Section 10. Laboratory Considerations

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10.1 Overview and General Guidance

This section contains information on the laboratory procedures performed in MTN-017.

As transmission of HIV and other infectious agents can occur through contact with contaminated needles, blood, blood products, rectal, and vaginal secretions, all study staff must take appropriate precautions when collecting and handling biological specimens. Sites must have appropriate written safety procedures in place before study initiation. Guidance on universal precautions available from the US Centers for Disease Control and Prevention can be found at the following website: http://www.cdc.gov/hai/

Laboratory procedures may be performed in the study site clinics or laboratories, approved commercial laboratories and in the MTN Laboratory Center (LC), including the MTN Pharmacology Core (Johns Hopkins University Clinical Pharmacology Analytical Lab or JHU CPAL). Table 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 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.

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-017 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 Standard Operating Procedures. 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 *
- Urine Dipstick *

*Must be approved by the MTN LC for study activation

Table 10-1
Overview of Laboratory Testing Locations, Specimens,
And Methods for MTN-017

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
Urine NAAT for Gonorrhea and Chlamydia	Local Lab	Urine	Kit Specific Transport Tube	GenProbe Aptima or GeneXpert
Dipstick Urinalysis	In Clinic	Urine	Plastic screw top cup	LC approved local methodology
Plasma archive	On-Site until notified by MTN LC	Plasma	EDTA tube 10mL	Network Lab Protocol
Plasma for Storage	On-site until notified by MTN LC	Plasma	EDTA tube 10mL	Network Lab Protocol
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 antibody screen and Western Blot	Local Lab or MTN Virology Core (WB only)	Plasma, serum or whole blood	EDTA or plain tube 4mL	FDA approved tests
Hepatitis B (HBsAg and HBsAb)	Local Lab	Serum or plasma	EDTA, plain or serum separator 4mL	Local Methodology
HSV-1 and HSV-2 IgG Serology	Local Lab	Serum	plain or serum separator tube 4mL	Local Methodology
Hep C Antibody	Local Lab	Serum	Plain or serum separator tube 4mL	Local Methodology
INR/PT *	Local Lab	Whole Blood	Light Blue (Na Citrate) 4mL	Local Methodology
Plasma for PK	JHU CPAL	Plasma	EDTA tube 10mL	JHU Protocol
PBMCs for PK	JHU CPAL	Whole Blood	CPT (Na Citrate) tube 3x8mL	JHU Protocol
Anal HSV 1 and 2	Local Lab	Anal Swab	Consult local lab requirements	Local Methodology
Anal NAAT for HPV	MTN LC	Anal Swab	PreservCyte (Digene HC2 Kit)	MTN LC protocol
Rectal NAAT for Gonorrhea and Chlamydia	Local, Regional or MTN LC	Rectal swab	Kit Specific Transport tube	GenProbe Aptima or GeneXpert
Rectal Sponge for PD	MTN LC	Rectal Sponge	5mL Cryotube	MTN LC protocol
Rectal Sponge for PK	JHU CPAL	Rectal Sponge	5mL Cryotube	MTN LC protocol
Rectal Sponge for Mucosal Immunology*	MTN LC	Rectal Sponge	5ml Cryotube	MTN LC protocol

Test	Testing Location	Specimen Type	Tube or Container and tube size (recommended)	Kit/Method
Rectal Biopsies for PD*	Local Lab	2-4 Rectal Biopsies	Biopsy Transport Media	MTN LC Protocol
Rectal Biopsies for PK*	JHU CPAL	2-5 Rectal Biopsies	1.8mL Cryovial	MTN LC protocol
Rectal Biopsy for Proteomics*	MTN LC	1 Rectal biopsy	1.8mL Cryovial	MTN LC protocol
Rectal Biopsy for Histology*	MTN LC	1 Rectal biopsy	2.0mL tube	MTN LC protocol
Rectal Biopsies for Mucosal T cell phenotyping*	Local Lab	7 Rectal biopsies	Biopsy Transport Media	MTN LC protocol
Rectal Biopsies for Mucosal gene expression array*	MTN LC	2 Rectal biopsies	1.8mL Cryovial with RNA*later*	MTN LC protocol

**Tissue subset only*

Volumes may vary depending on each site's testing platforms. Please confirm with the testing lab to determine minimum volume requirements. Sites are responsible for ensuring that specimen volumes do not exceed what is described in the informed consent process. The MTN LC may request details of collection containers and volumes for this purpose.

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

Table 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 / Plasma for Storage	EDTA	1x10mL	Spin 10 minutes at 1500xg (or double spin at 800xg). Aliquot and freeze.	Batch to MTN LC
Plasma for PK	EDTA	1x10mL	Spin 10 minutes at 1500xg. Aliquot and freeze within 8 hours of collection.	Batch to JHU-CPAL
PBMCs for PK	CPT (Na Citrate)	3x8mL	Isolate PBMCs per JHU Protocol. Process and freeze within 8 hours of collection.	Batch to JHU-CPAL
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
Urine and Rectal Swab for GC/CT	Viral Transport Media	Kit specific tube	Store at 2-30°C	Scheduled shipment to local lab or MTN LC
Rectal Sponge for PD	None	Merocel Sponge in 5mL Cryovial	Freeze at ≤-70°C within 2 hours of collection	Batch to MTN LC
Rectal Sponge for PK	None	Merocel Sponge in 5mL Cryovial	Freeze at ≤-70°C within 2 hours of collection	Batch to JHU-CPAL
Rectal Sponge for Mucosal Immunology*	None	Merocel Sponge in 5mL Cryovial	Freeze at ≤-70°C within 2 hours of collection	Batch to MTN LC
Rectal Biopsies for PD*	Biopsy Transport Media	50mL conical tube with 10mL media	Transport to testing lab within 30 minutes of collection.	Local Testing Lab
Rectal Biopsies for PK*	None	1.8mL Cryovial	Flash freeze and store at ≤-70°C within 2 hours of collection	Batch to JHU-CPAL
Rectal Biopsy for Proteomics*	None	1.8mL Cryovial	Flash freeze and store at ≤-70°C within 2 hours of collection	Batch to MTN LC
Rectal Biopsy for Histology*	10% Formalin	2.0mL tube	Store at room temperature	Scheduled shipment to MTN LC
Rectal Biopsies for Mucosal T cell phenotyping*	Biopsy Transport Media	50mL conical tube with 10mL media	Transport to testing lab within 30 minutes of collection.	Local Testing Lab
Rectal Biopsies for Mucosal gene expression array*	RNA*later*	1.8mL Cryovial	Store at 4ºC overnight (16-24 hours) then transfer to ≤-70°C.	Batch to MTN LC

**Tissue subset only*

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 Network Lab 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 Network Lab 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).

Questions related to use of LDMS in MTN-017 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

10.4.1 Off-Hours Contact Information

If you are locked out of your LDMS or are experiencing errors that prevent you from completing your LDMS lab work during off-hours, page LDMS User Support using the LDMS Web Pager

utility. Alternatively, you may e-mail the paging system directly at ldmspager1@fstrf.org. Please allow at least 15 minutes to get a response before sending another e-mail to the paging system.

Each site must export its LDMS data to Frontier Science (FSTRF) on a weekly basis. Exported data are used by the MTN SDMC to generate a monthly specimen repository report and to reconcile data entered in LDMS with data entered on study case report forms. Any discrepancies identified during the reconciliation are included in a monthly discrepancy report for 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.

Table 10-3
LDMS Specimen Management Guide to Logging in MTN 017 Specimens

The table below should be used as a guide when logging in MTN 017 specimens. Please use the LDMS codes listed below when logging in specimens for each test listed. Tests that are listed as local do not require that a sample be logged into the LDMS. The LDMS Tracking Sheet can be found on the MTN website (www.mtnstopshiv.org) under the MTN 017 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 ORAL or PK GEL
PBMCs for PK	BLD	CPS	24.0 ML	1	Cell count	CEL	CIO	MET	
Anal Swab for HPV	PAN	VTM	1 EA	1	1	EA	SWB	N/A	
Rectal Sponge for PD	REC	NON	1 EA	1	Weight	MG	SPG	N/A	PD
Rectal Sponge for PK	REC	NON	1 EA	1	Weight	MG	SPG	N/A	PK
Rectal Sponge for Mucosal Immunology*	REC	NON	1 EA	1	Weight	MG	SPG	N/A	MI
Rectal Biopsies for PK*	FSR	NON	5 EA	5	Weight	MG	BPS	N/A	PK
Rectal Biopsy for Gene Expression*	FSR	RNL	2 EA	2	1	EA	BPS	N/A	
Rectal Biopsy for Histology*	FSR	FOR	1 EA	1	1	EA	BPS	N/A	
Rectal Biopsies for PD* (Log each in separately)	FSR	BTM	1 EA	1	Weight	MG	BPS	N/A	PD
Rectal Biopsies for T cell Phenotyping*	FSR	BTM	7 EA	1	7	EA	BPS	N/A	PHENO
Rectal Biopsy for Proteomics*	FSR	NON	1 EA	1	1	EA	BPS	N/A	PRO

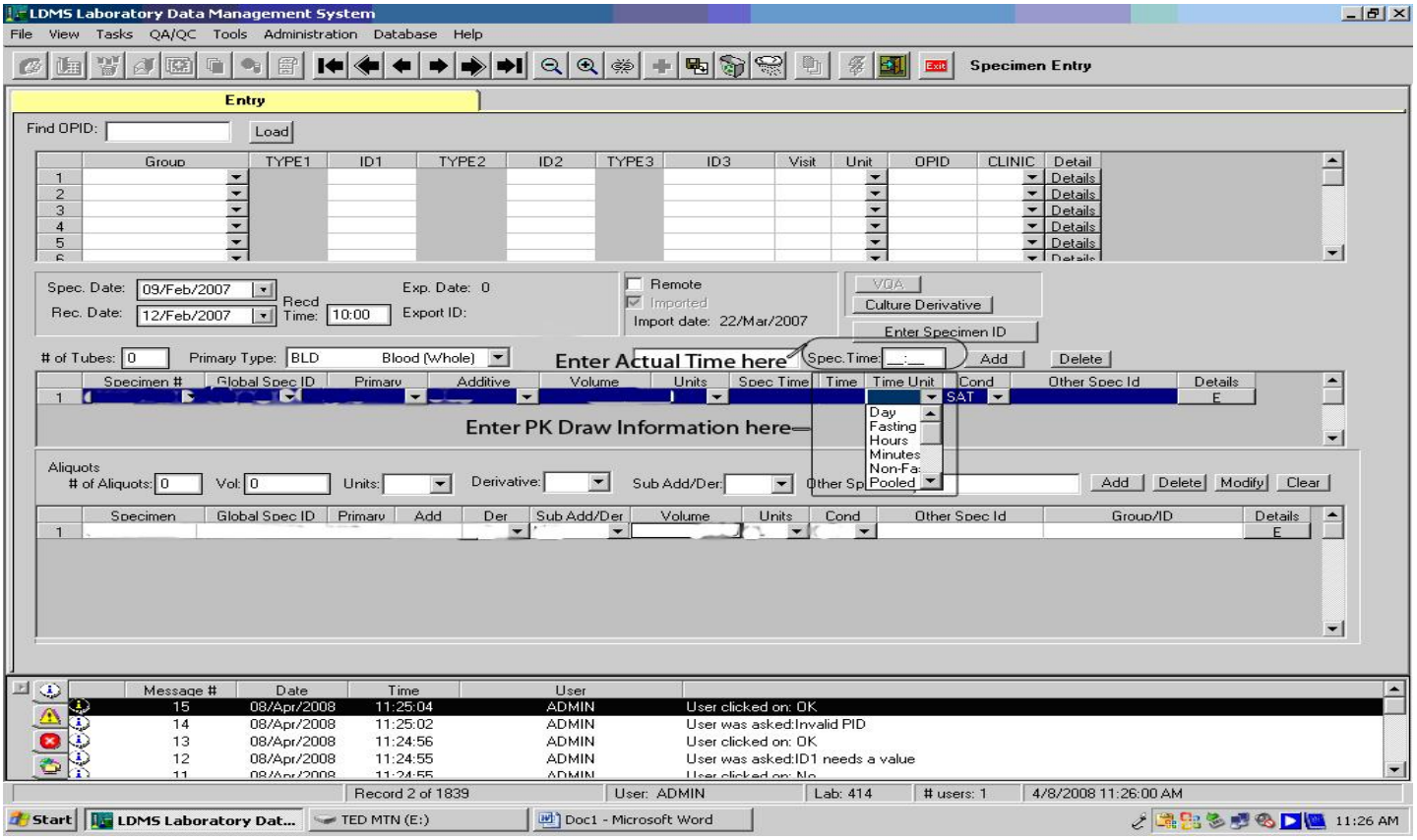
**Tissue subset only*

BLD: Whole Blood
BPS: Biopsy
BTM: Biopsy Transport Media
CEL: PBMCs, viable
CIO: Cells in other solution, Non-viable
CPS: Cell Preparation Tube SCI
EDT: EDTA
FOR: Formalin
FSR: Rectal biopsy by flexible sigmoidoscopy
MET: Methanol
NON: None
PAN: Perianal
PL1: Single spun Plasma
PL2: Double spun Plasma
REC: Rectal
RNL: RNAlater
SPG: Sponge
SWB: Swab
VTM: Viral Transport Media

10.4.2 Logging in PK Samples

- Enter the actual time in the Specimen Time area (See Image 1)
- When applicable enter the PK time point information (0 pre-dose, 1 hour, 2 hour, etc.) in Time and Time Unit area (See Image 1) 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. When doing multiple tests from one specimen, the correct order is separation of urine for the Chlamydia and Gonorrhea first and then the urine dipstick last.

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.
- Instruct the female participant not to clean the labia prior to specimen collection. Male participants should withdraw foreskin if present.
- Collect the first 15-60 mL of voided urine in a sterile collection cup. (Not mid-stream).
- Instruct the participant to screw the lid tightly onto the cup after collection.
- At visits when dipstick urinalyses is required, aliquot 5-10 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.

10.5.2 Urine Chlamydia and Gonorrhea Testing

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

If the site does not have access to these tests, they can send the samples to the LC for testing. Contact the LC (PKunjara@mwri.magee.edu) for shipping instructions and timeline for GC/CT (CT/NG) testing.

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 Gen-Probe UPT

1. Collect urine as noted above.
2. Open the UPT kit and remove the UPT and transfer pipette. Label the UPT with the participants PTID number and date.
3. Hold the UPT upright and firmly tap the bottom of the tube on a flat surface to dislodge any large drops from inside the cap.
4. Uncap the UPT and use the transfer pipette to transfer enough urine to fill the tube to the level indicated on the tube between the black lines. Do not under fill or overfill the tube.
5. Cap tightly and invert the tube 3-4 times to ensure that the specimen and reagent are mixed.
6. The specimen can now remain at 2-30°C for 30 days.
7. Place the transport tube in a biohazard zip-lock bag and transport to the local laboratory for testing. If samples are to be tested by the MTN LC, please ship samples bimonthly or sooner on ice pack. Refer to section 10.7.2 for shipping instructions.
8. The results are sent to the clinic and are reported on a STI Test Results CRF.

10.5.2.2 Instructions for transferring urine into the GeneXpert transport reagent tube

1. Collect urine as noted above.
2. Open the packaging of a disposable transfer pipette provided in the kit. Label the tube with the participants PTID number and date.
3. Remove the cap from the Xpert CT/NG Urine Transport reagent tube. Insert the transfer pipette into the urine cup so that the tip is near the bottom of the cup. Transfer approximately 7 mL of urine into the Xpert CT/NG Urine Transport reagent tube. The correct volume of urine has been added when the level reaches the black dashed line on the label.
4. Cap tightly and invert the tube 3-4 times to ensure that the specimen and reagent are mixed.
5. The specimen can remain at 2-30°C for 30 days.
6. Place the transport tube in a biohazard zip-lock bag and transport to the local laboratory for testing. If samples are to be tested by the MTN LC, please ship samples bimonthly or sooner on ice pack. Refer to section 10.7.2 for shipping instructions.
7. The results are sent to the clinic and are reported on a STI Test Results CRF.

10.5.3 Dipstick Urinalysis

Dip the urinalysis test strip into an aliquot of urine. Perform this test according to site SOPs and the package insert. Assess and record results for glucose, protein, leukocytes and nitrites. If leukocytes or nitrites are positive, perform a urine microscopy and a urine culture according to local SOP. To avoid overgrowth of bacteria, refrigerate specimen before and during transport to laboratory.

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

10.6 Blood Testing

The blood tests performed depend on the time point of the visit and potentially the clinical presentation of the participant. Perform all tests according to site SOPs and package inserts.

10.6.1 Specimen Collection and Initial Processing

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

After collection:

- Allow plain tubes (no additive or serum separator) to clot, then centrifuge per site SOPs.
- Lavender top tubes (additive = EDTA) should be gently inverted at least eight times after specimen collection to prevent clotting. If whole blood for hematology testing and plasma are to be taken from the same tube, the hematology must be completed before the tube is centrifuged and aliquoted. If whole blood is to be used for multiple tests, ensure that the tube is well mixed before removing any specimen.
- Light blue top tubes (additive = Na Citrate) are used for coagulation determinations. These tubes should be gently inverted at least 4 times after specimen collection to prevent clotting.
- CPT light blue tiger top tube (additive = Na Citrate) are used for PBMC isolation. These tubes should be gently inverted 8-10 times and stored upright at room temperature.

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.

10.6.2 HIV Testing

Although the HIV algorithm (Appendix II of the MTN 017 protocol) allows for EIA testing, rapid testing is recommended in order 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-017 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 Western Blot (WB) test will be performed on the original sample. If there is insufficient sample to perform WB, then additional blood must be collected. If the WB is negative or indeterminate, contact the LC for guidance.

For sites that no longer have local access to HIV WB testing, plasma samples may be shipped overnight on dry ice to the MTN Virology Core. Please notify the MTN LC (pkunjara@mwri.magee,edu) and Virology Core (ump3@pitt.edu) via e-mail with tracking number and details of each specimen prior to shipping. These samples will not be entered into LDMS.

Ship samples to MTN Virology Core:Urvi Parikh/ Kristen Cummings
University of Pittsburgh
3550 Terrace Street
S804 Scaife Hall
Pittsburgh, PA 15261
Phone # 412-648-3103
Fax # 412-648-8521

Plasma storage (section 10.6.9) is required for further Network Lab 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.

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

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.

10.6.3 Hematology Testing

Complete blood counts (CBC) with five-part differentials will be performed at all sites. Each of the following must be analyzed and reported:

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

These tests will be performed on EDTA whole blood per local site SOPs.

10.6.4 Liver and Renal Function Testing

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

Liver Function

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

Renal Function

- Creatinine

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

10.6.5 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. Enrolled participants considered positive should include 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 IgG test (such as EIA, MHA-TP, TPHA, TPPA, or FTA-ABS) and confirming 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 prior to enrollment in the study. If the RPR or VDRL is negative, this may indicate that the participant may have been previously treated, has an advanced latent disease, or the original test was a false positive. MTN LC recommends additional testing preferably using different antigens than the original treponemal IgG test so the participant can be correctly evaluated. If the second confirmation test is negative, the participant is not considered infected with syphilis. If the second confirmation test is positive, they cannot be enrolled at this time. Enrolled participants considered positive should include 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.

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-017 Protocol Safety Physicians (mtn017safetymd@mtnstopshiv.org).

10.6.6 Hepatitis B Surface Antigen, Hepatitis B Surface Antibody, and Hepatitis C Antibody

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

10.6.7 HSV Serology

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

10.6.8 INR/PT (Tissue Subset only)

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

10.6.9 Plasma Archive (baseline) and Plasma for Storage

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

For plasma archive and plasma for storage, use collection tubes with EDTA anticoagulant. Aliquot plasma into 2 ml cryovials, store at <-70°C, and batch onsite until the MTN LC study team requests shipping and/or testing.

1. If sample is collected and held at room temp, freeze plasma within 4 hours. If refrigerated or on ice after collection, freeze plasma within 24 hours.
2. If total whole blood volume is less than 2.0 mL, redraw as soon as possible.
3. Spin blood at room temperature in a centrifuge according to one of these techniques:
   - Single spun: Spin blood at 1500 x g for 10 minutes and remove plasma.
   - Double spun: Spin blood at 800 x g for 10 minutes, recover plasma and place in a tube to spin again at 800 x 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.

10.6.10 Blood for Tenofovir PK

Plasma Tenofovir

Collect blood into a labeled 10 mL EDTA Vacutainer tube using either an indwelling venous catheter or direct venipuncture. Document collection time and product regimen on the LDMS tracking sheet. For product regimen circle the regimen that represents the last product dispensation that occurred prior to specimen collection.

1. Mix blood sample with the anticoagulant using gentle inversions (8 to 10 times).
2. Centrifuge the sample at approximately 1500 x g for 10 minutes at 4°C. The centrifugation must be completed and sample placed in the freezer within 8 hours of blood collection.
3. Transfer plasma to appropriately labeled 2.0 mL cryovials in as many 1.0 mL aliquots as possible.
4. Log samples into LDMS (Table 10-3) and store at ≤-70°C until shipped to JHU CPAL.
5. One aliquot will be shipped to the JHU-CPAL and assayed for Tenofovir according to the shipping schedule, every other week or as needed. The first shipment should be initiated approximately two weeks after the first sample is collected. The remaining aliquots will be retained at the site until advised by the MTN-017 leadership group.
6. Shipping costs may be billed directly to the MTN LC World Courier or FedEx accounts provided by the MTN LC. Please be sure to reference MTN-017-9231 for all shipments.

Peripheral Blood Mononuclear Cells (PBMC) for Intracellular Tenofovir

Draw three 8 mL Cell Preparation Tubes (CPT) with Sodium Citrate (BD Cat# 362761) at each PK time point. Document collection time on LDMS tracking sheet. Specimens must be kept upright at room temperature (22-25°C) and processed within 8 hours. The following instructions were obtained from the Johns Hopkins University Clinical Pharmacology Analytical Lab SOP.

1. Invert CPT tubes gently to mix anticoagulant thoroughly.
2. Spin at 1800 x g for 20 minutes using a refrigerated centrifuge set at 20-25°C. Centrifuge temperatures may rise when spinning for extended amounts of time. Document start time of centrifugation on site developed processing worksheet. A worksheet template can also be provided by the MTN LC.
3. Gently invert the CPT tube 2 times, without disturbing the underlying gel, to suspend the PBMCs in the plasma. Transfer the cells, using a disposable transfer pipette, from the CPT tube to an appropriately labeled 15 mL conical tube.
4. Add PBS, using a serological pipette, to bring the total volume of the conical tube containing the cells up to the 12 mL mark on the tube. Cap the 15 mL conical tube and mix by gently inverting.
5. Centrifuge the conical tube at 400 x g for 15 min at 4°C. Remove and discard supernatant.
6. Resuspend each pellet in 3 mL PBS, pool all suspensions into a single 15 mL conical tube and make up the volume to 10 mL with PBS.
7. Transfer 0.2mL for cell count. Perform a viable cell count during step 8.
8. Centrifuge conical tube at 400 x g for 15 minutes at 2-8°C.
9. Remove and discard as much of the supernatant as possible without disturbing the cell pellet using a pipette.
10. Add 1.0mL of fresh cold 70% methanol (7 parts methanol and 3 parts distilled water). Vortex lightly to lyse cells completely. **NOTE:** Prepare fresh 70% methanol lysing solution each day and store at 2-8°C for at least 2 hours before use.
11. Transfer all contents to cryovial ~ 1.0ml.
12. When logging into LDMS (Table 10-3), although there were 3 CPT specimens collected, enter the primary specimen as 1 sample with a volume of 24ml since they are pooled at processing. Use LDMS to label and track all aliquots.
13. Place PBMC lysate directly into ≤-70°C freezer. Record time frozen on processing worksheet. This should be completed within 8 hours from collection.

14. The MTN LC will send instructions when shipping and/or testing is required.

Ship PK samples to JHU-CPAL:

James Johnson
Clinical Pharmacology Analytical Lab
Division of Clinical Pharmacology
The Johns Hopkins University School of Medicine
600 N. Wolfe Street, Osler 523
Baltimore, MD 21287
Lab Phone#: +1-410-955-9710 or +1-410-614-9978

10.7 Testing of Rectal Specimens

The tests performed on rectal specimens depend on the time point of the visit and potentially the clinical presentation of the participant. Perform all tests according to site SOPs and package inserts.

Rectal samples should be collected in the following order:

1. Anal swab for HSV 1/2
2. Anal swab for HPV
3. Rectal swab for GC/CT
4. Rectal sponges for PD and PK
5. Rectal samples for Tissue/Fluid subset only
6. Rectal sponge for mucosal immunology
7. Biopsies* for PK, PD, Proteomics, Histology, Mucosal T cell phenotyping, and mucosal gene expression array

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, Mucosal Gene Expression Array, Histology, PD, T Cell Phenotyping, and then Proteomics.

10.7.1 Anal HSV-1/2

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

10.7.2 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.
4. Specimens will be batch shipped quarterly to the MTN LC on dry ice.
5. Please notify Pam Kunjara by e-mail (pkunjara@mwri.magee.edu) prior to shipping. Include the date the shipment is scheduled to depart along with the LDMS batch file and the shipment tracking number.

Ship HPV samples to MTN LC:

Pam Kunjara
Microbicides Trials Network
204 Craft Ave. A540

Pittsburgh, PA 15213
Telephone: 412-641-6157/6393

10.7.3 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 the Gen-Probe Aptima NAAT Method or the Cepheid GeneXpert NAAT method by the local or regional laboratory.

If the site does not have access to these tests, they can send the samples to the LC for testing. Contact the LC (PKunjara@mwri.magee.edu) for shipping instructions and timeline for GC/CT testing.

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

10.7.3.1 Instructions for collection and transport of rectal swabs for GC/CT testing with Gen-Probe:

1. Collect specimen using the Gen-Probe Aptima Unisex Swab (blue swab).
2. Label the transport tube with the participants PTID number and date.
3. Remove the swab from the plastic transport tube and insert into the rectum according to the procedure outlined in the SSP for Clinical Considerations (Section 8) and rotate gently through 360 degrees and remove.
4. Immediately place the swab in the transport tube, break off shaft of swab and cap. The specimen can now remain at 2-30°C for 30 days.
5. Place the transport tube in a biohazard zip-lock bag and transport to the local laboratory for testing. If samples are to be tested by the MTN LC, please ship samples bimonthly or sooner on ice pack. Refer to section 10.7.2 for shipping instructions.
6. The results are sent to the clinic and are reported on a STI Test Results CRF.

10.7.3.2 Instructions for collection and transport of rectal swabs for GC/CT testing with GeneXpert:

1. Collect specimen using the Xpert collection swab.
2. Label the pink-capped transport tube with the participants PTID number and date.
3. Remove the swab and insert into the rectum according to the procedure outlined in the SSP for Clinical Considerations (Section 8) and rotate gently through 360 degrees and remove.
4. Immediately place the swab in the transport tube, break off shaft of swab and cap.
5. Cap tightly and invert or gently shake the tube 3-4 times to elute material from the swab. Avoid foaming.
6. The specimen can now remain at 2-30°C for 60 days.
7. Place the transport tube in a biohazard zip-lock bag and transport to the local laboratory for testing. If samples are to be tested by the MTN LC, please ship samples bimonthly or sooner on ice pack. Refer to section 10.7.2 for shipping instructions.
8. The results are sent to the clinic and are reported on a STI Test Results CRF.

10.7.4 Rectal Sponge for PK

1. Tare the weighing balance and ensure balance has been calibrated within the past year.
2. Remove a sponge (Merocel eye-wick Spears Fisher Scientific # NC0093269) from the box, wear gloves at all times when handling sponges.
3. Note: The rectal sponge for PD (Section 10.7.5) can also be collected simultaneously.
4. 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.

5. Place each sponge into an appropriately labeled 5mL cryovial, labeled with a unique participant identifier.
6. 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
7. Weigh the dry sponge + labeled cryovial and document the weight (pre-weight) on the LDMS Tracking Sheet.
8. The clinician will collect specimen using a pre-weighed sponge according to the procedures outlined in the SSP for Clinical Considerations (Section 8).
9. 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.
10. Weigh the cryovial and sponge after collection using the same analytical balance used for the pre-weights. Document the post-collection weight on the LDMS Tracking Sheet.
11. Complete the LDMS tracking sheet and submit to lab on ice for LDMS entry.
12. Log into LDMS (Table 10-3) and label specimen with LDMS label.
13. Freeze at ≤-70°C within 2 hours of collection until ready to ship.
14. Specimens may be batched and shipped on dry ice. Once the LC notifies the site to ship, use LDMS to create a shipping manifest.
15. Ship specimens to JHU-CPAL Monday through Wednesday for overnight delivery. See Section 10.6.10 for shipping address.

10.7.5 Rectal Sponge for PD (and Mucosal Immunology* for tissue subset)

1. Tare the weighing balance and ensure balance has been calibrated within the past year.
2. Remove two sponges (Merocel eye-wick Spears Fisher Scientific # NC0093269) from the box, wear gloves at all times when handling sponges.
3. Note*: If participant is not part of the tissue subset, rectal sponge for mucosal immunology is not collected; only one sponge is needed for PD. The rectal sponge for PK (Section 10.7.4) can also be collected simultaneously.
4. 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.
5. Place each sponge into an appropriately labeled 5mL cryovial, labeled with a unique participant identifier.
6. 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
7. Weigh the dry sponge + labeled cryovial and document the weight (pre-weight) on the LDMS Tracking Sheet.
8. The clinician will collect specimen using the pre-weighed sponges according to the procedures outlined in the SSP for Clinical Considerations (Section 8).
9. 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.
10. Transport the cryovials so that they can be weighed using the same balance that was used in the preparation of the sponges. Weigh the sponge + labeled tube and document the weight (post-weight) on the LDMS tracking sheet.
11. Cryovials must be transported on ice to the LDMS laboratory to allow storage within 2 hours of collection.
12. Log into LDMS (Table 10-3) and label specimen with LDMS label.
13. Place in a ≤-70°C freezer for storage until shipment is requested by the LC.
14. Record the time that the sample is introduced to the freezer on the LDMS Tracking Sheet.
15. Samples will be batched and shipped to the MTN LC on dry ice at end of study. Please see section 10.7.2 for shipping instructions.

10.7.6 Rectal Biopsies for PD (Tissue subset only)

1. Biopsies should be collected and placed into biopsy transport media immediately. (See Appendix 10-1 for ordering and preparing media).
2. Transport biopsies to lab within 15-30 minutes from time of collection.
3. Please refer to the Non-Polarized Colorectal Explant Culture SOP provided by the MTN LC for processing.
4. Record weights of biopsies onto the MTN 017 LDMS Tracking Sheet.
5. Supernatants collected during tissue culture should be logged into LDMS (Table 10-3) and stored frozen in cryovials at ≤-70°C until shipped for p24 analysis. See LDMS Culture Derivative SOP provided by MTN LC.
6. Supernatants for p24 analysis will be batched and shipped to the MTN LC on dry ice. Please see section 10.7.2 for shipping instructions.

10.7.7 Rectal Biopsies for PK (Tissue subset only)

1. Logistics permitting, biopsies should be delivered to the lab to allow freezing within two hours of collection.
2. Number cryovials 1-5 depending on how many biopsies are received with appropriate participant information.
3. Weigh each cryovial using an analytical balance – use the same analytical balance throughout the procedure. Document the weight of the labeled cryovial (pre-weight) on the LDMS Tracking Sheet.
4. Biopsies for PK may be collected in the same media as biopsies for PD or placed directly into the pre-weighed cryovial without media.
5. If biopsies are collected in media, using pointed forceps, pick up each individual biopsy and drain off excess medium by touching biopsy to side of collection vessel.
6. Transfer biopsy to a pre-weighed cryovial. Store only ONE biopsy per cryovial. Ensure biopsy sits at bottom of cryovial.
7. Weigh the cryovial containing the biopsy (post-weight). Document the weight of the cryovial containing the biopsy on the LDMS Tracking Sheet.
8. Freeze the cryovial containing the biopsy in Liquid Nitrogen or a dry ice-alcohol bath.
9. Log into LDMS (Table 10-3) and label specimen with LDMS label.
10. 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.
11. Specimens may be batched and shipped on dry ice. Once the LC notifies the site to ship, use LDMS to create a shipping manifest.
12. Ship specimens to JHU-CPAL Monday through Wednesday for overnight delivery. See section 10.6.10 for shipping address.

10.7.8 Rectal Biopsy for Histology (Tissue subset only)

1. Place one biopsy into a microtube filled with 10% formalin for shipping. These can be kept at room temperature.
2. Complete the LDMS tracking sheet and submit to lab for LDMS entry.
3. Log specimens into LDMS (Table 10-3), label specimen with LDMS label.
4. The tissue processing/embedding must occur within 72 hours of collection.
5. Batch ship the histology blocks quarterly to the MTN LC at room temperature. See section 10.7.2 above for shipping instructions.

10.7.9 Rectal Biopsy for Proteomics (Tissue subset only)

1. Place one biopsy into a cryovial and snap freeze at ≤-70°C within 2 hours of collection.
2. Complete the LDMS tracking sheet and submit to lab for LDMS entry.
3. Log the specimens into LDMS (Table 10-3) and label specimens with LDMS label.

4. Batch and ship specimens to the MTN LC on dry ice. The MTN LC will notify sites when to send the specimens. See section 10.7.2 for shipping address and e-mail notification.

10.7.10 Biopsies for Mucosal T Cell Phenotyping (Tissue subset only)

1. Label one 50 ml conical tube with the PTID, visit # and visit date.
2. Submerge 7 biopsies into the tube containing 12-15 mL of biopsy transport media (See Appendix 10-1 for ordering and preparing media). Keep refrigerated.
3. Complete the LDMS tracking sheet and submit to lab for LDMS entry and processing.
4. Log specimens into LDMS (Table 10-3) and process according to McGowan Lab SOP.

10.7.11 Mucosal Gene Expression Array (Tissue subset only)

1. Take two cryovials containing 1.5 mL of RNA*later* (Ambion, Invitrogen Cat #AM7020) and label it with PID, study visit and date.
2. Place one biopsy into each cryovial and submerge the tissue in the RNA*later* solution.
3. Store each vial containing one rectal biopsy in RNA*later* at 4ºC overnight (16-24 hours).
4. Complete the LDMS tracking sheet and submit to lab for LDMS entry.
5. Log the specimen into LDMS (Table 10-3) and label specimen with LDMS label.
6. Transfer vials from 4ºC to ≤-70°C. Each biopsy must be stored at ≤-70°C for a minimum of 24 hours prior to shipping.
7. Batch and ship specimens to the MTN LC on dry ice. The MTN LC will notify sites when to send the specimens. See section 10.7.2 for shipping address and e-mail notification.

10.8 Appendices

10.8.1 Section Appendix 10-1: Procedure for preparing reagents for MTN-017

Equipment:

- 2-8°C Refrigerator
- -20°C freezer
- Biological laminar flow hood
- Pipette Aid

Disposables:

- 50 mL conical tubes
- 10 mL serological pipettes
- 25 mL serological pipettes

Reagents:

1. RPMI (1x) 1640 w/HEPES w/L-glutamine, Invitrogen #22400-089 (500 mL) Freeze until needed.
2. Heat inactivated, certified Fetal Bovine Serum (FBS), Invitrogen #10082-147 (500 mL) or #10082-139 (100 mL). Aliquot into 50 mL tubes and freeze at -20^{o}C
3. Antibiotic/antimycotic (100x), Invitrogen #15240-104 (100 mL) aliquot 5 mL quantities into 10 mL size tubes and freeze at -20^{o}C

Procedure:

Biopsy transport media

Ingredients	100 mL
RPMI	90.5 mL
FBS (f.c. 7.5%)	7.5 mL
Antibiotics (f.c. 1%)	1.0 mL
Zosyn (50mg/ml)	1.0mL

1. Take precautions to maintain the sterility of the media and use sterile techniques with transferring.
2. Thaw the RPMI, FBS, and antibiotic/antimycotics.
3. Combine the above ingredients and mix
4. Dispense 10-12 mL aliquots into 50 ml tubes labeled Biopsy Transport Media and expiration date of 1 month from preparation.
5. Store at 4°C

RNA*later* (to be used for collecting biopsies for Mucosal Gene Expression Array)

Order RNA*later* Solution (Ambion, Invitrogen Cat #AM7020) 100 mL

1. Dispense 1.5 mL aliquots of the RNA*later* into cryovials.
2. Store at room temperature until used.