Section 10. Laboratory Considerations

10.1 Overview and General Guidance 10-1
10.2 Specimen Labeling 10-4
10.3 Procedures for Specimens that cannot be Evaluated 10-4
10.4 Use of LDMS 10-5
10.4.1 Logging in PK/PD Samples 10-7
10.5 Urine Testing 10-7
10.5.1 Specimen Collection 10-7
10.5.2 Urine Chlamydia and Gonorrhea Testing 10-7
10.5.2.1 Instructions for transferring urine into the GeneXpert transport reagent tube 10-7
10.5.3 Urine hCG 10-8
10.6 Pharyngeal Chlamydia and Gonorrhea Testing 10-8
10.7 Blood Testing 10-8
10.7.1 Specimen Collection and Initial Processing 10-8
10.7.2 HIV Testing 10-9
10.7.3 Hematology Testing 10-9
10.7.4 Liver and Renal Function Testing 10-10
10.7.5 Syphilis Testing 10-10
10.7.6 INR/PT 10-10
10.7.7 Plasma Archive/Storage 10-10
10.7.8 Blood for MIV-150 PK 10-11
10.8 Testing of Vaginal Specimens 10-11
10.8.1 Vaginal Testing for GC/CT/TV (Neisseria gonorrhea/Chlamydia trachomatis/Trichomonas) by NAAT 10-11
10.8.2 Vaginal Fluid for PK 10-11
10.9 Testing of Rectal Specimens 10-13
10.9.1 Anal HPV Typing (DNA PCR) 10-13
10.9.2 Rectal NAAT for Gonorrhea and Chlamydia 10-13
10.9.3 Rectal Sponges for PK and PD 10-14
10.9.4 Rectal Enema for PD/PK 10-14
10.9.5 Rectal Biopsies for PK 10-15
10.9.6 Rectal Biopsy for Histology 10-15
10.9.7 Rectal Biopsies for PD 10-15
10.9.8 Rectal Biopsies for Archive 10-16

10. Introduction

10.1 Overview and General Guidance

This section contains information on the laboratory procedures performed in MTN-037.

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 Colorado Antiviral Pharmacology Laboratory (CAVP). Table 10-1 lists for each test, the testing location, specimen type, specimen container and kit/method (if specified). Table 10-2 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-037 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 10-1
Overview of Laboratory Testing Locations, Specimens,
And Methods for MTN-037

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
Pharyngeal NAAT for Gonorrhea and Chlamydia	Local Lab	Pharyngeal swab	Kit Specific Transport Tube	GeneXpert
Urine NAAT for Gonorrhea and Chlamydia	Local Lab	Urine	Kit Specific Transport Tube	GeneXpert
Urine hCG	Local Lab	Urine	Plastic screw top cup	Not Specified
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
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	CAVP	Plasma	EDTA tube 10mL	CAVP Protocol
Vaginal NAAT for Gonorrhea, Chlamydia, and Trichomonas	Local Lab	Vaginal Swabs	Kit Specific Transport Tube	GeneXpert
Vaginal fluid for PK	CAVP	Vaginal Swab	Cryovial	CAVP Protocol
Anal HPV	MTN LC	Anal Swab	PreservCyte (Digene HC2 Kit)	MTN LC Protocol
Rectal NAAT for Gonorrhea and Chlamydia	Local Lab	Rectal Swab	Kit Specific Transport tube	GeneXpert
Rectal Sponge for PK	CAVP	Rectal Sponge	5mL Cryovial	CAVP Protocol
Rectal Sponge for PD	MTN LC	Rectal Sponge	5mL Cryovial	MTN LC Protocol
Rectal Enema for PD/PK	MTN LC	Rectal Enema	50mL Conical Tube	MTN LC Protocol
Rectal Biopsies for PK	CAVP	3 Rectal Biopsies	1.8mL Cryovial	CAVP 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 Archive	MTN LC	2 Rectal biopsies	1.8mL Cryovial	MTN LC Protocol

Notes: Volumes may vary depending upon each site's testing platforms. Please confirm with the testing lab to determine minimum volume requirements. 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 10-2
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 1500x*g* (or double spin at 800x*g*). Aliquot and freeze.	Batch to MTN LC
Plasma for PK	EDTA	1x10mL	Spin 10 minutes at 1500x*g*. Aliquot and freeze within 2 hours of collection.	Batch to CAVP
Vaginal Swab for PK	None	Swab in Cryovial	Record net weight of swab and freeze at ≤-70°C within 2 hours of collection	Batch to CAVP
Anal Swab for HPV	Viral Transport Media	Digene HPV collection kit	Freeze at ≤-70°C within 2 hours of collection	Scheduled shipment to MTN LC
Rectal Sponge for PD	None	Sponge in Cryovial	Freeze at ≤-70°C within 2 hours of collection	Batch to MTN LC
Rectal Sponge for PK	None	Sponge in Cryovial	Record net weight of sponge and freeze at ≤-70°C within 2 hours of collection	Batch to CAVP
Rectal Enema for PD/PK	None	50mL conical tube	Spin 10 minutes at 400x*g*. Aliquot supernatant and suspend pellet. Freeze supernatants and pellet within 2 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 ≤-70°C within 2 hours of collection	Batch to CAVP
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 Biopsy for Archive	None	2.0mL Cryovial	Record net weight of biopsies then flash freeze and store at ≤-70°C within 2 hours of collection	Batch to MTN LC

10.2 Specimen Labeling

All containers into which specimens are initially collected (e.g., urine collection cups, blood collection tubes) will be labeled with SCHARP-provided Participant ID (PTID) labels. The date 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 10-3) and labeled with LDMS-generated labels.

10.3 Procedures for Specimens that cannot be Evaluated

Specimen collection will be repeated (whenever possible) if samples cannot be evaluated per site SOPs. Site clinic and laboratory staff will monitor specimen collection, processing and management as part of ongoing quality assurance (QA) procedures and take action as needed to address any issues or problems. In cases where additional specimens need to be recollected either due to a laboratory error (lost, broken tube, clerical, etc.) or clinic error, a protocol deviation form may be required.

The 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.

10.4 Use of LDMS

The Laboratory Data and Management System (LDMS) is a program used for the storage and shipping of laboratory specimens. It is supported by the Frontier Science Foundation (FSTRF). LDMS must be used to track the collection, storage, and shipment of specimens in Table 10-3.
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-037 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 10-3
LDMS Specimen Management Guide to Logging in MTN-037 Specimens

The table below should be used as a guide when logging in MTN-037 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-037 study implementation materials.

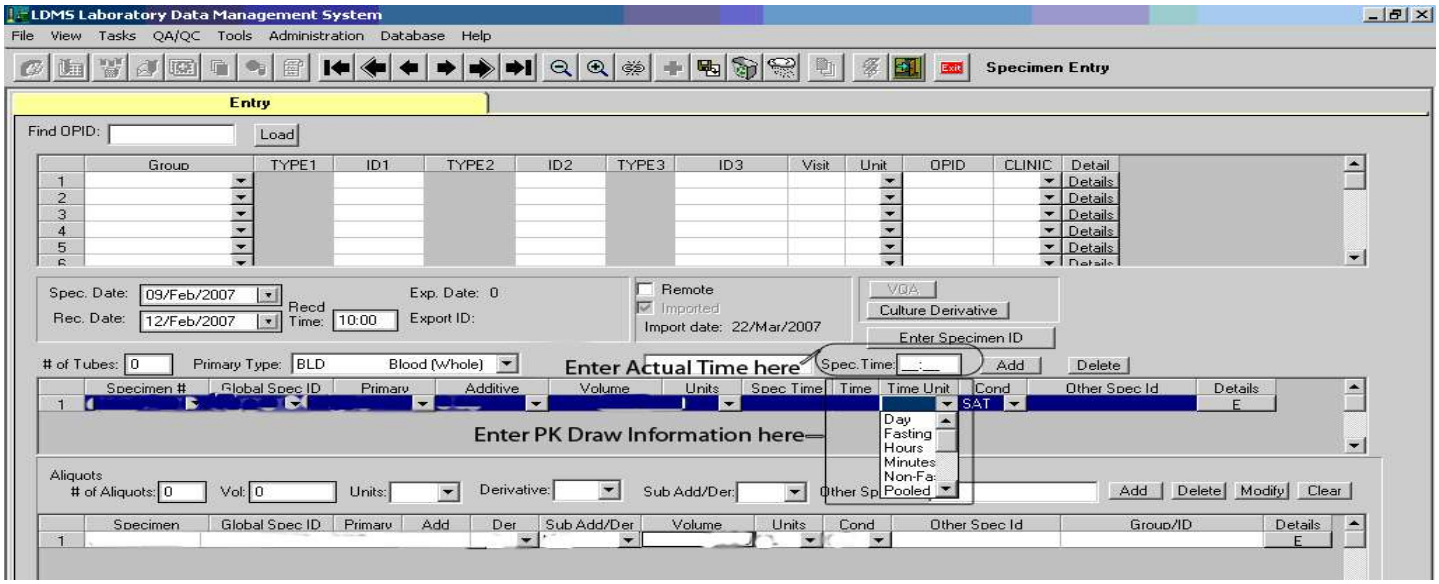
Test	Primary	Additive	Primary Volume	No. of Aliquots	Aliquot Volume	Units	Derv	Sub Add/ Derv	Other Spec ID
Plasma Archive or Storage	BLD	EDT	10.0 ML	4-5	1.0	ML	PL1/2	N/A	
Plasma for PK	BLD	EDT	10.0 ML	4-5	1.0	ML	PL1	N/A	PK
Vaginal Swab for PK	VAG	NON	1 EA	1	Net Weight	MG	SWB	N/A	
Anal Swab for HPV	ANL	VTM	1 EA	1	1	EA	SWB	N/A	
Rectal Sponge for PD	REC	NON	1 EA	1	Net Weight	MG	SPG	N/A	PD
Rectal Sponge for PK	REC	NON	1 EA	1	Net Weight	MG	SPG	N/A	PK
Test	**Primary**	**Additive**	**Primary Volume**	**No. of Aliquots**	**Aliquot Volume**	**Units**	**Derv**	**Sub Add/ Derv**	**Other Spec ID**
Rectal Enema for PD/PK	REC	NSL	10.0 ML	6+	1.0	ML	FLD	N/A	
				1	1.0	ML	PEN	NSL	
Rectal Biopsies for PK	FSR	NON	3 EA	3	Net Weight	MG	BPS	N/A	PK
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 Archive	FSR	NON	2 EA	2	Net Weight	MG	BPS	N/A	ARC

ANL: Anal
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
PAN: Perianal
PEN: Non-viable cells from non-blood specimen
PL1: Single spun Plasma
PL2: Double spun Plasma
REC: Rectal
RNL: RNAlater
RPM: RPMI
SPG: Sponge
SUP: Supernatant
SWB: Swab
TIS: Tissue
VAG: Vaginal Swab
VTM: Viral Transport Media

10.4.1 Logging in PK/PD 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/PD time point information (0 pre-dose, 1.0 hr, 2.0 hr, 3 hr, etc.) when applicable, otherwise leave blank.

IMAGE 1: LDMS Entry Screen



10.5 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.

10.5.1 Specimen Collection

- The participant should not have urinated within one hour prior to urine collection.
- Provide the participant with a sterile, plastic, preservative-free screw-top urine collection cup labeled with a SCHARP-provided PTID label.
- 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 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.

10.5.2 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.

10.5.2.1 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 a STI Test Results CRF.

10.5.3 Urine hCG

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

10.6 Pharyngeal Chlamydia and Gonorrhea Testing

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. 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 a STI Test Results CRF.

10.7 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.

10.7.1 Specimen Collection and Initial Processing

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

<u>After collection</u>:

- 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.

10.7.2 HIV Testing

Although the HIV algorithm (Appendix II of the MTN-037 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-037 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 10.7.9) 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.

10.7.3 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.

10.7.4 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.

10.7.5 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-037 Protocol Safety Physicians (mtn037safetymd@mtnstopshiv.org).

10.7.6 INR/PT

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

10.7.7 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 ≤-70°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 per one of these techniques:
    - Single spun: Spin blood at 1500×*g* for 10 minutes and remove plasma.
    - Double spun: Spin blood at 800×*g* for 10 minutes, recover plasma and place in a tube to spin again at 800×*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 (≥) 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.

10.7.8 Blood for MIV-150 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.

8. Mix blood sample with the anticoagulant using gentle inversions (8 to 10 times).
9. 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 2 hours of blood collection.
10. Transfer plasma to appropriately labeled 2.0 mL cryovials in as many 1.0 mL aliquots as possible.
11. Log samples into LDMS (Table 10-3) and store at ≤-70°C until batch shipped to CAVP.

Ship PK samples to CAVP (LDMS Lab 533)

Lane R. Bushman
CAVP
UC Denver-Skaggs School of Pharmacy and Pharmaceutical Sciences
V20-4410
12850 E. Montview Blvd
Aurora, CO 80045
Lab Phone#: (303) 724-6132

10.8 Testing of Vaginal Specimens

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

10.8.1 Vaginal Testing for GC/CT/TV (Neisseria gonorrhea/Chlamydia trachomatis/Trichomonas) by NAAT

Testing for chlamydia, gonorrhea, and trichomonas are performed at screening and when clinically indicated. Sites can choose to use the GeneXpert or Gen-Probe Aptima.

- Two swabs should be collected.
- 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 specimens at ambient temperature to the local laboratory.

10.8.2 Vaginal Fluid for PK

- Each day of collection of vaginal 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 to avoid 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 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. If the post-weight is smaller than the pre-weight, yielding a negative net weight, please confirm that the scale has been calibrated and quality control checks have been performed. If the negative net weight cannot be resolved please notify the MTN LC.
8. Within 2 hours, place the sample tubes in the freezer at ≤-70°C.
9. Log into LDMS (Table 10-3) 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** 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 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. If the post-weight is smaller than the pre-weight, yielding a negative net weight, please confirm that the scale has been calibrated and quality control checks have been performed. If the negative net weight cannot be resolved please notify the MTN LC.
5. Within 2 hours, place the sample tubes in the freezer at ≤-70°C.
6. Log into LDMS (Table 10-3) and batch ship to JHU CPAL (LDMS Lab 194) upon request.

10.9 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 HPV
2. Rectal swab for GC/CT
3. Rectal sponges for PK and PD
4. Rectal Enema for PD/PK
5. Biopsies* for PK, PD, Histology, and Archive

Table 10-2 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, Histology, PD, and then Archive.

10.9.1 Anal HPV Typing (DNA PCR)

The Qiagen Digene Female Swab Specimen Collection Kit (Anal Swab) Catalog Number: 5123-1220 should be used for specimen collection. Please use the swab provided in the Digene kit. Wooden shafted swabs are not acceptable for PCR testing and must not be used.

1. Once specimen is collected, insert the swab to the bottom of the transport tube containing media. Snap off the shaft at the score line, and then cap the tube securely.
2. Store specimens at ≤-70°C. To prevent caps from popping off specimen tubes that are shipped or stored frozen, cover caps with Parafilm® prior to shipment.
3. Log specimen into LDMS (Table 10-3) and label specimen with LDMS label.

10.9.2 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.

6. Collect specimen using the Xpert collection swab.
7. Label the pink-capped transport tube with the participants PTID number and date.
8. 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.
9. Immediately place the swab in the transport tube, break off shaft of swab and cap.
10. Cap tightly and invert or gently shake the tube 3-4 times to elute material from the swab. Avoid foaming.
11. The specimen can now remain at 2-30°C for 60 days.
12. Place the transport tube in a biohazard zip-lock bag and transport to the laboratory for testing.
13. The results are sent to the clinic and are reported on a STI Test Results CRF.

10.9.3 Rectal Sponges for PK and PD

- Each day of collection of rectal fluid for PK and/or PD, 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 and PD sponges must be collected within one hour of PK blood draw.
- Ensure that new or sterilized supplies are used for each sample as MIV-150 is very sensitive to cross-contamination.

14. Remove a sponge (Merocel eye-wick Spears Fisher Scientific # NC0093269) from the box, wear gloves at all times when handling sponges.
15. If collecting more than one sponge, using a permanent marker, identify each of the sponges by numbering the sponge shaft or using another unique identifier.
16. Place each sponge into an appropriately labeled 5mL cryovial, labeled with a unique participant identifier.
17. Mark the exterior of one cryovial with the same identifier used to label the sponge shaft. NOTE: ALWAYS REPLACE SAME SPONGE TO THE SAME VIAL
18. Weigh the dry sponge + labeled cryovial and document the weight (pre-weight) on the LDMS Tracking Sheet.
19. The clinician will collect specimen using a pre-weighed sponge according to the procedures outlined in the SSP for Clinical Considerations (Section 8).
20. Place the sponges back into the original weighed cryovial (by matching the color code of the sponge to the tube) and ensure that the cap is fully tightened.
21. Weigh the cryovial and sponge after collection using the same analytical balance used for the pre-weights. Document the post-collection weight and calculate the NET weight on the LDMS Tracking Sheet. If the post-weight is smaller than the pre-weight, yielding a negative net weight, please confirm that the scale has been calibrated and quality control checks have been performed. If the negative net weight cannot be resolved please notify the MTN LC.
22. Complete the LDMS tracking sheet and submit to lab on ice for LDMS entry.
23. Log into LDMS (Table 10-3) and label specimen with LDMS label.
24. Freeze at ≤-70°C within 2 hours of collection until ready to ship.
25. Specimens may be batched and shipped on dry ice. Once the LC notifies the site to ship, use LDMS to create a shipping manifest. Ship PK specimens to CAVP (LDMS Lab 533) and PD specimens to the MTN LC (LDMS Lab 414) Monday through Wednesday for overnight delivery.

10.9.4 Rectal Enema for PD/PK

1. Rectal enema should be kept on wet ice or refrigerated and processed within 2 hours of collection.
2. In a 50mL conical tube collect 10 mLs of the rectal enema. Spin at 400×*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 ≤-70°C within 2 hours of collection and track in LDMS. For organizational purposes, one aliquot of supernatant may be stored for PD and one for PK while the remaining aliquots can be stored as extra (EXT). Store pellet separately.
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 10-3) and batch ship to MTN LC (LDMS Lab 414) upon request.

10.9.5 Rectal Biopsies for PK

26. Logistics permitting, biopsies should be delivered to the lab to allow freezing within two hours of collection.
27. Up to 3 biopsies will be collected for PK analysis. Number cryovials 1-3 depending on how many biopsies are received with appropriate participant information.
28. 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.
29. Transfer biopsy to a pre-weighed cryovial. Store only ONE biopsy per cryovial. Ensure biopsy sits at bottom of cryovial.
30. Weigh the cryovial containing the biopsy (post-weight). Document the weight of the cryovial containing the biopsy and calculate the Net weight on the LDMS Tracking Sheet. If the post-weight is smaller than the pre-weight, yielding a negative net weight, please confirm that the scale has been calibrated and quality control checks have been performed. If the negative net weight cannot be resolved please notify the MTN LC.
31. Freeze the cryovial containing the biopsy in Liquid Nitrogen or a dry ice-alcohol bath.
32. Log into LDMS (Table 10-3) and label specimen with LDMS label.
33. Store the labeled cryovial containing the biopsy in a ≤-70°C freezer. Document the date/time the cryovial containing the biopsy was placed in the freezer.
34. Batch and ship on dry ice to CAVP (LDMS Lab 533) upon request.

10.9.6 Rectal Biopsy for Histology

35. Place one biopsy into a microtube filled with 10% formalin for shipping. These can be kept at room temperature.
36. Complete the LDMS tracking sheet and submit to lab for LDMS entry.
37. Log specimens into LDMS (Table 10-3), label specimen with LDMS label.
38. The tissue processing/embedding must occur within 72 hours of collection.
39. Batch ship the histology blocks to the MTN LC (LDMS Lab 414) at room temperature.

10.9.7 Rectal Biopsies for PD

40. Three biopsies for PD should be collected and placed into biopsy transport media immediately.
41. Transport biopsies to lab within 15-30 minutes from time of collection.
42. Please refer to the Non-Polarized Colorectal Explant Culture SOP provided by the MTN LC for processing.
43. Record weights of biopsies onto the MTN-037 LDMS Tracking Sheet.
44. Each biopsy will be logged into LDMS separately (Table 10-3)
45. Supernatants collected during tissue culture should be logged into LDMS as culture derivatives and stored frozen in cryovials at ≤-70°C until shipped for p24 analysis. See LDMS Culture Derivative SOP provided by MTN LC.
46. Supernatants for p24 analysis will be batched and shipped to the MTN LC (LDMS Lab 414) on dry ice.
47. Biopsies after completion of culture will be stored in RNAlater per the Non-Polarized Colorectal Explant Culture SOP and logged into LDMS as a culture derivative.

10.9.8 Rectal Biopsies for Archive

48. Logistics permitting, biopsies should be delivered to the lab to allow freezing within two hours of collection.
49. Up to 2 biopsies will be collected for archive. Number cryovials 1-2 depending on how many biopsies are received with appropriate participant information.
50. 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.
51. Transfer biopsy to a pre-weighed cryovial. Store only ONE biopsy per cryovial. Ensure biopsy sits at bottom of cryovial.
52. Weigh the cryovial containing the biopsy (post-weight). Document the weight of the cryovial containing the biopsy and calculate the NET weight on the LDMS Tracking Sheet. If the post-weight is smaller than the pre-weight, yielding a negative net weight, please confirm that the scale has been calibrated and quality control checks have been performed. If the negative net weight cannot be resolved please notify the MTN LC.
53. Log into LDMS (Table 10-3) and label specimen with LDMS label.
54. Store the labeled cryovial containing the biopsy in a ≤-70°C freezer. Document the date/time the cryovial containing the biopsy was placed in the freezer.
55. Batch and ship on dry ice to the MTN LC (LDMS Lab 414) upon request.