin blood at room temperature in a centrifuge according to one of these techniques:
 - Single spun: Spin blood at $1500\times g$ for 10 minutes and remove plasma.
 - Double spun: Spin blood at $800\times g$ for 10 minutes, recover plasma and place in a tube to spin again at $800\times g$ for 10 minutes, remove plasma.
4. Prepare as many 1.0 mL aliquots as possible with a total volume of aliquots greater than or equal (\geq) to 4ml
5. If less than 4 mL of plasma are available, store that plasma and inform the MTN LC for instruction.
6. If samples are hemolyzed, store the aliquots as per normal and enter comments in LDMS.

7. The MTN LC will send instructions to the site when shipping and/or testing is required.

9.6.10 Blood for Dapivirine PK

Collect blood into a labeled 10 mL EDTA Vacutainer tube using either an indwelling venous catheter or direct venipuncture. Record the collection time on to the LDMS tracking sheet.

1. Mix blood sample with the anticoagulant using gentle inversions (8 to 10 times).
2. Centrifuge the sample at approximately 1500×g for 10 minutes at 4°C. The centrifugation must be completed and sample placed in the freezer within 8 hours of blood collection.
3. Transfer plasma to appropriately labeled 2.0 mL cryovials in as many 1.0 mL aliquots as possible.
4. Store at ≤-70°C.
5. Log samples into LDMS (Table 9-3) and batch ship to JHU CPAL upon request.

Ship PK samples to JHU-CPAL (LDMS Lab 194)

James Johnson
John Hopkins School of Medicine
Clinical Pharmacology Analytical Lab at Bayview
4940 Eastern Avenue
MFL Center Tower Suite 6000 Rm. 621
Baltimore, MD 21224
Phone: 410-550-9703 or 410-550-9713

9.7 Testing of Vaginal and Cervical Specimens

Cervicovaginal specimens will be collected in the order and manner stated in the clinical considerations section of this SSP.

9.7.1 Papanicolaou (Pap) Test

At visits when Pap smears are indicated, ecto- and endocervical cells will be collected after all tissues have been visually inspected and all other required specimens have been collected. Specimen collection, slide preparation, slide interpretation, and QC procedures must be performed and documented in accordance with study site SOPs. There is no required external review of these procedures by the MTN.

9.7.2 Cervicovaginal Testing for GC/CT (*Neisseria gonorrhoea* and *Chlamydia trachomatis*) by NAAT

Testing for chlamydia and gonorrhoea is performed at screening and when clinically indicated. Sites can choose to use the GeneXpert or Gen-Probe Aptima.

- Swab the lateral wall of the vagina.
- Immediately place the swab in the transport tube, break off the shaft of the swab, and cap the tube.
- Transport the specimen at ambient temperature to the local laboratory.

9.7.3 Cervicovaginal Fluid for PK

- Each day of collection of cervicovaginal fluid for PK, perform QC that would be required for the analytical scale to accurately weigh samples to a weight of at least 0.1 milligrams. Do not turn off balance until weighing for the day is completed.
- PK swab must be collected within one hour of PK blood draw.
- Ensure that new or sterilized supplies are used for each sample as Dapivirine is very sensitive to cross-contamination.
- There are two methods to collecting and weighing the swab for PK. Collection may be obtained with a pre-cut swab or swab may be cut after collection. Please see instructions below. Sites may choose either method based on site preference.

Pre-cut Swab Collection Method

Materials for each collection:

- SCHARP label with PTID, visit number, and visit date
 - 2-mL Nalgene cryovial containing pre-cut Polyester-Tipped (Dacron) Swab
 - Hemostat, Ring Forceps, or Transfer Pipet (recommend 8 inches or longer)
 - Analytical scale (accurate to 0.1 milligrams)
1. Affix SCHARP label to the cryovial containing the pre-cut swab.
 2. Perform pre-weight measurement by weighing the labeled capped cryovial with pre-cut swab and record on the LDMS Tracking Sheet.
 3. Uncap the pre-weighed cryovial. Use a clean hemostat or forceps to clamp on to the shaft of the swab.
 4. Insert the hemostat/forceps/transfer pipet holding the swab to the posterior fornix for 10-20 seconds, rotating in a circular motion touching all walls to absorb as much fluid as possible.
 5. Immediately place swab into the cryovial after sampling and recap.
 6. Perform post-weight measurement by weighing the capped cryovial containing the absorbed swab tip and record on the LDMS Tracking Sheet.
 7. Calculate and record the NET weight on the LDMS Tracking Sheet.
 8. Within 2 hours, place the sample tubes in the freezer at $\leq -70^{\circ}\text{C}$.
 9. Log into LDMS (Table 9-3) and label specimen with LDMS label.
 10. Batch ship to JHU CPAL (LDMS Lab 194) upon request.

Post-cut Swab Collection Method

Materials for each collection:

- 2 SCHARP labels with PTID, visit number, and visit date
 - 2-mL Nalgene cryovial
 - Polyester-Tipped (Dacron) Swab
 - Zip-lock biohazard sample bag
 - Plastic cup (without lid) or similar lightweight container, placed on middle of scale, to contain items to be weighed. (Some balances have an optional basket.)
 - Scissors to cut swab shaft
1. Place identically-labeled SCHARP labels on the cryovial and the biohazard sample bag.
 2. Perform pre-weight. Handle items to be weighted with gloves.
 - a. Zero the cup or similar container on the scale.
 - b. Place the labeled 2-mL cryovial and the packaged sterile Dacron swab upright in the urine cup. (Make sure it is not leaning on a part of the scale.)
 - c. Record this pre-weight on the LDMS Tracking Sheet.
 - d. Place the cryovial and the packaged Dacron swab in a labeled biohazard sample bag.
 3. Sample collection

NOTE: **All** the items in the bag should return to the bag. Nothing will be thrown into the garbage.

 - a. Remove swab from packaging. Do **NOT** discard the packaging. Place all the packaging back into the bag.
 - b. Collect vaginal fluid holding the swab to the posterior fornix for 10-20 seconds, rotating in a circular motion touching all walls to absorb as much fluid as possible.
 - c. Place the swab in the cryovial and cut the swab shaft using scissors at the pivot point. Be sure to hold onto the shaft to avoid losing it. Do **NOT** discard the shaft!
 - d. Place the cut shaft in the specimen bag.
 - e. Screw the lid back on the cryovial and place sample in the bag with the swab packaging and the swab shaft.
 - f. Document the collection time on to the LDMS tracking sheet.
 4. Perform Post Weight:
 - a. Zero the cup or similar lightweight container on the scale.
 - b. Weigh the capped cryovial containing the absorbed swab tip, the swab packaging and the remainder of the swab shaft (Suggestion: Place the swab shaft into the packaging and have it upright during weighing.)

- c. Record post-weight on the LDMS Tracking sheet then calculate and record the NET weight.
5. Within 2 hours, place the sample tubes in the freezer at $\leq -70^{\circ}\text{C}$.
6. Log into LDMS (Table 9-3) and batch ship to JHU CPAL (LDMS Lab 194) upon request.

9.7.4 Cervicovaginal Lavage (CVL) for PD and PK

1. 10mL of normal saline should be used to lavage the cervix, fornices, and vaginal walls. Using a syringe collect all of the CVL and place into a 15mL conical tube.
2. CVL specimens are kept on wet ice or refrigerated and should be processed within 8 hours of collection.
3. All the CVL liquid will be spun at $800\times g$ for 10 minutes in the 15 mL conical collection tube.
4. Remove supernatant from the cell pellet and store as many 1 mL aliquots as possible in cryovials.
5. Re-spin the 15 mL conical tube containing cells for 10 minutes at $800\times g$.
6. Pull off and save any additional supernatant making sure not to remove any cells or debris.
7. Resuspend cell pellet in 0.5 mL normal saline and store in a cryovial.
8. Freeze all supernatants and cell pellet at $\leq -70^{\circ}\text{C}$ within 8 hours of collection and track in LDMS.
 - a. Supernatant aliquots may be stored separately for organizational purposes. Store one for PK, one for PD and the remaining aliquots as EXTRA.
 - b. Pellet should be stored separately.
9. If less than a total of 6 mL's (or less than 6 cryovials) of supernatant are recovered, contact the MTN LC.
10. Log into LDMS (Table 9-3) and batch ship PK samples to JHU CPAL (LDMS Lab 194) and MTN LC upon request.

Ship PD samples to MTN LC (LDMS Lab 414)
 Pamela Kunjara
 Magee-Womens Research Institute
 204 Craft Ave, Room A540
 Pittsburgh, PA 15213
 Phone# +1-412-641-6157
 Email: pkunjara@mwri.magee.edu

9.7.5 Cervical Biopsies for PK

1. Label two 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 (0.0000 g) or better. Document the pre-weight of each labeled cryovial on the LDMS tracking sheet.
3. Collect each biopsy and place directly into its designated pre-weighed cryovial. Store only ONE biopsy per cryovial. Ensure the biopsy sits at the bottom of cryovial.
4. Obtain the post-weight for each cryovial containing a biopsy using the same analytical scale and document the weight on the LDMS tracking sheet.
5. Immediately freeze the cryovial containing the biopsy in liquid nitrogen or a dry ice alcohol bath (dry ice with enough ethanol to make a slushy consistency).
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 the LC. The net-weights will be calculated by the formula in the worksheet and entered into the LDMS system.
9. Log samples into LDMS (Table 9-3) and batch ship to JHU CPAL (LDMS Lab 414) upon request.

9.8 Testing of Rectal Specimens

The tests performed on rectal specimens depend 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.

Rectal samples should be collected in the following order:

1. Anal swab for HSV 1/2
2. Rectal swab for GC/CT

3. Rectal microflora
4. Rectal swab for PK
5. Rectal sponge for Mucosal Safety
6. Rectal Enema for PD and PK
7. Biopsies* for PK, PD, Proteomics, Histology, Mucosal T Cell Phenotyping, and Mucosal Gene Expression Array

Table 9-2 gives a brief summary of how these rectal samples should be handled.

*If at any time the collection of biopsies is limited, submit for assays in order of importance – PK, Mucosal Gene Expression Array, Histology, PD, T Cell Phenotyping, and then Proteomics.

9.8.1 Anal HSV-1/2

Testing will be performed from an anal swab collected per local SOP

9.8.2 Rectal NAAT for Gonorrhea and Chlamydia

Note: Testing for Chlamydia and Gonorrhea is done at screening and when clinically indicated only. Product gel may cause interference during testing. Please be careful to avoid contact with gel when collecting specimen.

This testing will be done using either the GenProbe Aptima or the Cepheid GeneXpert NAAT method by the local or regional laboratory.

The laboratory that is performing the test must provide the clinic with the appropriate transport tube for the test being performed.

1. Collect specimen using the Xpert collection swab.
2. Label the pink-capped transport tube with the participants PTID number and date.
3. Remove the swab and insert into the rectum according to the procedure outlined in the SSP for Clinical Considerations (Section 8) and rotate gently through 360 degrees and remove.
4. Immediately place the swab in the transport tube, break off shaft of swab and cap.
5. Cap tightly and invert or gently shake the tube 3-4 times to elute material from the swab. Avoid foaming.
6. The specimen can now remain at 2-30°C for 60 days.
7. Place the transport tube in a biohazard zip-lock bag and transport to the local laboratory for testing.
8. The results are sent to the clinic and are reported on a STI Tests CRF.

9.8.3 Rectal Microflora

Rectal swabs will be collected for rectal microflora and sent to the MTN LC. Shipping instructions follow.

- Collect the specimen for microflora by rotating a flocked nylon swab several times over the lateral wall of the rectum. Insert the swab into a cryovial and snap the shaft of the tube off in order to screw on the top.
- The specimen may be kept refrigerated for up to 2 hours.
- Deliver the swab and the LDMS specimen tracking sheet to the local LDMS laboratory.
- Log the specimen into LDMS (Table 9-3) and label the specimen with LDMS labels.
- Freeze at $\leq -70^{\circ}\text{C}$ within 2 hours of collection.
- Specimens may be batched and shipped on dry ice to the MTN LC (LDMS Lab 414).

9.8.4 Rectal Swab for PK

- Each day of collection of rectal fluid for PK, perform QC that would be required for the analytical scale to accurately weigh samples to a weight of at least 0.1 milligrams. Do not turn off balance until weighing for the day is completed.
- PK swab must be collected within one hour of PK blood draw.
- Ensure that new or sterilized supplies are used for each sample as Dapivirine is very sensitive to cross-contamination.
- There are two methods to collecting and weighing the swab for PK. Collection may be obtained with a pre-cut

swab or swab may be cut after collection. Please see instructions below. Sites may choose either method based on site preference.

Pre-cut Swab Collection Method

Materials for each collection:

- SCHARP label with PTID, visit number, and visit date
 - 2-mL Nalgene cryovial containing pre-cut Polyester-Tipped (Dacron) Swab
 - Hemostat or Ring Forceps (recommend 8 inches or longer)
 - Analytical scale (accurate to 0.1 milligrams)
1. Affix SCHARP label to the cryovial containing the pre-cut swab.
 2. Perform pre-weight measurement by weighing the labeled capped cryovial with pre-cut swab and record on the LDMS Tracking Sheet.
 3. Uncap the pre-weighed cryovial. Use a clean hemostat/forceps/transfer pipet to clamp on to the shaft of the swab.
 4. Insert the hemostat/forceps/transfer pipet holding the swab to the mucosa for 2 minutes.
 5. Immediately place swab into the cryovial after sampling and recap.
 6. Perform post-weight measurement by weighing the capped cryovial containing the absorbed swab tip and record on the LDMS Tracking Sheet.
 7. Calculate and record the NET weight on the LDMS Tracking Sheet.
 8. Within 2 hours, place the sample tubes in the freezer at $\leq -70^{\circ}\text{C}$.
 9. Log into LDMS (Table 9-3) and label specimen with LDMS label.
 10. Batch ship to JHU CPAL (LDMS Lab 194) upon request.

Post-cut Swab Collection Method

Materials for each collection:

- 2 SCHARP labels with PTID, visit number, and visit date
 - 2-mL Nalgene cryovial
 - Polyester-Tipped (Dacron) Swab
 - Zip-lock biohazard sample bag
 - Plastic cup (without lid) or similar lightweight container, placed on middle of scale, to contain items to be weighed. (Some balances have an optional basket.)
 - Scissors to cut swab shaft
1. Place identically-labeled SCHARP labels on the cryovial and a biohazard sample bag.
 2. Perform pre-weight. Handle items to be weighted with gloves.
 - a. Zero the cup or similar container on the scale.
 - b. Place the labeled 2-mL cryovial and packaged sterile Dacron swab upright in the cup. (Make sure it is not leaning on a part of the scale.)
 - c. Record this pre-weight on the LDMS Tracking Sheet.
 - d. Place the cryovial and the packaged Dacron swab in a labeled biohazard sample bag.
 3. Sample collection

NOTE: **All** of the items in the bag should return to the bag. Nothing will be thrown into the garbage.

 - a. Remove swab from packaging. Do **NOT** discard the packaging. Place all of the packaging back into the bag.
 - b. Collect rectal fluid by holding the swab against the mucosa for 2 minutes.
 - c. Place the swab in the cryovial and cut swab shaft using scissors at the pivot point. Be sure to hold onto the shaft to avoid losing it. Do **NOT** discard the shaft.
 - d. Place the cut shaft in the specimen bag.
 - e. Screw the lid back on the cryovial and place sample in the bag with the swab packaging and the swab shaft.
 - f. Document the collection time (the time the swab was removed from the rectum) on to the LDMS tracking sheet.
 4. Perform Post Weight:

- a. Zero the cup or similar lightweight container on the scale.
 - b. Weigh the capped cryovial containing the absorbed swab tip, the swab packaging and the remainder of the swab shaft (Suggestion: Place the swab shaft into the packaging and have it upright during weighing.)
 - c. Record post-weight on the LDMS Tracking sheet then calculate and record the NET weight.
5. Within 2 hours, place the sample tubes in the freezer at $\leq -70^{\circ}\text{C}$.
 6. Log into LDMS (Table 9-3) and batch ship to JHU CPAL (LDMS Lab 194) upon request.

9.8.5 Rectal Sponge for Mucosal Safety

1. Tare the weighing balance and ensure balance has been calibrated within the past year.
2. Remove a sponge (Merozel eye-wick Spears Fisher Scientific # NC0093269) from the box, wear gloves at all times when handling sponges.
3. Place the sponge into an appropriately labeled 5mL cryovial.
4. Weigh the dry sponge + labeled cryovial using an analytical scale with a sensitivity rating of 0.1 milligrams (0.0000 g) or better. Document the weight (pre-weight) on the LDMS Tracking Sheet.
5. The clinician will collect specimen using a pre-weighed sponge according to the procedures outlined in the SSP for Clinical Considerations (Section 8).
6. Place the sponges back into the original weighed cryovial and ensure that the cap is fully tightened.
7. Weigh the cryovial and sponge after collection using the same analytical balance used for the pre-weights. Document the post-collection weight on the LDMS Tracking Sheet.
8. Complete the LDMS tracking sheet and submit to lab on ice for LDMS entry.
9. Log into LDMS (Table 9-3) and label specimen with LDMS label.
10. Freeze at $\leq -70^{\circ}\text{C}$ within 2 hours of collection until ready to ship.
11. Specimens may be batched and shipped on dry ice to the MTN LC (LDMS Lab 414).

9.8.6 Rectal Enema for PD and PK

1. Rectal enema should be kept on wet ice or refrigerated and processed within 8 hours of collection.
2. In a 15 or 50 mL conical tube collect 10 mLs of the rectal enema. Spin at $400\times g$ for 10 minutes.
3. Remove supernatant from the pellet and store as many 1 mL aliquots as possible in cryovials.
4. Resuspend cell pellet in 0.5 mL normal saline and store in a cryovial.
5. Freeze all supernatants and pellet at $\leq -70^{\circ}\text{C}$ within 8 hours of collection and track in LDMS.
 - a. Supernatant aliquots may be stored separately for organizational purposes. Store one for PK, one for PD and the remaining aliquots as EXTRA.
 - b. Pellet should be stored separately.
6. If less than a total of 6 mL's (or less than 6 cryovials) of supernatant are recovered, contact the MTN LC.
7. Log into LDMS (Table 9-3) and batch ship PK samples to JHU CPAL (LDMS Lab 194) and PD samples to MTN LC (LDMS Lab 414) upon request.

9.8.7 Rectal Biopsies for PK

1. Logistics permitting, biopsies should be delivered to the lab to allow freezing within two hours of collection.
2. Up to 5 biopsies will be collected for PK analysis. Number cryovials 1-5 depending on how many biopsies are received with appropriate participant information.
3. Weigh each cryovial using an analytical balance – use the same analytical balance throughout the procedure. Document the weight of the labeled cryovial (pre-weight) on the LDMS Tracking Sheet.
4. Biopsies for PK may be collected in the same media as biopsies for PD or placed directly into the pre-weighed cryovial without media.
5. If biopsies are collected in media, using pointed forceps, pick up each individual biopsy and drain off excess medium by touching biopsy to side of collection vessel.
6. Transfer biopsy to a pre-weighed cryovial. Store only ONE biopsy per cryovial. Ensure biopsy sits at bottom of cryovial.
7. Weigh the cryovial containing the biopsy (post-weight). Document the weight of the cryovial containing the biopsy on the LDMS Tracking Sheet.

8. Freeze the cryovial containing the biopsy in Liquid Nitrogen or a dry ice-alcohol bath.
9. Log into LDMS (Table 9-3) and label specimen with LDMS label.
10. Store the labeled cryovial containing the biopsy in a $\leq -70^{\circ}\text{C}$ freezer. Document the date/time the cryovial containing the biopsy was placed in the freezer.
11. Batch and ship on dry ice to JHU CPAL (LDMS Lab 194) upon request.

9.8.8 Rectal Biopsy for Mucosal Gene Expression Array

1. Two biopsies will be collected for mucosal gene expression. Take two cryovials containing 1.5 mL of RNA*later* (Ambion, Invitrogen Cat #AM7020) and label it with PID, study visit and date.
2. Place one biopsy into each cryovial and submerge the tissue in the RNA*later* solution.
3. Store each vial containing one rectal biopsy in RNA*later* at 4°C overnight (16-24 hours).
4. Complete the LDMS tracking sheet and submit to lab for LDMS entry.
5. Log the specimen into LDMS (Table 9-3) and label specimen with LDMS label.
6. Transfer vials from 4°C to $\leq -70^{\circ}\text{C}$. Each biopsy must be stored at $\leq -70^{\circ}\text{C}$ for a minimum of 24 hours prior to shipping.
7. Batch and ship specimens on dry ice to the MTN LC (LDMS Lab 414) upon request.

9.8.9 Rectal Biopsy for Histology

1. Place one biopsy into a microtube filled with 10% formalin for shipping. These can be kept at room temperature.
2. Complete the LDMS tracking sheet and submit to lab for LDMS entry.
3. Log specimens into LDMS (Table 9-3), label specimen with LDMS label.
4. The tissue processing/embedding must occur within 72 hours of collection. If the site does not have capacity to embed tissue, the specimens may be shipped to the MTN LC in real-time.
5. Batch ship the histology blocks to the MTN LC (LDMS Lab 414) at room temperature.

9.8.10 Rectal Biopsies for PD

1. Four biopsies for PD should be collected and placed into biopsy transport media immediately. (See Appendix 9-1 for ordering and preparing media). **NOTE:** Biopsies for PD and T Cell Phenotyping may be collected in the same vial and divided by the lab if the processing lab for both assays is the same.
2. Transport biopsies to MTN LC validated ex vivo challenge lab within 15-30 minutes from time of collection.
3. Please refer to the Non-Polarized Colorectal Explant Culture SOP provided by the MTN LC for processing.
4. Record weights of biopsies onto the MTN-026 LDMS Tracking Sheet.
5. Each biopsy will be logged into LDMS separately (Table 9-3)
6. Supernatants collected during tissue culture should be logged into LDMS as culture derivatives and stored frozen in cryovials at $\leq -70^{\circ}\text{C}$ until shipped for p24 analysis. See LDMS Culture Derivative SOP provided by MTN LC.
7. Supernatants for p24 analysis will be batched and shipped to the MTN LC (LDMS Lab 414) on dry ice.
8. Biopsies after completion of culture will be stored in RNA*later* per the Non-Polarized Colorectal Explant Culture SOP and logged into LDMS as a culture derivative.

9.8.11 Rectal Biopsies for Mucosal T Cell Phenotyping

1. Label one conical tube with the PTID, visit # and visit date.
2. Submerge 7 biopsies into the tube containing 10-12mL of biopsy transport media (See Appendix 9-1 for ordering and preparing media). Keep refrigerated. **NOTE:** Biopsies for PD and T Cell Phenotyping may be collected in the same vial and divided by the lab if the processing lab for both assays is the same.
3. Complete the LDMS tracking sheet and submit to lab for LDMS entry and processing.
4. Log specimens into LDMS (Table 9-3).
5. **For Pitt and Bangkok:** Place specimens in the refrigerator and allow to rest overnight prior to processing per the McGowan Lab SOP.

For UAB only: Ship specimen **FedEx Priority Overnight** on ice packs to the McGowan lab for processing.

- Do not place specimen directly on ice pack. Ice pack may cause media to freeze. At minimum place a paper towel in between the ice pack and specimen.
- Notify Aaron Siegel by e-mail providing the LDMS batch file and FedEx tracking number.

Ship T Cell Phenotyping samples to McGowan Lab (LDMS Lab 414)

Attn: Aaron Siegel
Magee-Womens Research Institute
204 Craft Ave, Room B530G
Pittsburgh, PA 15213
Phone# +1-412-641-7767
Email: siegela@mwri.magee.edu

9.8.12 Rectal Biopsy for Proteomics

1. Logistics permitting, biopsy should be delivered to the lab to allow freezing within two hours of collection.
2. Weigh one cryovial using an analytical balance – use the same analytical balance throughout the procedure. Document the weight of the labeled cryovial (pre-weight) on the LDMS Tracking Sheet.
3. Transfer biopsy to a pre-weighed cryovial. Ensure biopsy sits at bottom of cryovial.
4. Weigh the cryovial containing the biopsy (post-weight). Document the weight of the cryovial containing the biopsy on the LDMS Tracking Sheet.
5. Log into LDMS (Table 9-3) and label specimen with LDMS label.
6. Store the labeled cryovial containing the biopsy in a $\leq -70^{\circ}\text{C}$ freezer. Document the date/time the cryovial containing the biopsy was placed in the freezer.
7. Batch and ship specimens to the MTN LC (LDMS Lab 414) on dry ice.

9.9 Appendices 9-1

Procedure for preparing reagents for MTN-026

Equipment:

- 2-8°C Refrigerator
- -20°C freezer
- Biological laminar flow hood
- Pipette Aid
- Pipettors (100 - 250 µL volume)

Disposables:

- cryovials
- 15 mL conical tubes
- 50 mL conical tubes
- 10 mL serological pipettes
- 25 mL serological pipettes
- Pipette tips (100 - 250 µL volume)

Reagents:

1. RPMI (1x) 1640 w/HEPES w/L-glutamine, Invitrogen #22400-089 (500 mL) stored at 2-8°C.
2. Heat inactivated, certified Fetal Bovine Serum (FBS), Invitrogen #10082-139 (100 mL). Aliquot into 7.5 mL volumes and freeze at -20°C
3. Antibiotic/antimycotic (100x), Invitrogen #15240-104 (100 mL) aliquot 1 mL quantities and freeze at -20°C
4. Zosyn® [piperacillin sodium, 2g / tazobactam sodium, 0.25g] (Wyeth, NDC. # 0206-8852-16 or equivalent)
 - Place powder into a 50mL conical tube and add 45mL of D-PBS. Vortex to mix (50mg/mL, final concentration).
 - Aliquot 150 µL into labeled cryovials and store the single use aliquots at -20°C.

Procedure:

Biopsy transport media

<u>Ingredients</u>	<u>100 mL</u>
RPMI	91.5 mL
FBS (f.c. 7.5%)	7.5 mL
Antibiotics/antimycotic (f.c. 1%)	1.0 mL

1. Take precautions to maintain the sterility of the media and use sterile techniques while transferring.
2. Bring the RPMI, FBS, and antibiotic/antimycotic to room temperature.
3. Combine the above ingredients and mix.
4. Dispense 10-12 mL aliquots into 15 mL conical tubes labeled Biopsy Transport Media with expiration date of 1 month from preparation.
5. Store at 4°C for up to one month.
6. On day of visit, thaw 1 aliquot of Zosyn and add 100-120 µL (1%) to the 10-12 mL aliquot of biopsy transport media. Media must be used within 48 hours of addition of Zosyn.