

## Section 9. Laboratory Considerations

---

<b>9.</b>	<b>Introduction .....</b>	<b>9-1</b>
<b>9.1</b>	<b>Overview and General Guidance.....</b>	<b>9-2</b>
<b>9.2</b>	<b>Specimen Labeling.....</b>	<b>9-4</b>
<b>9.3</b>	<b>Procedures for Specimens that cannot be Evaluated.....</b>	<b>9-4</b>
<b>9.4</b>	<b>Use of LDMS.....</b>	<b>9-5</b>
9.4.1	Logging in PK Samples.....	9-6
9.5.1	Specimen Collection .....	9-7
9.5.2	Urine Chlamydia and Gonorrhea Testing .....	9-7
9.5.3	Dipstick Urinalysis.....	9-8
9.5.4	Urine Culture.....	9-8
<b>9.6</b>	<b>Pharyngeal Chlamydia and Gonorrhea Testing.....</b>	<b>9-8</b>
<b>9.7</b>	<b>Blood Testing.....</b>	<b>9-8</b>
9.7.1	Specimen Collection and Initial Processing .....	9-8
9.7.2	HIV Testing .....	9-8
9.7.3	Hematology Testing .....	9-9
9.7.4	Liver and Renal Function Testing .....	9-9
9.7.5	Syphilis Testing.....	9-9
9.7.6	Hepatitis B Surface Antigen and Hepatitis C Antibody .....	9-10
9.7.7	INR/PT .....	9-10
9.7.8	Plasma Archive/Storage .....	9-10
9.7.9	Blood for Dapivirine PK.....	9-11
<b>9.8</b>	<b>Testing of Rectal Specimens.....</b>	<b>9-11</b>
9.8.1	Anal HSV-1/2 .....	9-11
9.8.2	Rectal NAAT for Gonorrhea and Chlamydia .....	9-11
9.8.3	Rectal Microbiome .....	9-12
9.8.4	Rectal Swab for PK.....	9-12
9.8.5	Rectal Enema for PD/PK .....	9-12
9.8.6	Rectal Biopsies for PK .....	9-13
9.8.7	Rectal Biopsy for Mucosal Gene Expression Array .....	9-13
9.8.8	Rectal Biopsy for Histology .....	9-13
9.8.9	Rectal Biopsies for PD .....	9-13
9.8.10	Rectal Biopsy for Proteomics.....	9-13

### 9. Introduction

## 9.1 Overview and General Guidance

This section contains information on the laboratory procedures performed in MTN-033.

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 known as JHU CPAL). Table 9-1 lists for each test the testing location, specimen type, specimen container and kit/method (if specified). Table 9-2 details 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 for ensuring 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 for 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-033 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 (SOPs). 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-033**

Sites are responsible for ensuring 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
Pharyngeal NAAT for Gonorrhea and Chlamydia	Local Lab	Pharyngeal swab	Kit Specific Transport Tube	GeneXpert
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	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	Plasma, serum or whole blood	EDTA or plain tube 4mL	FDA approved tests
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
Anal HSV 1 and 2	Local Lab	Anal Swab	Consult local lab requirements	Local Methodology
Rectal fluid for Microbiome	MTN LC	Rectal Swab	Cryovial	MTN LC Protocol
Rectal NAAT for Gonorrhea and Chlamydia	Local Lab	Rectal Swab	Kit Specific Transport tube	GeneXpert
Rectal Swab for PK	JHU CPAL	Rectal Swab	Cryovial	JHU CPAL Protocol
Rectal enema effluent for PD/PK	MTN LC	Rectal Enema	50mL Conical Tube	MTN LC Protocol
Rectal Biopsies for PK	JHU CPAL	6 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	3 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 on the 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
Rectal Microbiome	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 Enema for PD/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 $4^{\circ}\text{C}$ 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 20mL media	Transport to local testing lab within 30 minutes of collection.	Supernatants batched to MTN LC
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 compromising 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](mailto: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 shipment 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).

The site 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. The site will be responsible for backing up its LDMS data (frequency determined by site) locally and exporting its data to FSTRF (at least weekly).

The 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. The site is expected to resolve all discrepancies within two weeks of receipt of the report. The MTN LC is responsible for reminding the site to adhere to the two-week timeframe and for following up with the site if it does 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-033 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](mailto: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-033 Specimens**

The table below should be used as a guide when logging in MTN-033 specimens for each test listed. Tests that are listed as “local lab” and specimens are not stored and are not required to be logged into the LDMS. The LDMS Tracking Sheet can be found on the MTN website ([www.mtnstopshiv.org](http://www.mtnstopshiv.org)) under the MTN-033 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
Rectal Swab for Microbiome	REC	NON	1 EA	1	1.0	EA	SWB	N/A	MB
Rectal Swab for PK	REC	NON	1 EA	1	Net Weight	MG	SWB	N/A	PK
Rectal Enema for PD/PK	REC	NSL	10mL	6+	1.0	ML	FLD	N/A	
				1	1.0	ML	PEN	NSL	
Rectal Biopsies for PK	FSR	NON	6 EA	6	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	
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 Biopsy for Proteomics	FSR	NON	1 EA	1	Net Weight	MG	BPS	N/A	PRO

BLD: Whole Blood

BPS: Biopsy

BTM: Biopsy Transport Media

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

RPM: RPMI

SUP: Supernatant

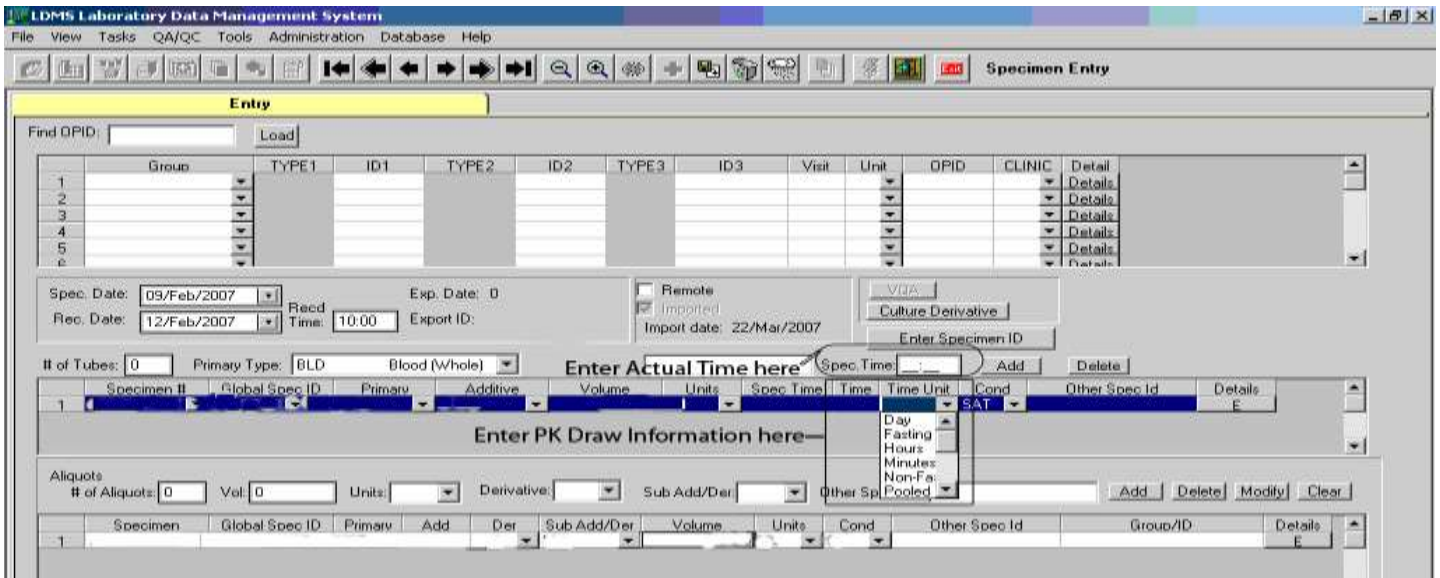
SWB: Swab

TIS: Tissue

#### 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, 0.5 hr, 1.0 hr, 1.5 hr, 2.0 hr, 2.5 hr, etc.) when applicable, otherwise leave blank.

IMAGE 1: LDMS Entry Screen



#### 9.4.2 Urine Testing

The urine tests performed during 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.

#### 9.4.3 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.
- 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 are 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 Urine Chlamydia and Gonorrhea Testing

This testing will be done using 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.

##### 9.5.2.1 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 the STI Test Results CRF.

### 9.5.3 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.

### 9.5.4 Urine Culture

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

## 9.6 Pharyngeal Chlamydia and Gonorrhea Testing

**Note:** Testing for Chlamydia and Gonorrhea is done at screening and when clinically indicated only.

This testing will be done using only 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. Use the Xpert CT/NG Vaginal/Endocervical Specimen Collection kit to collect pharyngeal samples.
2. Remove the sterile swab from the kit and swab each lateral posterior wall, including tonsillar crypts and the pharyngeal arc.
3. Place the swab in the kit transport tube, break off shaft of swab and cap.
4. Cap tightly and invert or gently shake the tube 3-4 times to elute material from the swab. Avoid foaming.
5. The specimen can now remain at 2-30°C for 60 days.
6. Place the transport tube in a biohazard zip-lock bag and transport to the laboratory for testing.
7. The results are sent to the clinic and are reported on the STI Test Results CRF.

## 9.7 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.

### 9.7.1 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.

### 9.7.2 HIV Testing

Although the HIV algorithm (Appendix II of the MTN-033 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-033 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](mailto: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 9.7.8) 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.

### 9.7.3 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.

### 9.7.4 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.

### 9.7.5 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-033 Protocol Safety Physicians ([mtn033safetymd@mtnstopshiv.org](mailto:mtn033safetymd@mtnstopshiv.org)).

#### **9.7.6 Hepatitis B Surface Antigen and Hepatitis C Antibody**

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

#### **9.7.7 INR/PT**

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

#### **9.7.8 Plasma Archive/Storage**

Plasma archive/storage is required at Enrollment. 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/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 specimen 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. Log samples into LDMS (Table 9-3) and store at  $\leq -70^{\circ}\text{C}$ .
7. If samples are hemolyzed, store the aliquots as per normal and enter comments in LDMS.

### 9.7.9 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. Log samples into LDMS (Table 9-3) and store at ≤-70°C until batch shipped to JHU CPAL.

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

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

### 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 swab for microbiome
4. Rectal swab for PK
5. Rectal Enema for PD/PK
6. Biopsies\* for PK, PD, Proteomics, Histology, 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, 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 only 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 participant's 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 7) 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 laboratory for testing.
8. The results are sent to the clinic and are reported on the STI Test Results CRF.

### 9.8.3 Rectal Microbiome

One rectal swab will be collected for rectal microbiome and sent to the MTN LC.

1. 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.
2. The specimen may be kept refrigerated for up to 2 hours.
3. Deliver the swab and the LDMS specimen tracking sheet to the local LDMS laboratory.
4. Log the specimen into LDMS (Table 9-3) and label the specimen with LDMS labels.
5. Freeze at  $\leq -70^{\circ}\text{C}$  within 2 hours of collection.

### 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.

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 equivalent to hold cut swab (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 or equivalent device to hold the shaft of the swab.
  4. Insert the swab and hold against 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.

### 9.8.5 Rectal Enema for PD/PK

1. Rectal enema should be kept on wet ice or refrigerated and processed within 8 hours of collection.
2. In a 50mL 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. Store one vial each of supernatant for PD and PK, the remaining vials may be stored as extra.
6. If less than a total of 6 mLs (or less than 6 cryovials) of supernatant are recovered, contact the MTN LC.
7. Log into LDMS (Table 9-3).

### 9.8.6 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 6 biopsies will be collected for PK analysis. Number cryovials 1-6 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.

### 9.8.7 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  $4^{\circ}\text{C}$  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  $4^{\circ}\text{C}$  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.

### 9.8.8 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.

### 9.8.9 Rectal Biopsies for PD

1. Three biopsies for PD should be collected and placed immediately into biopsy transport media provided by the ex vivo lab.
2. Transport biopsies to lab within 15-30 minutes from time of collection.
3. Biopsies should be processed according to the Non-Polarized Colorectal Explant Culture SOP.
4. Record weights of biopsies onto the MTN-033 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 p24 analysis.
7. 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.

### 9.8.10 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.