Section 11. Laboratory Considerations

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11 Introduction

This section contains information on the laboratory procedures performed in MTN-039.

11.1 Overview and General Guidance

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 or network laboratories and in the MTN Laboratory Center (LC). Table 11-1 lists for each test the testing location, specimen type, specimen container and kit/method (if specified). Table 11-2 specifies specimen collection for storage and shipment.

Regardless of whether tests are performed in clinic or laboratory settings, study staff that perform 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 specimens 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-039 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 11-1
Overview of Laboratory Testing Locations, Specimens,
And Methods for MTN-039

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 or Aptima Combo 2
Urine NAAT for Gonorrhea and Chlamydia	Local Lab	Urine	Kit Specific Transport Tube	GeneXpert or Aptima
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
Hepatitis B (HBsAg)	Local Lab	Serum or plasma	EDTA, plain or serum separator tube 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	PK Lab	Plasma	EDTA tube 10mL	PK Protocol
Vaginal NAAT for Gonorrhea, Chlamydia, and Trichomonas	Local Lab	Vaginal Swabs	Kit Specific Transport Tube	GeneXpert or Aptima
Cervicovaginal fluid for PK	PK Lab	Cervicovaginal Swab	Dacron Swab in Cryovial	PK Protocol
Cervicovaginal fluid for PD	MTN LC	Cervicovaginal Swab	Dacron Swab in Cryovial	MTN LC Protocol
Cervicovaginal fluid for Microflora and Gram Stain	MTN LC	Vaginal Swab	Starplex Starswab Anaerobic Transporter 2 Slides	MTN LC Protocol
Rectal NAAT for Gonorrhea and Chlamydia	Local Lab	Rectal Swab	Kit Specific Transport tube	GeneXpert or Aptima Combo 2
Rectal fluid for PK	PK Lab	Rectal Swab	Cryovial	PK Protocol
Rectal fluid for PD	MTN LC	Rectal Swab	Cryovial	MTN LC Protocol
Rectal fluid for Microbiome	MTN LC	Rectal Swab	Cryovial	MTN LC Protocol
Rectal Biopsies for PK	PK Lab	1 Biopsy – EVG	1.8mL Cryovial	PK Protocol
		1 Biopsy – TFV/TFV-DP	1.8mL Cryovial	PK Protocol
		1 Biopsy – Backup	1.8mL Cryovial	PK Protocol

		8 Biopsies – MMC	Biopsy Transport Media	PK 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	4 Rectal Biopsies	Biopsy Transport Media	MTN LC Protocol
Rectal Biopsies for T cell Phenotyping	MTN LC	4 Rectal Biopsies	Biopsy Transport Media	MTN LC Protocol
Rectal Biopsy for Proteomics	MTN LC	1 Biopsy	1.8mL Cryovial	MTN LC Protocol
Rectal Biopsy for Metabolomics	MTN LC	1 Biopsy	1.8mL Cryovial	MTN LC Protocol

Note: 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 contains no additive.
- Purple top tubes contain EDTA.
- Light Blue top tubes contain Na Citrate.

Table 11-2
Overview of Specimens for Storage and Shipment

Specimen and Subsequent Testing	Additive	Recommended Tube type or size	Processing and Storage	Ship to:
Plasma Archive / Storage	EDTA	1x10mL	Spin 10 minutes at 1500x*g*. Aliquot and freeze.	Batch to MTN LC
Plasma for PK	EDTA	1x10mL	Spin 10 minutes at 1500x*g*. Aliquot and freeze within 1 hour of collection.	Batch to PK Lab
Cervicovaginal fluid for PK	None	Dacron Swab in Cryovial	Record net weight of swab and freeze at ≤-70°C within 2 hours of collection	Batch to PK Lab
Cervicovaginal fluid for PD	None	Dacron Swab in Cryovial	Record net weight of swab and freeze at ≤-70°C within 2 hours of collection	Batch to MTN LC
Cervicovaginal Microflora	CTK	Starplex Starswab Anaerobic Transporter	Store at 4°C.	Overnight to MTN LC
Vaginal Smear for Gram Stain	None	2 slides	Store at room temperature.	Overnight to MTN LC
Rectal fluid for PD	None	Dacron Swab in Cryovial	Record net weight of swab and freeze at ≤-70°C within 2 hours of collection	Batch to MTN LC
Rectal fluid for PK	None	Dacron Swab in Cryovial	Record net weight of swab and freeze at ≤-70°C within 2 hours of collection	Batch to PK Lab
Rectal fluid for Microbiome	None	Flocked Nylon Swab in cryovial	Freeze at ≤-70°C within 2 hours of collection	Batch to MTN LC
Rectal Biopsies for PK (3)	None	1.8mL Cryovial	Record net weight of biopsies then flash freeze and store at ≤-70°C within 1 hour of collection	Batch to PK Lab
Rectal Biopsies for PK MCC Isolation (8)	Biopsy Transport Media	50mL conical tube with 20mL media	Transport refrigerated to local testing lab within 30 minutes of collection for local processing.	Batch to PK Lab

Rectal Biopsies for PD (4)				Batch to MTN LC
Rectal Biopsy for Histology	10% Formalin	2.0mL tube	Store at room temperature	Overnight to MTN LC
Rectal Biopsies for T cell Phenotyping (4)	Biopsy Transport Media	10mL conical tube with 10mL media	Transport refrigerated to local testing lab.	Overnight to MTN LC
Rectal Biopsy for Proteomics	None	1.8mL Cryovial	Flash freeze and store at ≤-70°C within 1 hour of collection.	Batch to MTN LC
Rectal Biopsy for Metabolomics	None	1.8mL Cryovial	Flash freeze and store at ≤-70°C within 1 hour of collection.	Batch to MTN LC

11.2 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 11-3) and labeled with LDMS-generated labels.

11.3 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
- 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 have any question regarding time windows or collection processes, call LC staff (Pam Kunjara at +1-412-641-6393/6157 or e-mail:PKunjara@mwri.magee.edu as soon as possible for guidance.

11.4 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 11-3.
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).

If using the desktop version of LDMS, each site must export its LDMS data to FSTRF on a weekly basis. For web-based LDMS users, the data is exported automatically. 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 actions 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-039 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 11-3
LDMS Specimen Management Guide to Logging in MTN-039 Specimens

The table below should be used as a guide when logging in MTN-039 specimens for each test listed. Tests that are listed as "local lab" and specimens that 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-039 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	N/A	
Plasma for PK	BLD	EDT	10.0 ML	4-5	1.0	ML	PL1	N/A	PK
Cervicovaginal fluid for PD	CVF	NON	1 EA	1	Net Weight	MG	SWB	N/A	PD
Cervicovaginal fluid for PK	CVF	NON	2 EA	2	Net Weight	MG	SWB	N/A	PK
Cervicovaginal fluid for Microflora and Gram Stain	VAG	CTK	1 EA	1	1	EA	SWB	N/A	MS
		NON	1 EA	2	1	EA	SLD	GRS	
Rectal fluid for PD	REC	NON	1 EA	1	Net Weight	MG	SWB	N/A	PD
Rectal fluid for PK	REC	NON	2 EA	2	Net Weight	MG	SWB	N/A	PK
Rectal fluid for Microbiome	REC	NON	1 EA	1	1	EA	SWB	N/A	MS
Rectal Biopsies for PK	FSR	NON	3 EA	3	Net Weight	MG	BPS	N/A	PK
Rectal Biopsies for MMC Isolation	FSR	BTM	8 EA	2	0.5	ML	CIO	MET	MMC
Rectal Biopsy for Histology	FSR	FOR	1 EA	1	1	EA	BPS	N/A	
Rectal Biopsies for PD x4 (Log each in separately)	FSR	BTM	Net Weight MG	1	500	UL	SUP	RPM	PD
				1	1	ML	TIS	RNL	
Rectal Biopsy for T cell Phenotyping	FSR	BTM	4 EA	1	4	EA	BPS	N/A	PHENO

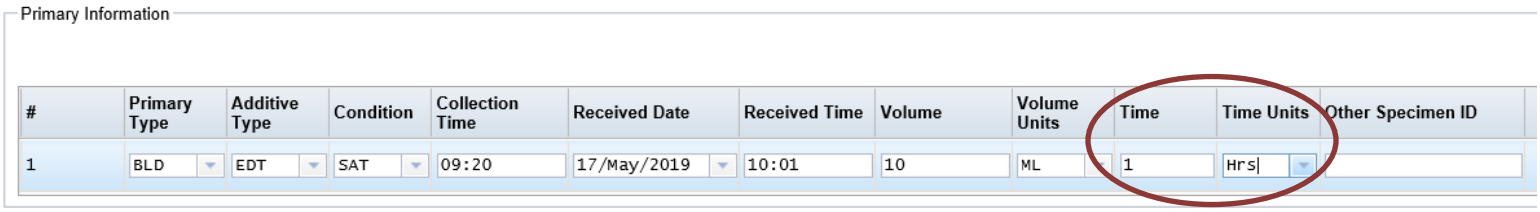
Rectal Biopsy for Proteomics	FSR	NON	1 EA	1	Net Weight	MG	BPS	N/A	PRO
Rectal Biopsy for Metabolomics	FSR	NON	1 EA	1	Net Weight	MG	BPS	N/A	MB

BLD: Whole Blood
BPS: Biopsy
BTM: Biopsy Transport Media
CTK: Culture Transport Kit
CVF: Cervicovaginal Fluid
EDT: EDTA
FOR: Formalin
FSR: Rectal biopsy by flexible sigmoidoscopy
GRS: Gram Stain
NON: None
PL1: Single spun Plasma
REC: Rectal
RNL: RNAlater
RPM: RPMI
SLD: Slide from a primary sample
SUP: Supernatant
SWB: Swab
TIS: Tissue
VAG: Vaginal Swab

11.4.1 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, 1.0 hr, 2.0 hr, 4.0 hr, 6.0 hr etc.) when applicable, otherwise leave blank.

IMAGE 1: LDMS Entry Screen



11.5 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.

11.5.1 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.

11.5.2 Urine Chlamydia and Gonorrhea Testing

This testing will be done using either the Cepheid GeneXpert NAAT method or FDA approved Aptima assay 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.

11.5.2.1 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 participant's 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.

11.5.2.2 Instructions for transferring urine into the Aptima transport tube

8. Collect urine as noted above.
9. Open the UPT kit and remove the UPT and transfer pipette. Label the UPT with the participants PTID number and date.
10. 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.
11. 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.
12. Cap tightly and invert the tube 3-4 times to ensure that the specimen and reagent are mixed.
13. The specimen can now remain at 2-30°C for 30 days.
14. Place the transport tube in a biohazard zip-lock bag and transport to the local laboratory for testing.
15. The results are sent to the clinic and are reported on a STI Tests CRF.

11.5.3 Urine hCG

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

11.6 Pharyngeal Chlamydia and Gonorrhea Testing

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

This testing will be done using either the Cepheid GeneXpert NAAT method or Aptima Combo 2 assay 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.

11.6.1.1 Instructions for using the Xpert Vaginal/Endocervical Collection kit

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 up to 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.

11.6.1.2 Instructions for using the Aptima Multitest Swab Specimen Collection kit

1. Partially peel open the swab package. Remove the swab. Do not touch the soft tip or lay the swab down. If the soft tip is touched, the swab is laid down, or the swab is dropped, use a new Aptima Multitest Swab Specimen Collection Kit.
2. Hold the swab, placing your thumb and forefinger in the middle of the swab shaft covering the score line. Do not hold the swab shaft below the score line.
3. Carefully insert the swab into the throat ensuring contact with bilateral tonsils (if present) and the posterior pharyngeal wall, then withdraw the swab without touching the inside of the cheeks or tongue.
4. While holding the swab in the same hand, unscrew the cap from the tube. Do not spill the contents of the tube. If the contents of the tube are spilled, use a new Aptima Multitest Swab Specimen Collection Kit.
5. Immediately place the swab into the transport tube so that the score line is at the top of the tube.
6. Carefully break the swab shaft at the score line against the side of the tube. Immediately discard the top portion of the swab shaft.
7. Tightly screw the cap onto the tube.
8. Transport and store the swab at 2-30°C for up to 60 days.
9. Place the transport tube in a biohazard zip-lock bag and transport to the laboratory for testing.
10. The results are sent to the clinic and are reported on a STI Test Results CRF.

11.7 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.

11.7.1 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.

11.7.2 HIV Testing

Although the HIV algorithm (Appendix II of the MTN-039 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-039 protocol). If the test isd 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 an 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 9.7.9) 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.

11.7.3 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.

11.7.4 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.

11.7.5 Syphilis Testing

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

1. 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.

2. Perform syphilis assessment using a specific FDA approved treponemal test (such as EIA, MHA-TP, TPHA, TPPA, or FTA-ABS) and confirming positive test results with a non-treponemal assay (RPR or VDRL). If the confirmatory non-treponemal assay is reactive at screening visit, appropriate clinical management action must be taken. 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-039 Protocol Safety Physicians (mtn039safetymd@mtnstopshiv.org).

11.7.6 Hepatitis B Surface Antigen

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

11.7.7 INR/PT

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

11.7.8 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.

Collected samples held at room temp should be processed and plasma frozen within 4 hours. If refrigerated or on ice after collection, freeze plasma within 24 hours. If total whole blood volume is less than 2.0 mL, redraw specimen as soon as possible.

1. For plasma archive/storage, use collection tubes with EDTA anticoagulant.
2. Spin blood at room temperature at 1500×g for 10 minutes and remove plasma.
3. Prepare as many 1.0 mL aliquots as possible with a total volume of aliquots greater than or equal (≥) to 4ml.
4. Store at ≤-70°C.
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.

11.7.9 Blood for 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. Processing must be completed within 1 hour of collection time.

16. Mix blood sample with the anticoagulant using gentle inversions (8 to 10 times).
17. Centrifuge the sample at approximately 1500×*g* for 10 minutes at 4°C.
18. Transfer plasma to appropriately labeled cryovials in as many 1.0 mL aliquots as possible. If less than 4 mL of plasma are available, store that plasma and inform the MTN LC for instruction.
19. Log samples into LDMS (Table 11-3) and store at ≤-70°C.
20. Batch ship PK samples to PK Lab upon request.

11.8 Testing of Cervicovaginal Specimens

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

11.8.1 Cervicovaginal Testing for GC/CT/TV (Neisseria gonorrhea/Chlamydia trachomatis/Trichomonas) by NAAT

Testing for chlamydia, gonorrhea, and Trichomonas is performed at screening and when clinically indicated. Sites can choose to use the GeneXpert or FDA approved 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.

11.8.2 Cervicovaginal Fluid for PK and PD

- Each day of collection of vaginal 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 swabs must be collected within one hour of PK blood draw, transported on ice, and stored frozen within 2 hours of collection.
- Two swabs must be collected when PK testing is required.
  1. When collecting more than one swab, using a permanent marker, identify each of the swabs by numbering the shaft or using another unique identifier.
  2. Place each swab into an appropriately labeled cryovial, labeled with a unique participant identifier.
  3. Mark the exterior of one cryovial with the same identifier used to label the swab shaft. NOTE: ALWAYS REPLACE SAME SWAB TO THE SAME VIAL
- Ensure that new or sterilized supplies are used for each sample to avoid cross-contamination.
- All PK and PD swabs will be logged into LDMS.
- Upon authorization from the MTN LC, PK swab will be batch shipped to the PK Lab and PD swabs will be batch shipped to the MTN LC.
- There are two methods to collecting and weighing each swab for PK/PD. 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.

<u>Pre-cut Swab Collection Method</u>

Materials for each swab 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.
   - If collecting more than one swab, using a permanent marker, identify each of the swabs by numbering the shaft or using another unique identifier.
   - Place each swab into an appropriately labeled cryovial, labeled with a unique participant identifier.
   - Mark the exterior of one cryovial with the same identifier used to label the swab shaft. NOTE: ALWAYS REPLACE SAME SWAB TO THE SAME VIAL
2. Perform pre-weight measurement by weighing the labeled capped cryovial with pre-cut swab and record on the LDMS Tracking Sheet.
3. Sample collection
   a. Uncap the pre-weighed cryovial. Use a clean hemostat, forceps, or transfer pipette to clamp or extend the shaft of the swab.
   b. 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.
   c. Immediately place swab into the cryovial after sampling and recap.
   d. Document collection time on the LDMS tracking sheet.
4. Perform post-weight measurement by weighing the capped cryovial containing the absorbed swab tip and record on the LDMS Tracking Sheet.
5. Calculate and record the NET weight on the LDMS Tracking Sheet.
6. Within 2 hours, place the sample tubes in the freezer at ≤-70°C.

<u>Post-cut Swab Collection Method</u>

Materials for each swab 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.
   - If collecting more than one swab, using a permanent marker, identify each set of the supplies using unique identifiers.
     NOTE: ALWAYS REPLACE SAME SWAB TO THE SAME VIAL and keep each set together.
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.
5. Within 2 hours, place the sample tubes in the freezer at ≤-70°C.

11.8.3 Cervicovaginal Fluid for Microflora and Gram Stain

Vaginal swabs will be collected for microflora culture. Dried vaginal fluid smears will be prepared for Gram staining and assessment for bacterial vaginosis at the MTN LC. The primary slide will be shipped to the MTN LC with the swab for microbiota culture, and the backup will be archived on site until written notification is received from the *Statistical Center for HIV*AIDS Research & Prevention (SCHARP) that the slide may be discarded.

Materials

- Completed SCHARP Labels
- Starplex Starswab Anaerobic collection and (glass) transport kit (provided by MTN LC)
- Pencil
- 2 slides with frosted end, primary and backup (provided by MTN LC)
- Slide holder for individual slide (provided by MTN LC) to send with culture
- Slide box (provided by MTN LC) to store backup slides until end of study or when requested
- Shipping box (if applicable)

1. Use the Starplex Starswab Anaerobic collection and transporter kit. The kit comes with 2 sterile Dacron swabs and a glass transport tube.
2. Collect the specimen for culture by rotating 2 Dacron swabs several times over the lateral wall of the vagina. Do not collect culture swabs in the exact same area that another sample was collected (i.e: If the PK swab was collected first, then collect in a different location in the vagina preferably closer to the introitus).
3. Insert **one** of the swabs into the tube, slowly pushing the swab half way into the gel, not to the bottom of the tube. Break off the shafts of the swabs (at perforation point) and secure the cap tightly.The specimen may be kept at controlled room temperature for up to 4 hours. After four hours, the specimen must be refrigerated.
4. Use a pencil to write the PTID and specimen collection date on the frosted end of the slide. This is the side of the slide that the specimen is to be applied.
5. Use the second swab to prepare the gram stain slides. Roll the swab across each of the slides. Do not place the swab in saline, transport medium, or any transport container prior to slide preparation.

6. A SCHARP-provided PTID label is to be placed on the underside of the slides (on the frosted end, under the pencil markings); write the specimen collection date in indelible ink (e.g. Sharpie pen) on each label.

7. Allow the specimens to air-dry on the slides. Do not heat-fix.
8. Deliver the Starplex tube, gram stain slides, and the LDMS specimen tracking sheet to the local LDMS laboratory.
9. Log the specimen into LDMS (Table 11-3) and label the Starplex tube and slides with LDMS labels. Place the LDMS label on the frosted end of the slide on top of the pencil markings (same side as sample).

10. The primary slides will be positioned in a plastic slide holder and sent to the MTN LC along with the swab for microflora. (See shipping instructions below).
11. Store the secondary slide in the slide box location assigned in LDMS at room temperature. (This is a backup slide in case the first is lost, broken, or unreadable).
12. Use LDMS to generate a shipping manifest (i.e. batch file) for the specimens to be shipped to lab 414.
13. Ship the Starplex tube and one vaginal smear for Gram stain the same day of collection by overnight courier.
14. Into a biohazard specimen ziplock bag, place the Starplex tube with absorbent material (e.g. paper towels) and the case holding the corresponding Gram stain slide. If shipping multiple participant visits in same shipment, each participant visit should have its own specimen ziplock bag. Place the specimen ziplock bag(s), ice packs, and a copy of the manifest in a cardboard box lined with Styrofoam.
15. Use diagnostics packing code 650, UN3373 labels.
16. Confirm the address is correct (see below). The Research Institute is not open for weekend deliveries. Therefore, specimens collected on Friday must be sent to the hospital address for delivery on Saturday.

    If sending **<u>Monday through Thursday</u>**, send to:
    May Beamer
    Magee-Womens Research Institute
    204 Craft Ave, Room A530
    Pittsburgh, PA 15213
    Phone# 412-641-6041

    If sending on **<u>Friday</u>** for Saturday delivery ***(<u>Select Saturday delivery</u>)***, and send to:
    May Beamer, C/O Safety and Security

Magee-Womens Hospital of UPMC
300 Halket St.
Pittsburgh, PA 15213
Phone # 412-641-4191 (contact number for Safety and Security)
** Be sure to select Saturday delivery on the Fed Ex label

Notify the MTN LC via email (hillierlab@mwri.magee.edu) when the shipment has been picked up from the site by the courier/shipping company. Attach an electronic copy of the shipping manifest (i.e the LDMS batch file) to the email notification and include the following information: name of courier/shipping company, shipment tracking number, number of boxes shipped, date of shipment, and expected date of arrival.

11.9 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. Rectal swab for GC/CT
2. Rectal swab for PK and PD
3. Rectal swab for Microbiome
4. Biopsies* for PK, PD, Histology, T Cell Phenotyping (flow cytometry), Proteomics, and Metabolomics

Table 11-2 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, T Cell Phenotyping (flow cytometry), Metabolomics, and then Proteomics.

11.9.1 Rectal NAAT for Gonorrhea and Chlamydia

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

This testing will be done using either the Cepheid GeneXpert NAAT method or Aptima Combo 2 assay 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.

11.9.1.1 Instructions for using the Xpert Vaginal/Endocervical Collection kit

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 Manual section on Clinical Considerations 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 a STI Test Results CRF.

11.9.1.2 Instructions for using the Aptima Multitest Swab Specimen Collection kit

1. Partially peel open the swab package. Remove the swab. Do not touch the soft tip or lay the swab down. If the soft tip is touched, the swab is laid down, or the swab is dropped, use a new Aptima Multitest Swab Specimen Collection Kit.
2. Hold the swab, placing your thumb and forefinger in the middle of the swab shaft covering the score line. Do not hold the swab shaft below the score line.
3. Carefully insert the swab into the rectum about 1-2 inches (3-5 cm) past the anal margin and gently rotate the swab for 5 to 10 seconds. Withdraw the swab without touching the skin.
4. While holding the swab in the same hand, unscrew the cap from the tube. Do not spill the contents of the tube. If the contents of the tube are spilled, use a new Aptima Multitest Swab Specimen Collection Kit.
5. Immediately place the swab into the transport tube so that the score line is at the top of the tube.
6. Carefully break the swab shaft at the score line against the side of the tube. Immediately discard the top portion of the swab shaft.
7. Tightly screw the cap onto the tube.
8. The specimen can now remain at 2-30°C for 60 days.
9. Place the transport tube in a biohazard zip-lock bag and transport to the laboratory for testing.
10. The results are sent to the clinic and are reported on a STI Test Results CRF.

11.9.2 Rectal Swab 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 swabs must be collected within one hour of PK blood draw, transported on ice, and stored frozen within 2 hours of collection.
- Two swabs must be collected when PK testing is required.
    1. When collecting more than one swab, using a permanent marker, identify each of the swabs by numbering the shaft or using another unique identifier.
    2. Place each swab into an appropriately labeled cryovial, labeled with a unique participant identifier.
    3. Mark the exterior of one cryovial with the same identifier used to label the swab shaft. NOTE: ALWAYS REPLACE SAME SWAB TO THE SAME VIAL
- Ensure that new or sterilized supplies are used for each sample to avoid cross-contamination.
- All PK and PD swabs will be logged into LDMS.
- Upon authorization from the MTN LC, PK swab will be batch shipped to the PK Lab and PD swabs will be batch shipped to the MTN LC.
- There are two methods to collecting and weighing each swab for PK/PD. 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.

<u>Pre-cut Swab Collection Method</u>

Materials for each swab 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.
    - If collecting more than one swab, using a permanent marker, identify each of the swabs by numbering the shaft or using another unique identifier.

- Place each swab into an appropriately labeled cryovial, labeled with a unique participant identifier.
- Mark the exterior of one cryovial with the same identifier used to label the swab shaft.
  NOTE: ALWAYS REPLACE SAME SWAB TO THE SAME VIAL

2. Perform pre-weight measurement by weighing the labeled capped cryovial with pre-cut swab and record on the LDMS Tracking Sheet.
3. Sample collection
   a. Uncap the pre-weighed cryovial. Use a clean hemostat, forceps, or transfer pipette to extend the shaft of the swab.
   b. Insert the swab and hold against the mucosa for 2 minutes.
   c. Immediately place swab into the cryovial after sampling and recap.
   d. Document collection time on the LDMS tracking sheet.
4. Perform post-weight measurement by weighing the capped cryovial containing the absorbed swab tip and record on the LDMS Tracking Sheet.
5. Calculate and record the NET weight on the LDMS Tracking Sheet.
6. Within 2 hours, place the sample tubes in the freezer at ≤-70°C.

Post-cut Swab Collection Method

Materials for each swab collection:

- 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.
   - If collecting more than one swab, using a permanent marker, identify each set of the supplies using unique identifiers.
     NOTE: ALWAYS REPLACE SAME SWAB TO THE SAME VIAL and keep each set together.
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. Insert the swab and hold against the mucosa for 2 minutes.
   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 ≤-70°C.

11.9.3 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 11-3) and label the specimen with LDMS labels.
5. Freeze at ≤-70°C within 2 hours of collection.
6. Samples will be batch shipped to the MTN LC upon request.

11.9.4 Rectal Biopsies for PK

For this study up to 11 biopsies will be needed for tissue PK analysis. Three will be flash frozen for EVG, TFV and TFV-DP, and back-up. The remaining 8 will be collected in transport media and processed for MMC isolation. Please follow the instructions below. Logistics permitting, biopsies should be delivered to the lab to begin MMC isolation within 30 minutes of collection and allow flash freezing within one hour of collection.

11.9.4.1 Instructions for isolating MMCs for PK

Required Materials and Equipment

1. Collagenase Type II, Sigma C1764 Collagenase powder may be stored in pre-weighed aliquots at -20°C if required.
2. DNase I (0.083U/mL), New England Biolabs cat. no.M0303S
3. D-PBS
4. Complete RPMI (cRPMI): 500 mL
   a. RPMI 1640 (Invitrogen, Cat. # 22400-105) - 445 mL
   b. Fetal Bovine Serum (Invitrogen, Cat. # 10082-147 or equivalent) - 50 mL
   c. Antibiotic/Antimycotic (Invitrogen, 100 x, Cat. # 15240-104) - 5 mL
   d. Store at 4°C for up to 60 days
5. Zosyn (or generic) solution (50mg/mL stock)
   a. Dissolve powder from 1 vial (2.25g) of Zosyn (or generic) in 45 mL sterile D-PBS.
   b. Divide into 1 mL aliquots in sterile 2mL cryovials and store at -20°C until use.
6. cRPMI+Zosyn
   a. Prepare cRPMI and add Zosyn stock to a final concentration of 0.5 mg/mL.
   b. For 100 mL medium, add 1 mL Zosyn stock.
   c. Store at 4°C for up to seven days.
7. MACS wash buffer
   a. D-PBS (Invitrogen, Cat. #14190-250) – 498 mL
   b. 0.5M EDTA (Invitrogen, Cat. # 15575-020) – 2 ml
   c. Store at 4°C for up to 90 days.
8. 37°C incubator shaker
9. 70 µm Nylon Mesh Strainers, Becton Dickinson cat. no. 352350
10. 50 mL conical bottom sterile centrifuge tubes
11. 15 mL conical bottom sterile centrifuge tubes
12. 2.0 mL cryovials
13. Pipet aid
14. Serological pipettes – 5 mL, 10 mL, 25 mL
15. Micropipettes and pipet tips – ranging from 2 µL to 1000 µL capacity

16. Flow cytometer or microscope and hemocytometer (using Trypan Blue Exclusion Method) for cell counting
17. 70% Methanol (ice-cold)
18. Parafilm

Procedure: Follow either the manual method or semi-automated method based on site capacity.
Manual method

1. Receive biopsies from clinic in cRPMI+Zosyn
2. Decant biopsies in petri dish
3. Transfer biopsies – up to 30 – in 50 mL conical bottom tube containing 30 mL cRPMI+Zosyn + 15 mg Collagenase Type II + 12.45 µL DNase. Close tube tightly and seal cap-tube junction with parafilm.
4. Affix tubes horizontally in incubator-shaker and agitate at 200 rpm for 30 minutes at 37°C with agitation (200 rpm).
5. Strain this digested suspension through a 70 µm cell strainer into a new, appropriately labeled 50 mL conical tube to separate free lymphocytes from tissue matrix.
   Note: Cell strainer pore size may vary in a study-specific manner. The filter may get clogged with tissue debris. If this happens, get a fresh filter, decant the retentate from the clogged filter into the new one, and continue filtration. It may require multiple filters to complete one filtration. Discard used filter and residue into a biohazard container.
6. Using two 200 µL pipette tips, disrupt/shear undigested pieces of tissue on the filter. Rinse filter with 20 mL cRPMI. Close tube. This is the first collection.
7. Transfer undigested tissue from filter into a fresh 50 mL conical containing 30 mL cRPMI+Zosyn + 15 mg Collagenase type II + 12.45 µL DNase. Close tube tightly and seal with parafilm.
8. Repeat steps 4 - 6. This is the second collection.
9. Repeat step 7
10. Repeat steps 4 – 6. This is the third collection.
11. Discard remaining tissue residue in biohazard container.
12. Centrifuge digested cell collections obtained in steps 6, 8 and 10 at 800 x g for 5 min at 4°C. Decant supernatants
13. Resuspend cell pellets in 3 mL each MACS buffer and pool all suspensions into a 15 mL conical tube.
14. Make volume up to 10 mL with MACS buffer and mix well.
15. Count cells using a flow cytometer or microscope/hemocytometer, or another validated counting procedure.
16. Record total cell count in sample log sheet.
17. Total cells should be noted in terms of 10^6 cells. For example, if the total cell count is 8 x 10^5 cells, it should be recorded as 0.8 x 10^6.
18. Centrifuge cells at 800 x g for 5 min at 4°C. Decant supernatant.
19. Add 1 mL ice-cold 70% Methanol to pellet and lyse cells by mixing with a 1000 µL pipet and vortexing. Divide into two equal aliquots in 2 mL cryovials and store at -80°C. Methanol volumes and number of aliquots are study-specific and may vary.
20. Cell count and lysate volume information must be entered in the aliquot details comments field. Click on the details tab for the first aliquot. Enter numerical information about lysate volume (steps 19) and total cell count (steps 17) into the comments field. Enter only numbers. For example, if 8x10^5 total cells were lysed in 1 mL 70% MeOH, information should be entered as – 1, 0.8x10^6. Please adhere precisely to this syntax to enable automated analysis.

Semi-automated method

1. Aliquot 9 mL cRPMI+Zosyn into a 50 mL conical tube.
2. Remove one aliquot of Collagenase II powder (5 mg) and resuspend in 1 mL cRPMI+Zosyn.
3. Transfer this collagenase suspension to the 9 mL medium in the conical tube.
4. Add 4.15 µL DNase I to the collagenase mix to create digest solution.
5. Warm to 37°C for use.
6. Transfer 5 mL digest solution to GentleMACS digestion tube (C-tube).
7. Receive rectosigmoid biopsies from clinic in cRPMI+Zosyn. Decant into petri dish.

8. Transfer biopsies – up to 30 – to the C-tube containing digestion medium. Close C-tube tightly and insert upside down into sleeve of GentleMACs Dissociator. Insert heating element around C-tube.
9. Digest using tumor dissociation program 37_h_TDK_03 with heater activated. Dissociation will occur at 37°C for 1 h.
10. Filter dissociated sample through a 40 µm BD filter into a sterile 50 mL conical tube.
    Note: Filter pore size may vary in a study-specific manner. The filter may get clogged with tissue debris. If this happens, get a fresh filter, decant the retentate from the clogged filter into the new one, and continue filtration. It may require multiple filters to complete one filtration. Discard used filter and residue into a biohazard container.
11. Add 40 mL MACS buffer to filtrate, mix well and centrifuge at 800 x g for 10 minutes at 4°C.
12. Remove supernatant. Resuspend pellet in 1 mL wash buffer.
13. Remove 20 µL aliquot and count cells using flow cytometer of microscope/hemocytomer or alternate validated counting procedure.
14. Record total cell count in sample log sheet.
15. Total cells should be noted in terms of 10^6 cells. For example, if the total cell count is 8 x 10^5 cells, it should be recorded as 0.8 x 10^6.
16. Transfer cell suspension to sterile 15 mL conical tube. Make volume up to 10 mL with MACS buffer.
17. Centrifuge 800 x g for 5 min at 4°C. Remove supernatant.
18. Add 1 mL ice-cold 70% methanol to pellet. Lyse cells by mixing with a 1000 µL pipette and vortexing. Divide into two equal aliquots in 2 mL cryovials and store at -80°C. Methanol volume and number of aliquots are study specific and may vary.
19. Cell count and lysate volume information must be entered in the aliquot details comments field. Click on the details tab for the first aliquot. Enter numerical information about lysate volume (steps 18) and total cell count (steps 15) into the comments field. Enter only numbers. For example, if 8x10^5 total cells were lysed in 1 mL 70% MeOH, information should be entered as – 1, 0.8x10^6. Please adhere precisely to this syntax to enable automated analysis.

11.9.4.2 Instructions for freezing biopsies for PK

1. Number cryovials 1-3 depending on how many biopsies are received with appropriate participant information.
2. 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.
3. Transfer biopsy to a pre-weighed cryovial. Store only ONE biopsy per 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. Freeze the cryovial containing the biopsy in Liquid Nitrogen or a dry ice-alcohol bath.
6. Log into LDMS (Table 11-3) and label specimen with LDMS label.
7. 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.
8. Batch and ship on dry ice to the PK Lab upon request.

11.9.5 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 (Table11-3), label specimen with LDMS label.
4. The tissue processing/embedding must occur within 72 hours of collection. If processing is unavailable on site, biopsies may be shipped at room temperature to the MTN LC.
5. Batch ship the histology blocks to the MTN LC (LDMS Lab 414) at room temperature.

11.9.6 Rectal Biopsies for PD

21. Four (4) biopsies for PD should be collected and placed into biopsy transport media immediately.
22. Transport biopsies to lab immediately. Processing must begin within 30 minutes from time of collection.
23. Please refer to the Non-Polarized Colorectal Explant Culture SOP provided by the Gastrointestinal Mucosal Immunology (GIMI) Lab for processing.
24. Record weights of biopsies onto the MTN-039 LDMS Tracking Sheet.
25. Each biopsy will be logged into LDMS separately (Table 11-3)
26. Supernatants collected during tissue culture should be logged into LDMS and stored frozen in cryovials at ≤-70°C until shipped on dry ice to the MTN LC (LDMS Lab 414) for p24 analysis.
27. Biopsies after completion of culture will be stored in RNAlater and frozen at ≤-70°C per the Non-Polarized Colorectal Explant Culture SOP and logged into LDMS.

11.9.7 Rectal Biopsied for Mucosal T Cell Phenotyping (flow cytometry)

1. Label one conical tube with the PTID, visit # and visit date.
2. Submerge 4 biopsies into the tube containing biopsy transport media (See Appendix 11-1 for ordering and preparing media). Keep refrigerated. NOTE: Biopsies for PD and T Cell Phenotyping (flow cytometry) 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 11-3).
5. For Pitt specimens place in the refrigerator and allow to rest overnight prior to processing. For UAB only: Ship specimen FedEx Priority Overnight on ice packs to the GIMI 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 the GIMI lab by e-mail (mtnmcgowanlab@mtnstopshiv.org) providing the LDMS batch file and FedEx tracking number.

   Ship T Cell Phenotyping (flow cytometry) samples to GIMI Lab (LDMS Lab 414)
   Attn: Ashley Myerski
   Magee-Womens Research Institute
   204 Craft Ave, Room B530G
   Pittsburgh, PA 15213

   Phone# +1-412-641-7508/6959
   Email: myerskia@mwri.magee.edu

11.9.8 Rectal Biopsy for Proteomics and Metabolomics

1. Weigh one cryovial for each biopsy using an analytical balance – use the same analytical balance throughout the procedure.
2. 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 11-3) and label specimen with LDMS label.
6. Store each labeled cryovial containing the biopsy separately in a ≤-70°C freezer within 1 hour of collection. Document the date/time the biopsy was placed in the freezer.
7. Batch and ship specimens to the MTN LC (LDMS Lab 414) on dry ice