

Section 9 Laboratory Consideration

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9. Introduction

This section provides information and instructions for site clinical and laboratory staff related to the processing, storing, shipping and testing of MTN-034 laboratory specimens. Additional information for collection specimens from participant can be found in SSP Section 7 Clinical Considerations.

9.1 Overview and General Guidance

MTN-034 Clinical research sites will complete the MTN-034 Laboratory Activation Checklist prior to study activation. This will document MTN Laboratory Center (LC) approval of readiness for activation as described in the MTN Manual of Operational Procedures, Section 14.9.

As transmission of HIV and other infectious agents can occur through contact with contaminated needles, blood, and blood products, 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 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 Colorado Antiviral Pharmacology Laboratory (CAVP), Clinical Pharmacology Analytical Laboratory (CPAL), and MTN Virology Core (at the University of Pittsburgh). Table 9-1 and table 9-2 highlight specimen, storage and shipment requirements. Table 9-3 is an overview of the testing to be performed at each visit.

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

All site laboratories will be monitored by the MTN LC which will utilize information from DAIDS monitoring groups (pSMILE, IQA, VQA, etc.) to monitor and certify laboratories for testing. Please refer all questions related to laboratory testing to the MTN LC using the following email address: mtnnetworklab@mtnstopshiv.org

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. Similarly, the MTN LC must be notified 1 week before any reference (normal) range changes are made.

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 endpoint data derived from laboratory testing will be considered acceptable to all regulatory authorities across study sites.

This section of the 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) for specimen management, processing, and testing.

Table 9-1: Overview of Laboratory Testing Locations, Specimens, and Methods for MTN-034

Test	Testing Location	Specimen Type	Tube or Container and tube size (recommended)	Kit or Method
Urine pregnancy test (hCG)	In clinic	Urine	Plastic screw top cup	One-Step or Combo hCG Quidel Quick Vue, or Fisher HealthCare Sure-Vue Urine hCG kit
Dipstick ¹ and/or Culture ¹	Local lab	Urine	Plastic screw top cup	Local methodology
CBC & Platelet	Local Lab	Whole Blood	EDTA 4 mL tube	Local methodology
Chemistries (Creatinine for creatinine clearance calculation)	Local Lab	Serum or Heparinized plasma	Plain or serum separator 4 mL	Local methodology
HIV-1/2 Rapid Test	Clinic/Local Lab	Plasma or whole blood	EDTA 4 mL	At least one FDA approved test
HIV Confirmation	Local Lab	Plasma	EDTA 4 mL	CE approved Geenius
HIV-1 RNA PCR ²	Local Lab	Plasma	EDTA tube	LC approved method
Hepatitis testing for HBsAG	Local Lab	Serum	plain or serum separator, 4 mL	Local methodology
HSV-2 Antibody	MTN LC	Serum or plasma	Plain tube or EDTA 4 mL	MTN LC methodology
Dry Blood Spots (Blood PK) for Truvada	UCT ³	Plasma	EDTA 4 mL tube	UCT methodology
Syphilis Serology	Local Lab	Serum or Plasma	EDTA tube, plain or serum separator, 4 mL	Local methodology
Plasma Archive/Storage	Clinic/Local Lab	Plasma	EDTA 10 mL tube 2.0 mL cryovials	MTN LC procedure
Vaginal Swab for biomarkers (clinician & self-collected)	MTN LC	Vaginal Swab	2.0 mL cryovial	MTN LC procedure
Cervical NAAT for Gonorrhea and Chlamydia	Local lab	Cervical swab (supplied with kit)	Kit specific Transport tube	Gen-Probe Aptima, GeneXpert, or LC approved test
Trichomonas Rapid Test	Local lab or in clinic	Vaginal swab (supplied with kit)	Sterile tube with no additives	OSOM kit
Vaginal pH	In clinic	Vaginal swab	N/A	pH Indicator Strips (range 3.6 to 6.1)
Vaginal swabs for qPCR (microbiota)	LC	Vaginal flocked swabs	2.0 mL cryovial	MTN LC procedure
Vaginal smear Gram-stain	LC	Vaginal Swab	Slides	MTN LC procedure
Used Vaginal Ring for PK residual assessment	Parexel	Used VR	Biohazard labeled ziplock bag	Parexel procedure
Vaginal saline wet preparation (for BV and/or KOH wet mount)	In clinic/ local lab	Vaginal swab	Tube with 6 drops of saline	Microscopy with MTN LC procedure
Cervical Cytobrush Flow Cytometry (Zimbabwe only)	Local Lab	Cytobrush	Screw top Conical tube	MTN LC procedure
CVL for biomarkers	LC	Cervicovaginal Lavage	15mL conical tube 2 mL cryovials	MTN LC procedure
Cervical swab for biomarkers	LC	Cervical swab	2.0 mL cryovial	MTN LC procedure
CD4+ T Cell Count ²	Local Lab	Whole Blood	EDTA	Local methodology

¹ Perform only if clinically indicated per local SOP.

² Performed on participants who have positive HIV rapid tests in the follow-up HIV testing algorithm and for post seroconversion follow-up when applicable.

³ Division of Pharmacology, Faculty of Health Sciences, University of Cape Town (UCT)

Table 9-2: Overview of Specimens for Storage and Shipment

Specimen and subsequent testing	Collection type and size	Aliquot type tube or other (min supernatant volume to be stored)	Processing	Ship to:	Shipping schedule
Plasma for Archive /Storage	EDTA 10 mL	1 mL into 2 mL cryovials (total of 4ml of plasma)	Freeze plasma at $\leq -70^{\circ}\text{C}$ within 4 hours of draw	LC	Store frozen at site until notified by LC
HSV-2 Antibody	Plain tube or EDTA 4 mL	2 mL cryovial (total of 1 mL of serum or plasma)	Freeze at $\leq -70^{\circ}\text{C}$ within 4 hours after collection	LC	Store frozen at site until conclusion of study
Dry Blood Spots (DBS)	Filter paper card	1 Whatman Protein Saver Card #903 (Fill 5 spots, 50 μL per spot)	Fill all 5 spots, let air dry ≥ 2 hours, Store 2-8 $^{\circ}\text{C}$	UCT	Ship weekly with desiccant and humidity card
Vaginal Swab for qPCR (microbiota)	Flocked swab	2 mL cryovials (3 flocked swabs)	Freeze at $\leq -70^{\circ}\text{C}$ within 8 hours	LC	Store frozen at site until notified by LC
Vaginal smear Gram-stain	Glass slides	2 slides	Roll vaginal swab on slides, let air dry.	LC	1 slide goes to LC when requested. Store 2nd slide.
Vaginal Swab for Biomarkers	Polyester swab	2 mL cryovial (1 swab)	Freeze at $\leq -70^{\circ}\text{C}$ within 4 hours	LC	Store frozen at site until notified by LC
Cervical Swab for biomarkers	Polyester swab	2 mL cryovial (1 swab)	Freeze at $\leq -70^{\circ}\text{C}$ within 4 hours	LC	Store frozen at site until notified by LC
Cervicovaginal Lavage (CVL)	15 mL conical tube	1 mL supernatant aliquots in 2 mL cryovials, with a minimum of 6 aliquots	Freeze at $\leq -70^{\circ}\text{C}$ within 8 hours of collection	LC	Store frozen at site until notified by LC
Used Vaginal Ring for PK residual assessment	None	Ziplock bag	Blot dry and place used VR in bag	FARMOVS	Store ambient temperature, ship weekly

Table 9-3: Overview of Laboratory Tests by visit for MTN-034

		Vis1 SCR	Vis 2 ENR	Vis 4 & 5	Vis 6	Vis 7 & 8	Vis 9	Vis 11-12	Vis 13	Vis 14 & 15	Vis 16	Vis 18 & 19	Vis 20	Vis 21 & 22	Vis 23
		70 days to ENR	Day 0	Wks 4 & 8	Wk 12	Wks 16 & 20	Wk 24	Wks 28 & 32	Wk 36	Wks 40 & 44	Wk 48	Wks 52 & 56	Wk 60	Wks 64 & 68	Wk 72
Pelvic Examination (PE)		X	X	*	X	*	X	*	X	*	X	*	X	*	X
LABORATORY															
URINE	hCG	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	Urine dipstick and/or culture	*	*	*	*	*	*	*	*	*	*	*	*	*	*
BLOOD	HIV-1 testing	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	HSV-2 antibody		X	*	X	*	X	*	X	*	X	*	X	*	X
	HBsAG	X													
	Plasma archive		X												
	Plasma storage				X		X		X		X		X		X
	Blood creatinine and creatinine clearance	X	*	*	*	*	X	*	*	*	X	*	*	*	X
	CBC with platelets	X	*	*	*	*	X	*	*	*	X	*	*	*	X
	Syphilis serology	X	*	*	X	*	X	*	X	*	X	*	X	*	X
Dry Blood Spot (when on Truvada)			X	X	X	X	X	X	X	X	X	X	X	X	
PELVIC	Rapid Trichomonas test	X	*	*	X	*	X	*	X	*	X	*	X	*	X
	Saline wet mount for BV	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	KOH wet mount for yeast	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	Vaginal pH	*	X	*	X	*	X	*	X	*	X	*	X	*	X
	Vaginal swabs (3) for qPCR (microbiota)		X		X		X		X		X		X		X
	Vaginal Gram Stain		X		X		X		X		X		X		X
	Vaginal swab for biomarkers ¹		X		X		X		X		X		X		X
	Clinician collected			X	X	X	X	X	X	X	X	X	X	X	X
	Self-collected			X	X	X	X	X	X	X	X	X	X	X	X
Cervico- vaginal	NAAT for GC/CT	X	*	*	X	*	X	*	X	*	X	*	X	*	X
	CVL for biomarkers		X		X		X		X		X		X		X
	Cervical swab for biomarkers		X		X		X		X		X		X		X
	Cervical Cytobrush for Flow Cytometry (designated site only)		X		X		X		X		X		X		X
STUDY PRODUCT / SUPPLIES															
Collection of study VR(s) or tablets				X	X	X	X	X	X	X	X	X	X	X	X

X= required, *= if indicated and/or per local standard of care,
¹= If there is a Pelvic Exam, then the clinician will collect this sample. If there is not a PE, then the participant will self-collect the sample.

9.2 Specimen Labeling

All specimen containers including microscope slides that are collected (e.g., urine collection cups, blood collection tubes) will be labeled with SCHARP-provided Participant ID (PTID) label. The finished label(s) should have the PTID number, visit number, date, time, and collector's initials (on collection tubes). All handwritten information should be neatly written using an indelible ink 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. Refer to Table 9.4 below for tests that will be entered into LDMS and labeled with LDMS-generated labels.

9.3 Procedures for Specimens that cannot be evaluated

Specimen collection will be repeated (whenever possible) if samples cannot be evaluated (tested) 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 LC must be notified in the following cases

- Any time a participant must return to the clinic for specimen collection.
- When PK specimens are missed.
- Insufficient blood volume is collected for the plasma archive.
- Any time specimen integrity has been compromised or samples improperly handled.
- Any situation that may indicate a laboratory related protocol deviation.

If site staff has any question regarding the collection processes, notify the LC staff as soon as possible for guidance.

9.4 Use of Laboratory Data and Management System (LDMS)

LDMS is a program used for the storage and shipping of laboratory specimens and supported by the Frontier Science Foundation (FSTRF). LDMS must be used at all sites to track the collection, storage, and shipment of the sample types described in Table 9-4.

Detailed instructions for use of LDMS are provided at: <https://www.ldms.org/>.

All sites will be required to maintain the current version 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). Backup and export are not required if sites are running Web LDMS.

LDMS records are used by the MTN Statistical Data and Management Center (SDMC) to generate monthly specimen discrepancy reports. These are used to resolve data entry errors when comparing information between LDMS and study case report forms (CRF). Sites are expected to resolve all discrepancies within two weeks of receipt of the report. MTN LC, while collaborating with the SDMC, will monitor this process and assist sites as needed to meet that timeline. All corrective action is documented in paper-based clinic and/or laboratory records as appropriate and entered in the details section of LDMS.

Questions related to use of LDMS in MTN-034 may be directed to MTN LC (or LDMS Technical User Support). Usual business hours for LDMS User Support are 7:00 am - 6:00 pm (US Eastern Time) 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

Logging in Samples into the LDMS Specimen Management Module (see Figure 9-1):

- Enter the actual time of specimen collection in the Specimen Time area.
- Enter all specimen codes to populate the specimen receiving area (see section 'A')
- To make aliquots for each specimen in section 'A', fill out section 'B'
- If more than one Intravaginal Ring is returned on a visit, refer to instructions in [Section 9.7.7](#)

Figure 9-1: LDMS Entry Screen

A

Group	TYPE1	ID1	TYPE2	ID2	TYPE3	ID3	Visit	Unit	OPID	CLINIC	Detail
1 Practice Group	PID	12345678-9	PROTOCOL	034	ID3						Details
2											Details
3											Details
4											Details
5											Details
6											Details

Spec. Date: 27/Mar/2018 Exp. Date:
 Rec. Date: 27/Mar/2018 Recd Time: 11:00 Export ID:
 Remote Imported Import date:
 Enter Specimen ID

of Tubes: Primary Type: BLD Other Spec ID: RPS Spec. Time: 08:05

Specimen #	Global Spec ID	Primary	Additive	Volume	Units	Spec Time	Time	Time Unit	Cond	Other Spec Id	Details
1	414V18002540	BLD	EDT	10.00 ML		08:05			SAT	RPS	DB

When required, Enter Other Spec ID here

Enter actual collection time here

Insert specimen information here

B

of Aliquots: 0 Vol: 0 Units: Derivative: Sub Add/Der: Other Spec ID:

Specimen	Global Spec ID	Primary	Add	Der	Sub Add/Der	Volume	Units	Cond	Other Spec ID	group/ID	Der
1	414V18002541	BLD	EDT	PL1	N/A	1.00 ML		SAT	RPS	Practice Group/034	RDB
2	414V18002541	BLD	EDT	PL1	N/A	1.00 ML		SAT	RPS	Practice Group/034	RDB
3	414V18002541	BLD	EDT	PL1	N/A	1.00 ML		SAT	RPS	Practice Group/034	RDB
4	414V18002541	BLD	EDT	PL1	N/A	1.00 ML		SAT	RPS	Practice Group/034	RDB
5	414V18002541	BLD	EDT	PL1	N/A	0.50 ML		SAT	RPS	Practice Group/034	RDB

Can modify volume when needed

Enter aliquot information here: In this example: # of Aliquots=5, Vol=1, Units=ML, Der=PL1, Sub Add/DER=NA

Table 9-4 should be used as a guide when logging in MTN-034 specimens. Please use the LDMS codes listed below when logging in specimens for each test listed. Tracking sheets can be found in the Study Implementation Materials section on the MTN-034 webpage.

WEB LDMS

Sites that switch to Web LDMS should use the condition code “LLT” (Local Lab Testing) for any samples entered into LDMS for local testing and not stored per protocol, such as RNA samples. This replaces “Never Store” from windows LDMS.

Contact the LC with any questions about MTN samples management related to switching to Web LDMS.

Table 9-4 LDMS Specimen Management Guide for MTN-034 Specimens

Test	Primary Specimen	Primary Additive	Other Spec ID	Primary Volume	No. of Aliquots	Aliquot Volume	Units	Derivative	Sub Add/ Derv
Plasma Archive (at enrollment only)	BLD	EDT	RPS	10.0 ML	4-5	1.0	ML	PL1/2	N/A
Plasma Storage (Routine Plasma Storage)	BLD	EDT	RPS	10.0 ML	4-5	1.0	ML	PL1/2	N/A
Seroconversion Plasma Storage	BLD	EDT	CON	15.0 ML	4-5	1.0	ML	PL1/2	N/A
Post HIV Seroconverter Confirmation (drawn when needed 1 month after initial confirmation tests) Plasma Storage	BLD	EDT	SER	15.0 ML	4-5	1.0	ML	PL1/2	N/A
Dry Blood Spot (DBS)	BLD	EDT		4.0 ML	1	250*	UL	DBS	N/A
HSV-2 antibody	BLD	NON		4.0 ML	1	1.0	ML	SER	N/A
Vaginal Swabs for qPCR (Microbiota)	VAG	NON		3 EA	3	1.0	EA	FLS	N/A
Vaginal Gram Stain	VAG	NON		2 EA	2	1.0	EA	SLD	GRS
Vaginal Swab for Biomarkers	VAG	NON		1 EA	1	1.0	EA	SWB	N/A
CVL for Biomarkers (saving supernatant & cell pellet)	CVL	NSL		~10 ML	6+	1.0	ML	FLD	N/A
					1	0.5	ML	CEN	NSL
Cervical Swab for Biomarkers	CER	NON		1 EA	1	1.0	EA	SWB	N/A
Cervical Cytobrush for Flow Cytometry (Zimbabwe only)	CER	RPM		1 EA	1	1.0	ML	CTB	N/A
Used Vaginal Ring for Residual PK	IVR	NON	^	1 EA	1	1.0	EA	IVR	N/A
Test	Primary	Additive	Other Spec ID	Primary Volume	No. of Aliquots	Aliquot Volume	Units	Derv	Sub Add/ Derv

*DBS aliquot volume: 50 µL per spot (fill all 5 circles) for a total of 250 µL on 1 Whatman Protein Saver Card (log in 1 card)

^ If 2 rings are returned in one visit, place '1st IVR' or '2nd IVR' in Other Spec ID. See instructions in [section 9.7.7](#)

Table 9-5 LDMS Codes

BLD: Whole Blood	FLD: Fluid (supernatant)	PL1 or PL2: Single or double spun plasma
CER: Cervix	FLS: Flocked Swab	RPM: RPMI Transport Media
CVL: Cervical Vaginal Lavage	GRS: Gram Stain	RPS: Plasma Archive or Storage (other spec ID)
CEN: Cell Pellet	IVR: Used Intravaginal Ring	SER: Seroconverter (other Spec ID)
CON: Seroconversion (other spec ID)	N/A: Not Applicable	SER: Serum
CTB: Cytobrush	NON: No Additive	SWB: Swab
DBS: Dry Blood Spot	NSL: Normal Saline	SLD: Slide
EDT: EDTA	ML: Milliliter (mL)	UL: Microliter (µL), also called Lambda (λ)
		VAG: Vaginal Swab

9.5 Urine Testing for Pregnancy, Dipstick Urinalysis, and Culture

The urine tests performed at each study visit will depend on the time point of the visit and the clinical presentation of the participant. At study visits when urine testing is required, a single specimen will be collected and then aliquoted for each test when possible. When performing multiple tests from one specimen, the correct order is first pregnancy testing, and then the urine dipstick/culture (if clinically indicated). Collect urine specimens before collecting any pelvic specimens. Heavy menses may interfere with testing – sites should use discretion and contact the MTN LC if there are questions.

9.5.1 Specimen Collection

- The participant should not have urinated within one hour prior to urine collection.
- Provide the participant with a sterile, plastic, preservative-free screw-top urine collection cup labeled with a SCHARP-provided PTID label.
- Participant should not clean her labia prior to specimen collection (the cleaning agent may adversely affect other samples taken).
- If testing is *only* for urinalysis, culture, and/or pregnancy test, then collect midstream urine.
- Instruct the participant to screw the lid tightly onto the cup after collection.

9.5.2 Pregnancy Testing

The only pregnancy kits that can be used for this study are: Quidel QuickVue One-Step hCG urine, Quidel QuickVue Combo hCG urine/serum pregnancy, or Fisher HealthCare Sure-Vue Urine hCG test. Perform the test according to site SOPs and the package insert.

If the urine pregnancy test cannot adequately be interpreted because of interfering factors, for example excess blood or extreme cloudiness due to amorphous material, the sample can be spun down and the urine supernatant can be used. If the test continues to have interferences such as gross hemolysis making the test difficult to read, then another urine sample will need to be collected.

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

If a participant becomes pregnant, follow directions in SSP section 5.7 (Modified Procedures for Participants Who Become Pregnant).

9.5.3 Urine dipstick and/or Culture

Perform only if clinically indicated and/or by local standard of care. Instruct the participant to collect a midstream urine sample.

9.6 Blood Specimens: Collection, Processing and Testing for HIV, Chemistry, Hematology, Hepatitis, Herpes, Syphilis, Plasma Archive/Storage, Dry Blood Spots

The blood tests performed at each study visit vary depending 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.

All sites submitting safety results to SCHARP will need to submit with SCHARP determined units. Refer to the MTN-034 CRF Completion Guidelines for required analytes units. If your labs testing units are different, then the results will need converted into universal units determined by SCHARP. Sites can convert units by using a SCHARP tool found on ATLAS:
(<https://atlas.scharp.org/cpas/project/Collaborators/Lab%20Unit%20Conversion%20Tool/begin.view>).

In addition, if MTN-protocol related reference ranges change during the study, the LC point of contact must be notified at least 1 week before the changes are implemented.

9.6.1 Specimen Collection and Initial Processing

Label all required tubes with a SCHARP-provided PTID label immediately after a phlebotomy collection.

- Collect venous blood collection tubes (red or gold top SST) and invert 5-8 times, allow samples to clot, then centrifuge per site SOPs (manufacturer directions) for spinning tubes to yield serum for proper testing. Typical serum tests are: chemistries, syphilis, HBsAg, and HSV.
- Lithium Heparin (Light Green Top) may be the specimen of choice for chemistry tests (Creatinine). Invert 8 times directly after collecting specimen.
- EDTA tubes (purple top) should be gently inverted at least 8 times after specimen collection to prevent clotting. EDTA tubes are used for CBC with platelets, HIV testing, and plasma

archive/storage. If whole blood for hematology testing and plasma is to be taken from the same tube, hematological tests 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.

Note: Use local laboratory guidelines for the type of tube required. 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.

Order of draw: Red (no additive) ⇒ Tiger or Gold (SST, gel, clot activator) ⇒ light green (Lithium Heparin) ⇒ Lavender (EDTA)

9.6.2 HIV Testing: Rapid testing, Confirmatory tests including CD4, RNA, and DNA

EDTA plasma (*whole blood and serum may also be acceptable for local testing*) will be tested for HIV using tests that have been validated at the study site. All tests and associated QC procedures must be documented on local laboratory log sheets or other laboratory source documents. Successful ratings for proficiency testing such as College of American Pathologists (CAP) must be maintained.

HIV infection status will be assessed according to the HIV testing algorithm as presented in Appendix 9-1 in this section (Appendix II and III of the MTN-034 protocol). The first specimen drawn for HIV testing is considered 'Sample 1', and the confirmatory specimen drawn is considered 'Sample 2'.

At any time point where HIV rapid testing is performed, two different rapid tests will be used:

- The first rapid test will be the (Fourth-Generation) CE-marked Alere HIV Combo rapid test, Alere product number 7D2846 (20 tests) or 7D2847 (100 tests), or an LC-approved third generation test.
 - Note: There are several different Alere products with similar names and formulations. It is imperative to obtain the correct kit.
- The second rapid test will be an FDA approved (Third-Generation) test, either OraQuick or Unigold. Note that there are FDA and non-FDA approved versions of these kits – please ensure that only the FDA-approved version is obtained.

In cases of potential kit shortages, sites must contact the MTN LC for guidance on backup kit selection. A backup third-generation test may be used if the fourth-generation kit is unavailable; the LC must be notified via email if this occurs and may send additional guidance.

Study participants may report potential exposures to HIV that would increase the likelihood they are acutely infected during screening, enrollment or post enrollment study visits. Participants may also present with signs and symptoms that are consistent with acute infection. In these cases, even if the participant has 2 negative rapid test results, site clinicians may request an HIV-1 RNA to be performed which may detect infection before other tests. For RNA tests done at the clinician's discretion for suspected acute infection, sites must notify the MTN Virology core with an HIV Query form (Appendix 9-2). The Virology core will track these cases and send guidance as needed.

SCREENING/ENROLLMENT Visit:

Sample 1 will be used to perform two different rapid HIV tests at screening and enrollment:

- If both rapid tests are negative, the participant will be considered HIV-uninfected.
- If both are positive, the participant will be considered HIV-infected, and ineligible for enrollment into this study.
- If the rapid tests are discordant, i.e., one rapid test is positive and one is negative:
 - The participant will be ineligible for enrollment into this study.
 - Inform the MTN LC by submitting a query form (Appendix 9-2) to mtnvirology@mtnstopshiv.org. The MTN LC will send guidance within 1 business day.

Sample 2: MTN LC recommends when Sample 1 is positive or is discordant, that a Sample 2 is drawn.

- In common circumstances, guidance from the MTN LC will be to collect 'Sample 2' blood and perform a Geenius confirmatory test and plasma viral load (HIV RNA PCR). The participant may

not wish to have Sample 2 drawn, in which they can seek alternative local HIV confirmation testing. If the participant agrees to Sample 2, site may immediately proceed with these tests as part of HIV status determination as long as a query form is also submitted on the same day. Please note, the participant has not completed enrollment procedures, so blood or plasma may not be stored for future testing at this time.

FOLLOW-UP Visits:

Sample 1 will be used to perform two different rapid HIV tests at each follow up visit.

- If the rapid tests are negative, the participant will be considered HIV-uninfected.
- If both rapid tests are positive, then 'Sample 2' blood will be a separate blood draw on the same day.
- If tests are discordant, then 'Sample 2' blood will be drawn on the same day.

Sample 2 Testing:

- Upon documentation of the first positive Rapid HIV test, the following Sample 2 procedures must be performed regardless of whether or not they are scheduled to be completed:
- Plasma collection for:
 - HIV confirmation (Geenius testing)
 - Storage (Plasma Storage Seroconversion)
 - Viral Load utilizing HIV-1 RNA PCR (Polymerase Chain Reaction).
- Whole Blood for:
 - CD4+ T Cell Testing
 - CBC with platelets
- Blood creatinine for creatinine clearance
- Collection of PK and biomarker specimens (if the visit did not have a pelvic exam, one will need to be performed to acquire biomarker samples: CVL, vaginal and cervical swabs).

Note: If the site is unable to collect the sample because the participant is unwilling or other reason at that visit, they should try to recall the participant back to the clinic as soon as possible.

- Notify the MTN LC using the query form (Appendix 9-2). **Do not wait** for MTN approval or MTN LC response to the query form to proceed with Geenius, CD4, and HIV RNA viral load testing.
- If the Geenius is positive for HIV-1, HIV-1 infection is considered confirmed for study purposes per the algorithm.
- If the Geenius is negative, indeterminate or indicates potential HIV-2 infection, notify the MTN LC using the query form (Appendix 9-2) and use the results of the HIV RNA viral load to determine the need for further testing. A viral load result above the limit of detection will be considered positive and the Geenius will be repeated on a new sample taken approximately 1 month later for confirmation (called the Post HIV Seroconverter Confirmation sample). A viral load result below the limit of detection will be considered negative; based on this result, the participant will be considered HIV-uninfected. A viral load result of "detected, below the limit of detection" may require further guidance before HIV status is finalized.

Post HIV Seroconverter Confirmation Testing:

- Collected during the next monthly visit (~1 month after the initial Geenius confirmation testing and positive HIV RNA results occurs).
- Collect blood for the repeat Geenius testing and for post seroconversion sample testing (CD4+ T-Cell, HIV RNA and plasma storage even though seroconversion is not yet confirmed at this point). HIV RNA and CD4+ T Cell testing should proceed immediately.

HIV DNA testing will only be used in rare circumstances where HIV infection status cannot be determined from Geenius and HIV RNA viral load results (for example, if Geenius is indeterminate with one major band such as p24, and HIV RNA viral load is detected but below the limit of detection). Samples for DNA testing can only be collected with approval from the MTN LC.

Kit inventories should be monitored closely, and re-supply orders placed at least 8-12 weeks in advance of actual need (or longer if needed per site procurement policies and procedures). Notify the MTN LC immediately if any kit inventory or quality control problems are identified, so that appropriate action can be taken.

Additional HIV Testing Guidance:

Fourth-Generation rapids

- Fourth-Generation rapids contain two test bands: one for antigen and one for antibody.
- For purposes of the testing algorithms, if either test line is present, the result is positive.
- Testing logs need to differentiate Antigen positive, Antibody positive, Dual positive, and negative.
- The Quality Control must include antigen and antibody positive samples. Sites will use the Alere Combo control (Cat# 7D2252). Alternate controls require LC approval.

Geenius

- Sites must maintain printouts of Geenius reports that include the PTID, Visit code, sample date and testing date.
- QC must be run each week study participants are tested, in addition to requirements in the package insert.
- The LC suggests labelling cassettes so that the label can be seen in the image displayed on the Geenius reports.
- Manual reading of test cassettes cannot be used to interpret or report results in MTN-034. In the event that the Reader Instrument is down, sites must revert to backup.
- If the Geenius reader indicates a line is 'present', but a line is not visibly seen for either participant or EQA testing, follow the instructions below and contact the LC immediately.

HIV-1 Indeterminate results

- Staff members should be observing the cassette when they place it in the instrument.
- If no bands are visible on the cassette but the reader gives an indeterminate or positive result, the technician can cancel the run and re-read the cassette. This must be done within the allowable read time for the cassette.
 - If the second reading is negative, this result can be accepted.
 - If the second reading is still indeterminate:
 - Accept the result
 - Proceed with the algorithm and notify the LC as required for enrolled participants.

HIV-2 positive or indeterminate results

- HIV-2 Positive results: if the Geenius reports an HIV-2 positive result, contact the LC with an HIV query form. The LC will send guidance on a case by case basis. Wait for guidance from LC before proceeding with the algorithm.
- HIV-2 Indeterminate results:
 - If no bands are visible on the cassette but the reader gives an indeterminate result, the technician can cancel the run and re-read the cassette. This must be done within the allowable read time for the cassette.
 - If the second read is negative, this result can be accepted.
 - If the second read is still indeterminate:
 - Accept the result
 - Notify the LC as required for enrolled participants. The LC will send guidance on a case by case basis. Wait for guidance from LC before proceeding with the algorithm.

Note: HIV-2 is rare in the countries where REACH is conducted, but all HIV-2 positive or indeterminate results must be evaluated. In cases of HIV-2 positive or indeterminate results, product should continue to be held and the MTN-034 PSRT consulted on further product use management, including progression to permanent discontinuation if HIV-2 infection is confirmed, and clinical care.

Commercial versus in-house HIV controls

- In-house HIV rapid test controls may be used instead of commercial controls when described in an SOP and is not in violation of package insert. Note that the Orasure Oraquick kit does not allow for use of in-house controls.

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

9.6.3 CD4 T-Cell

CD4+ T-Cell Count

CD4+ T-Cell counts are performed for participants in accordance to the follow-up testing required in the HIV Algorithm, and per MTN-034 protocol Section 7.5.1.

Site laboratories will test EDTA whole blood by flow cytometry for absolute CD4+ T Cell counts per local SOPs. Testing will be performed on FDA approved instruments per site SOPs and package inserts. Sites must participate in United Kingdom External Quality Assurance (UKNEQAS) programs and be approved by the Immunology Quality Assurance (IQA) group to perform this testing.

9.6.4 HIV PCR

HIV RNA PCR

HIV RNA PCR (viral load) testing is only performed for participants in the follow-up HIV testing algorithm, and during post-seroconversion follow up, if applicable, per protocol Section 7.5.1. On a case-by-case basis, sites may perform HIV RNA PCR on screening participants with discordant rapid test results, per guidance by the MTN LC.

All sites will participate in the Viral Quality Assurance (VQA) program. HIV RNA viral loads will be performed on EDTA plasma using methods approved by the MTN LC. All testing will be performed according to site SOPs and package inserts.

After research sites obtain ethics approval of LoA #03, to Protocol Version 2.0, HIV-1 RNA PCR or HIV-1 genotyping may be performed at additional/alternate time points as requested by the site IoR/designee or at the discretion of the Laboratory Center (LC). The LC may request samples be shipped to the LC or request local testing.

HIV DNA PCR

HIV DNA PCR can only be used in circumstances where HIV infection status cannot be determined from Geenius confirmation and HIV RNA viral load results. Do not collect unless advised to do so by MTN-LC.

9.6.5 Chemistry (Creatinine) and Hematology (CBC and Platelets)

Testing will be performed per the local standard of care.

- Creatinine: Renal function will be evaluated using the participant's height in conjunction with the Revised Schwartz Estimate formula (also called the Bedside IDMS-traceable Schwartz GFR). The REACH (MTN-034) Safety Lab Calculator is located on the MTN-034 webpage (in the [Study Implementation Materials](#) section). Either serum or lithium heparin plasma are acceptable for testing.

Note: Baseline height may be used for the Creatinine Clearance calculation.
Estimated Glomerular Filtration Rate (eGFR) = Calculated Creatinine Clearance in MTN-034
$$\text{eGFR (mL/min/1.73 m}^2\text{)} = (0.413 \times \text{Height in cm}) / \text{Blood Creatinine in mg/dL}$$

If site lab units are not in mg/dL, then convert using this tool:

<https://atlas.scharp.org/cpas/project/Collaborators/Lab Unit Conversion Tool/begin.view>

- Hematology: EDTA whole blood is used to test for:
 - Hemoglobin
 - Hematocrit
 - Mean Corpuscular Volume
 - Platelets

- White blood cell count with differential
 - Absolute neutrophil count
 - Absolute lymphocyte count
 - Absolute monocyte count
 - Absolute eosinophil count
 - Absolute basophil count

9.6.6 Hepatitis and Herpes testing

- **Hepatitis Testing:** Serum is generally the specimen of choice for HBsAG and will be collected and performed according to local laboratory SOPs.
- **Herpes simplex virus type 2:** Draw a plain tube (preferred) or EDTA tube. Centrifuge tube and aliquot 1 mL of supernatant into cryovial and freeze at $\leq 70^{\circ}\text{C}$ within 4 hours after collection. MTN-LC will contact site when and where samples are to be shipped.

9.6.7 Syphilis Testing

Syphilis testing is performed using serum (or EDTA plasma if acceptable) and can be performed in one of two ways:

1. Rapid Plasma Reagin (RPR) 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 results must have a titer reported. For reactive RPR tests observed during screening, a confirmatory test is performed, and appropriate clinical management action must be taken. These participants will not be eligible for enrollment in the study. MTN LC recommends for 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 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. These participants are not eligible for enrollment in the study. If the RPR or VDRL is negative, this may indicate prior treatment, late latent disease, or a false positive test. 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 confirmatory test is negative, the participant is not considered infected with syphilis. If the second confirmatory 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-034 Protocol Safety Physicians (mtn034safety@mtnstopshiv.org).

RPR tests may be performed on either serum or plasma (EDTA or heparinized). Serum is preferred for syphilis confirmatory tests. However, other sample types may be allowed according to the particular tests package insert. All testing and QC procedures must be performed and documented in accordance with study site SOPs.

9.6.8 Plasma Archive and Storage

'Plasma Archive' in MTN-034 will only be used for enrollment storage.

All stored plasma will fall under one of these categories: Routine Plasma Storage, Plasma Storage for HIV Algorithm Seroconversion, or Plasma Storage for Post HIV Seroconverter Confirmation.

Table 9-6 shows the four types of stored plasma including minimum volumes:

Table 9-6 Volume Guide for Plasma Storage

Plasma Specimen	Draw volume	Optimal Plasma Required
Enrollment Plasma Archive ('RPS' Spec ID) at Visit 2	~10 mL	4 mL
Routine Plasma Storage ('RPS' Spec ID) at Visit 6, 9, 13,16, & 23	~10 mL	4 mL
Seroconversion Plasma Storage ('CON' Spec ID)	~15 mL	6 mL
Post HIV Seroconverter Confirmation Plasma ('SER' Spec ID)	~15 mL	6 mL

Other SPEC ID in LDMS:

To simplify shipping procedures, MTN-034 will identify three types of plasma storage in LDMS in the "other SPEC ID" field. For sites using the LDMS tracking sheets, this will be identified on that form. For sites not using the LDMS tracking sheets, a mechanism will be required for the clinic to relay the type of archive to the LDMS laboratory.

Sites will enter:

- RPS for Enrollment Plasma (Archive) and Routine Plasma Storage recorded on the Specimen Storage CRF.
- CON for Seroconversion Plasma Storage recorded on the HIV Confirmatory Results CRF (blood taken after an HIV rapid test become + or ±).
- SER for Post HIV Seroconverter Confirmation Plasma recorded on the Seroconverter Laboratory Results CRF.

The "other SPEC ID" field is free text and the three letter codes will need to be entered exactly. This information will be tracked in the LDMS reconciliations and will show a discrepancy if the information in LDMS does not match the information recorded on the CRF. (See table 9-5)

For Plasma Archive and Routine Plasma Storage:

Use collection tubes with EDTA anticoagulant.

- Prepare as many 1 mL aliquots as possible. Store at $\leq -70^{\circ}\text{C}$ and batch onsite until the MTN LC study team requests shipping and/or testing.
- SCHARP PTID labels and LDMS will be used to track the specimens.
- If sample is collected and held at room temp, freeze plasma within 4 hours. If refrigerated or placed on ice after collection, freeze plasma within 24 hours.
- Spin blood at room temperature in a centrifuge according to one of these techniques:
 - Single spun*: Spin blood at 1200-1500xg RCF (Relative Centrifugal Force) for 10 minutes and remove plasma.
 - Double spun*: Spin blood at 800xg for 10 minutes, place plasma in a tube to spin again at 800xg for 10 minutes, remove plasma.

* Always follow the collection tube manufacturer instructions for acceptable centrifuge RCF.

- Prepare as many 1.0 mL aliquots as possible with a total volume of aliquots ideally greater than 3.5 mL.
- If total volume is less than 3.0 mL, redraw as soon as possible and notify the LC (do not wait for a response to recall the participant).
- If samples are hemolyzed, store the aliquots as per normal procedure and enter a comment in LDMS.
- The MTN LC will send instructions to the site when shipping and/or testing is required.
- Any positive HIV test results after enrollment is to be considered an indication for plasma storage. Store plasma for all post enrollment positive HIV results.

Short draws / missed collections:

- If plasma collection is missed or less than 3.0 mL for plasma storage, and the participant has left the premises, the participant is to be called back to obtain the required amount of plasma if an enrolment visit (Plasma Archive), PUEV, or HIV endpoint related. In these cases, notify the LC but do not wait for a response to recall the participant. On all other visits, contact the LC for guidance.

Leftover Specimens: Leftover specimens may be temporarily stored for site QA purposes and problem resolution for all participants. This process must be described in an SOP or on-site policy that indicates how long the samples will be stored. Local guidelines and regulations must be followed in these situations. Only specimens from participants who have consented to long-term storage may be stored longer for future research. Sites that save these specimens for long-term storage must have a plan to identify which participants have consented to this. Contact the management team for assistance as needed.

Procedures for plasma storage when a participant has discordant or positive rapids at a visit where routine plasma storage is also required (Visits 6, 9, 13, 16, 20, 23 and Termination Visits):

For sites conducting venipuncture for HIV rapid testing:

1. Optimally, store 4 mL of plasma (plasma archive at enrollment or plasma storage for other follow-up visits) with the LDMS code "RPS" from the first collection where specimen was drawn for rapid HIV tests
2. If any HIV Rapid test becomes positive, then collect 'Sample 2' and store an additional 6 mL of plasma (Seroconversion Plasma Storage) with the LDMS code "CON". Use this specimen for Geenius, HIV viral load. CD4 testing is also done on whole blood from this draw.

For sites conducting Fingerstick HIV rapid testing:

1. If the fingerstick HIV rapid test results become positive, then collect 'Sample 2 specimens' and store 6 mL of plasma (Seroconversion Plasma Storage) with the code "CON" in LDMS. Use this collection for Geenius, HIV viral RNA and CD4 testing.
2. The total plasma stored should remain 6 mL. Site does not need to draw a separate tube for routine plasma storage.
3. On the Specimen Storage CRF, mark "not required" for item "Plasma storage" and add a note that item plasma storage was not required due to HIV confirmatory plasma storage in the text field "Reason plasma not stored/not required."

9.6.9 Dry Blood Spots (DBS)

DBS will be used for determining the concentrations of tenofovir, emtricitabine and/or tenofovir diphosphate.

Procedure for Dried Blood Spot processing, Storage, and Shipping

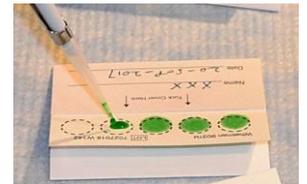
Supplies:

- 4 mL (smaller volume tubes acceptable) EDTA tube Blood Collection
- *Whatman Protein Saver Card #903 (Whatman 10534612 or Fisher Scientific #05-715-121)
- Gas-impermeable plastic sealable storage bag (LasecSA) or Whatman Plastic Zipper Seal Sample Bags (Whatman 10548232 or Fisher Scientific#50-853-570)
- Desiccant pack (Gel Silica Sachets – 1 gm) (LasecSA or Whatman WB100003 or Fisher Scientific#09-923-360)
- Humidity indicator cards (Multisorb Des Manufacture # MS200032, DESCO Industries #13870 or Fisher Scientific # NC0281067 or NC9511648)
- Whatman card drying rack (VWR catalogue # 89015-592)
- Power free (preferable) Latex or nitrile gloves
- Water proof marker
- 10-100 µL or 20-200 µL micropipette and appropriate tips with filters. Sites should check with local suppliers for appropriate tips for their micropipette

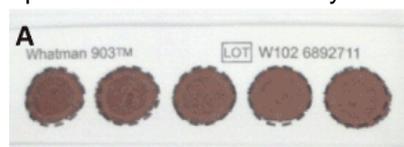
*Note: This procedure REQUIRES the use of this exact type of filter paper for sampling.

DBS Processing:

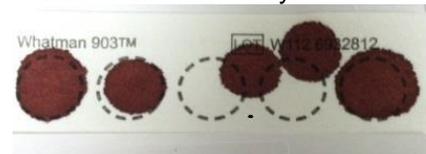
1. Gently invert the EDTA tube (8 to 10 times) to mix the blood thoroughly.
2. Within 4 hours (and preferably keeping the blood collection tube on ice), blood must be pipetted onto the filter paper spots in the card.
3. Pipette exactly 50 μ L of the whole blood into each single spot on a PTID labeled Whatman Protein Saver 903 Card.
 - Performed with a calibrated 50 μ L pipette and a disposable pipette tip using the wet tip technique (pre-wet the tip by aspirating liquid into the tip once and then dispensing all liquid out first, do this two to three times before dispensing into the circles).
 - Do not touch the filter paper with your hands.
 - Do not touch the card with the pipette tip.
 - Leaving the card slightly tilted may be beneficial for blood absorption.
 - Slowly expel blood from the tip and touch the drop to the paper, allowing the blood to absorb. Care must be taken when applying larger volumes of blood to ensure the spots do not run outside of the circle.
 - A single tip may be used to load the card.
 - Do not touch the DBS circle once blood is applied.
4. All five spots on the card should be filled (each spot 50 μ L).
 - If a difficult (short volume) blood draw was encountered, a minimum number of 50 μ L spots is 3.



Examples of DBS done correctly:



Examples of DBS done incorrectly:



5. Sample Drying:

- Allow the blood spot to air dry without the card flap covering the spots in a clean, dry place (i.e. biosafety cabinet, drying racks) that is protected from rodents, insects and direct sunlight for at least 2 hours (drying overnight may be necessary in areas with higher humidity).
 - i. Do not heat, stack or allow DBS to touch other surfaces during the drying process.
- Once confirmed to be completely dry, tuck in the flap of the Whatman Protein Saver 903 Card as indicated on the card to protect the samples from contamination.
- Store the card in a PTID labelled sealed plastic bag with a desiccant pack (sachet of desiccant).
- Do not store more than one card per bag.
- Store with a humidity indicator card with each sampled card.

6. Specimen Storage:

- If processing does not occur immediately, EDTA tubes are required to be refrigerated directly after being drawn.
- DBS spots are stable for up to 7 days at ambient temperature, 2 months at 4.0°C, and 6 months at -20°C.
- DBS processing sites are to store plastic bags (containing DBS, desiccant, and humidity cards) between 2-8 °C or freeze them at <-20°C.
- Make sure the bags are sealed tightly to prevent deterioration due to moisture.

7. DBS Specimen Shipping:

- Plastic Sample Bags containing DBS, desiccant, and humidity cards are stored in a 2-8 °C refrigerator.
 - Ship using dry ice or a thermal box with 2 to 4 (depending what's adequate for the season) gel cold packs and a single use temperature monitor, or ship in a validated temperature controlled refrigerated box (such as a returnable and reusable Credo system).
 - If DBS cards were stored in <-20°C °C freezer, then ship in a transport container with dry ice.
 - Sample Bags will be shipped weekly to the Division of Clinical Pharmacology, University of Cape Town (LDMS 499)
 - If weekly shipments are delayed for any reason or are not received at the testing lab in a timely manner, the LC must be notified.
 - If MTN LC or sites determine that refrigeration storage and/or shipping is not adequate for DBS stability, then DBS bags will be stored in a <-20°C freezer and shipped on dry ice.
- Inspect DBS specimens for a change in humidity and replace or add additional desiccants prior to shipping; document any changes relating to the humidity indicator.

Weekly shipments:

Each site has the responsibility to ensure that DBS samples are shipped weekly and are received by University of Cape Town. DBS samples must be shipped within a week of collection.

Total approximate testing timeline:

- Site Collection to shipment: 7 days
- Site shipment to UCT: 2 days
- Specimen testing and provision of results to site: 21 days
- Site processing of results before visit: 1-2 days

Note: The COVID pandemic may make shipping impossible or unadvisable because of flight limitations. Discuss any potential shipping pauses with the MTN LC or MTN-034 Management Team.

9.7 Vaginal Samples: Gram Stain, Microbiota (qPCR), Vaginal Fluid pH, Vaginal Wet Mount, Trichomonas, CT/GC, Biomarkers, and IVR.

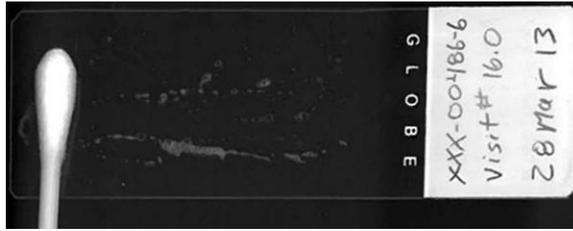
Refer to Pelvic Exam checklist of this SSP manual for further information on the required sequence of specimen collection and diagnostic procedures to be performed during study pelvic exams.

9.7.1 Gram Stains on Vaginal Fluid

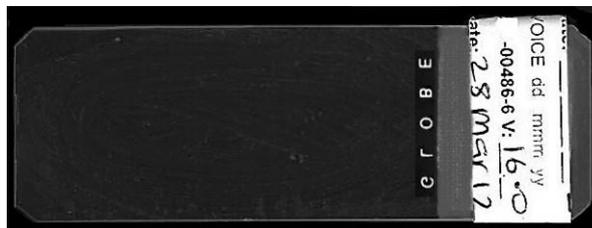
Dried vaginal fluid smears will be prepared for Gram staining and assessment for bacterial vaginosis at the MTN LC. Two slides (one designated as primary and the other as secondary) will be prepared using 1 swab. Both slides will be entered into LDMS. The primary slide will be shipped to the MTN LC and the secondary 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.

Instructions for slide preparation and shipping are provided below:

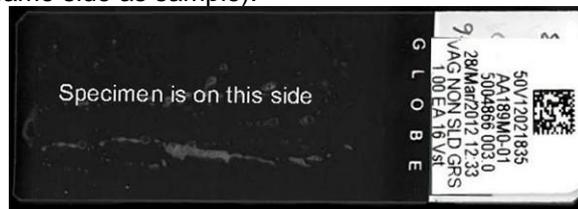
1. Use a pencil to write the PTID and specimen collection date on one side of the frosted end of the slide. This is the side of the slide that the specimen is to be applied.



2. Immediately following specimen collection from the lateral vaginal wall via 3 turns of a swab (polyester or cotton), roll the swab across each of the slides. (Be sure to collect the specimen from opposite the vaginal wall used for the wet mount specimen collection.) Do not place the swab in saline, transport medium, or any transport container prior to slide preparation.
3. 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. pen) on each label.



4. Allow the specimens to air-dry on the slides. Do not heat-fix.
5. Vaginal smears for gram stain are to be logged into LDMS (specimen type = VAG) and label the 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).



6. The primary slides will be stored in a slide storage box and sent to MTN LC upon request. If possible, gram stain slides will be shipped with other samples that are to be sent to the Magee-Womens Research Institute. (See shipping instructions below next section).
7. 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).

9.7.2 Microbiota: Vaginal Swab for qPCR

Vaginal fluid will be collected for quantitative PCR of key organisms in the vaginal microbiota and tested at the MTN LC at Magee-Womens Research Institute.

Procedure:

- Three flocked swabs will be collected. MTN LC will provide the swabs.
- Attach separate SCHARP labels to each of the 3 cryovials (2 mL size).
- Label should indicate vaginal q-PCR to distinguish this specimen from other vaginal specimens.
- Collect vaginal fluid by rotating flocked swabs several times over the lateral wall of the vagina. Do not collect swabs in the exact same area that another sample was collected (i.e. If the biomarker swab was collected first, collect the swab for microbiota in a different location in the vagina.) Do not collect near the cervix.
- Place each of the swabs into separate 2-mL cryovials, break off swab shaft, and cap the vial.
- Immediately refrigerate or place vials on ice and freeze at $\leq -70^{\circ}\text{C}$ within 8 hours of the sample collection.

- Mark these samples on the LDMS tracking sheet and transport to the LDMS laboratory.
- The sample will be labeled and tracked using LDMS.
- MTN LC will notify the sites when to ship samples for testing by the LC.

Ship to:
 May Beamer
 Magee-Womens Research Institute
 Room A530
 204 Craft Avenue
 Pittsburgh, PA 15213 USA
 Email: mbeamer@mwri.magee.edu
 Phone: 412-641-6041

9.7.3 Vaginal Fluid pH

Vaginal fluid pH will be assessed as part of on-site evaluations for bacterial vaginosis. pH Indicator Strips (pH range 3.6 to 6.1) with brand names S/P Cardinal Health, Baker-pHIX, Whatman, or Machery-Nagel must be used at all sites.

Vaginal fluid pH swab (polyester or cotton) may be collected in any of 2 ways depending on if a speculum is used at that particular visit:

1. Obtained by the clinician during the pelvic examination
2. Collected by the clinician in a non-speculum exam

Note: a speculum is not required for pH sample collection.

Vaginal Fluid pH Procedure:

1. Swab onto the pH strip (Do not insert the pH strip into the vagina).
2. Match the resulting color of the indicator strip to the color scale provided with the strips to determine the pH value.
3. Record the pH value directly onto the STI Test Results CRF. It is not necessary to record pH values onto laboratory log sheets or other source documents prior to recording values onto the CRF.

Note: If wet mount testing is required (if indicated), then the pH swab will be used for that testing.

9.7.4 Vaginal Fluid Wet Mount testing for BV and Yeast (KOH) at visits 6, 9, 13, 16, and 20

Wet mount procedures for this study are only performed if indicated, and consists of two different preparations:

1. Potassium Hydroxide (KOH) prep
2. Saline prep

These procedures are for diagnosis of bacterial vaginosis and candidiasis as summarized in Table 9-6 below.

If wet prep slides are read in-clinic by clinical staff, results may be recorded directly on to STI Test Results CRF. If slides are read by lab staff (either in the local laboratory or a designated in-clinic lab area), results must be recorded on laboratory log sheets or other laboratory source documents and then transcribed onto the CRF.

The MTN LC requires all wet mount readers are assessed by the LC for competency of the wet mount tests; therefore, the MTN LC will administer a web-based proficiency test approximately every six months. The MTN LC will post wet mount slides on the MTN web pages for this purpose every 6 months; results will be entered directly on the website (contact: Michele Austin: maustin@mwri.magee.edu). The MTN LC will report results back to the Laboratory Manager and also specify any corrective action that may be needed based on the results. Contact the MTN LC for additional information and guidance on performing and documenting the proficiency testing. Also,

notify the MTN LC when new laboratory staff is hired, so that appropriate training can take place prior to such staff performing wet mounts for study purposes.

Table 9-6 Summary of Wet Prep Assessments and Diagnostic Criteria

Assessment	Saline Prep	KOH Prep
Whiff Test	Not applicable	Positive if fishy amine odor detected
Yeast	Positive if pseudohyphae and/or budding yeast are observed. Pseudohyphae and budding yeast may be obscured by epithelial cells. These cells will be lysed by KOH, thus pseudohyphae and budding yeast that are not observed in a saline prep may be seen in the KOH prep.	Positive if pseudohyphae or budding yeast are observed.
Clue Cells	Individual cells rather than clusters of cells should be examined. Positive if at least 20% clue cells observed. Cells must be completely covered with bacteria (<i>Gardnerella vaginalis</i> and/or anaerobic GNR) to be counted as clue cells.	Not applicable (clue cells are lysed by KOH)

Note: Bacterial vaginosis will be diagnosed based on the presence of any three of the following Amsel's criteria: homogenous vaginal discharge, vaginal pH greater than 4.5, positive whiff test, at least 20% clue cells

Prepare and examine wet prep slides according to study site SOPs as follows:

- Use a pencil to write the PTID and specimen collection date on one side of the frosted end of two microscope slides. Affix a SCHARP-provided PTID label to the other side of the slides (on the frosted end, under the pencil markings) and write the specimen collection date in indelible ink (e.g. Sharpie pen) on each label.
- *Using the same swab as the pH, smear the vaginal fluid specimen onto each slide. Alternatively, the swab may be placed in a glass or plastic tube with approximately six drops (100 µL) sterile physiologic saline to allow for non-immediate slide preparation. In this case, vaginal fluid specimens should be smeared onto the two slides upon receipt from the collecting clinician.
- Apply one drop of 10% KOH to one slide and immediately perform whiff test for a “fishy” amine odor. Then apply cover slip.
- Apply one drop of sterile physiologic saline to the second slide, mix with the vaginal fluid specimen, and then apply cover-slip. Examine immediately at 100X total magnification for epithelial cells, budding yeast, and pseudohyphae. Examine at 400X magnification to determine whether observed epithelial cells are clue cells and quantitate the cells. Clue cells are irregularly bordered squamous epithelial cells that are completely covered with bacteria (*Gardnerella vaginalis*). Clue cells must comprise at least 20 percent of the observed epithelial cells in order for the saline prep to be considered positive for clue cells.
- Examine the KOH slide at both 100X and 400X magnification for yeast and pseudohyphae.

* If you cannot use the same swab as the pH (e.g. due to contamination or swab does not have enough material on it), then collect a new swab from the lateral vaginal wall and make a note of this on the CRF.

9.7.5 Rapid Test for Trichomonas

This testing will be done using the OSOM Rapid *Trichomonas* test (manufactured by Sekisui Diagnostics formally Genzyme) with vaginal swabs per site SOPs approved by the MTN LC. The kit provides rayon swabs for this test.

- Affix a SCHARP-provided PTID label to a clean glass or plastic tube with a cap.
- Collect specimen using kit-provided swab from the lateral vaginal wall (fluids also may be collected from the posterior fornix; avoid collecting specimens from the cervix).

- Immediately place the swab in the labeled tube, break off the shaft of the swab, and cap the tube.
- Testing is expected to be performed during the participant visit. However, specimens may be stored at room temperature for 24 hours or refrigerated for 36 hours before testing.

9.7.6 Vaginal Swab for Biomarkers

Biomarkers will be evaluated to determine the impact the intravaginal rings and drugs may have on innate immune mediators, cytokines, or other safety concerns. Vaginal fluids are either clinician collected from the posterior fornix or participant self-collected.

Procedures for Biomarker Vaginal Swab:

Clinician Collected Swabs will occur at visits 2, 6, 9, 13, 16, 20 and 23.

- Collect vaginal fluid using a Dacron (Polyester) swab from the posterior fornix.
- Place the swab in a labeled 2.0 mL cryovial.
- Break off the swab shaft by using a finger over cryovial opening making sure swab does not fly out, discard swab shaft, and cap the vial tightly.
- Freeze at $\leq -70^{\circ}\text{C}$ within 4 hours of collecting the sample.

Self-Collected Vaginal Swab at visits 4,5,7,8,12,12,14,15,18,19,21 and 22.

- Ensure that new, clean, or sterilized supplies (gloves, swabs, vials, ziplock bags, etc.) are used for each sample.
- Before starting procedure, pre-label the cryovial and ziplock bag with a SCHARP label.
- Instruct the participant to self-collect the vaginal fluid as per SSP Clinical Section 7.9.
- Within 4 hours, place the cryovials in the freezer at $< -70^{\circ}\text{C}$.

9.7.7 Testing of Intravaginal Ring (IVR), Remnant content analysis

Used rings will be analyzed for residual levels of Dapivirine and will be collected every month while participants are using IVR's. The used rings may contain vaginal secretions and therefore treated as a biohazard. Contact the LC if rings are defective or inserted briefly and removed for various reasons. These rings may be destroyed at the site via biohazard procedures.

Step 1: Wear lab coat, gloves, and protective face guards when performing this step. The clinician will remove the used ring and place in a clean container* with tap water. Move the ring around in the water or swirl the container to remove vaginal material. Take the ring out of the water and blot dry with paper towels or gauze. The ring should be dry before storing in pouch. Dispose of blotting materials and contaminated water according to your institution biohazard policy.

Important Notes:

*Use a disposable container or a reusable container that was sterilized or cleaned using 10% bleach solution for 20 minutes.

If the ring is removed by the participant prior to the clinic visit and will not be reinserted, then the used ring is still prepared for residual drug analysis. After the used ring is taken out of the participant's bag (container they returned the ring in), follow directions starting with step 1.

Step 2: Site staff will place the ring into a new ziplock bag to store the rings. Label the bag with the participant ID number and visit number. Add a biohazard sticker if one is not already attached to the pouch, making sure not to cover the identifier information.

Step 3: Store the used ring within the biohazard labeled bag at room temperature.

Step 4: Log all rings into LDMS.

- In the event that 2 rings are dispensed at the same visit and returned together, ring order will be recorded when entering rings in LDMS.
 - If ring order can be determined, enter under *Other Spec ID* with '1st IVR' or '2nd IVR'; additional details may be added in comment fields.
 - If ring order cannot be determined, enter "UNK" in the *Other Spec ID*.

Step 5: On a weekly basis, used rings will be sent to FARMOVS (LDMS lab#: 671) for testing. Each site has the responsibility to ensure that IVR's are shipped weekly and are received by FARMOVS. If weekly shipments are delayed for any reason or are not received at the testing lab in a timely manner, LC must be notified,. **Returned IVRs must be shipped within a week of collection.**

Total approximate testing timeline:

- Site ring collection to shipment: 7 days
- Site shipment to FARMOVS: 2 days
- Specimen testing and provision of results to site: 14 days
- Site processing of results before visit: 1-2 days

Note: While dapivirine rings are expected to be returned on schedule throughout the study, if any ring is returned ≥ 6 months after dispensation, please contact the management team for guidance before sending the ring for residual drug analysis.

9.8 Cervicovaginal Lavage (CVL) for Biomarkers, Aliquot storage, and Cell Pellet.

CVL aliquots will be collected, processed, and used for testing of biomarkers, supernatant storage, and preservation of the cell pellet.

9.8.1 Collection procedure for CVL

Sample Collection and Transport: see Clinical SSP section 7.5.3.

9.8.2 Processing of the CVL Cell Pellet and Supernatant for biomarker and storage

The following steps are performed in conjunction with the collection of CVL:

1. CVL specimens are kept on wet ice or refrigerated and should be processed within 8 hours of collection.
2. All the CVL liquid will be spun at $800\times g$ for 10 minutes in the 15 mL conical collection tube.
3. Remove supernatant from the cell pellet and save fluid in cryovials.
4. Re-spin the 15 mL conical tube containing cells for 10 minutes at $800\times g$.
5. Pull off and save any additional supernatant making sure not to remove any cells or debris.
6. Store all supernatant in as many 1 mL aliquots as possible in 2 mL cryovials, assuring there are at least 1 aliquot for biomarker testing. A minimum of 5 back-up aliquots is also required to be stored (mark as 'Extra CVL').
7. Freeze all aliquots at $\leq -70^{\circ}\text{C}$ within 8 hours of collection and track in LDMS.
8. If less than a total of 6 mL's (or less than 6 cryovials) of supernatant are recovered, contact the MTN LC.
9. Cell pellets will be suspended in 0.5 mL normal saline in a plastic cryovial and frozen at $\leq -70^{\circ}\text{C}$ within 8 hours of collection.
10. The MTN LC will send instructions to the site when shipping is required.

9.9 Collection of Cervical Specimens for Biomarker, GC/CT, and Flow Cytometry

9.9.1 Swab for Biomarkers

Clinician collected swabs will occur at visits 2, 6, 9, 13, 16, 20 and 23.

- a. Collect cervical fluid using a Dacron (polyester) swab from the endocervical canal.
- b. Place the swab in a labeled 2.0 mL cryovial.
- c. Break off the swab shaft by using a finger over cryovial opening making sure swab does not fly out, discard swab shaft, and cap the vial tightly.
- d. Freeze at $\leq -70^{\circ}\text{C}$ within 4 hours of collecting the sample.

9.9.2 NAAT Gonorrhea and Chlamydia Testing

Sites can choose to use the Gen Probe Aptima, Cepheid GeneXpert or a method that has been approved by MTN-LC.

Collect cervical sample (1 manufacturer recommended swab) and transport to the local laboratory according to the specific manufacturer's recommendations.
Testing will be done at the local laboratories according to the site SOP.

9.9.3 Cytobrush for Flow Cytometry (Zimbabwe only)

Supplies for Cytobrush Collection, processing and testing:

- Cytobrush
- Specimen transport tube: 4 or 5 mL with screw top conical tubes
- Transport Media (tRPMI):
 - 10.0% FBS solution of RPMI-1640: Prepare quantity that best fits laboratory needs.
 - Store at 2-8°C and has a 30-day shelf life.
- Required provisions for testing will be supplied by MTN-LC

Specimen Collection Procedure:

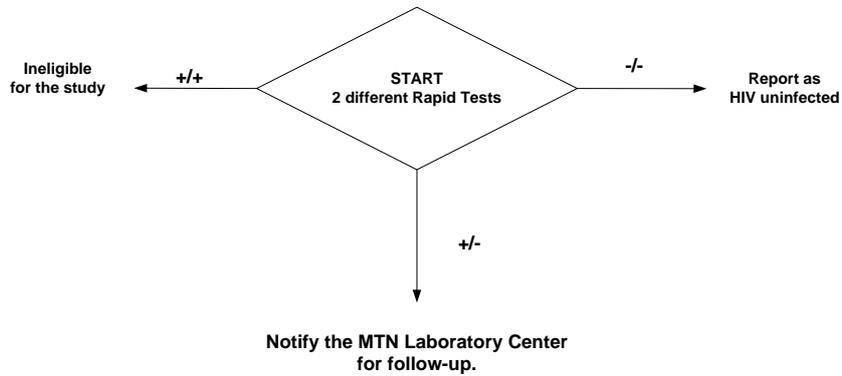
1. Collect sample using cytobrush by inserting into the cervical os and perform two – 360° turns.
2. Immediately place cytobrush into appropriately labeled screw cap transport vial containing at least 3 mL of tRPMI.
3. Break off or use scissors to cut shaft so the cytobrush will fit into the vial.
4. Keep on ice packs, wet ice or refrigerate until processed.

Laboratory Processing Procedure:

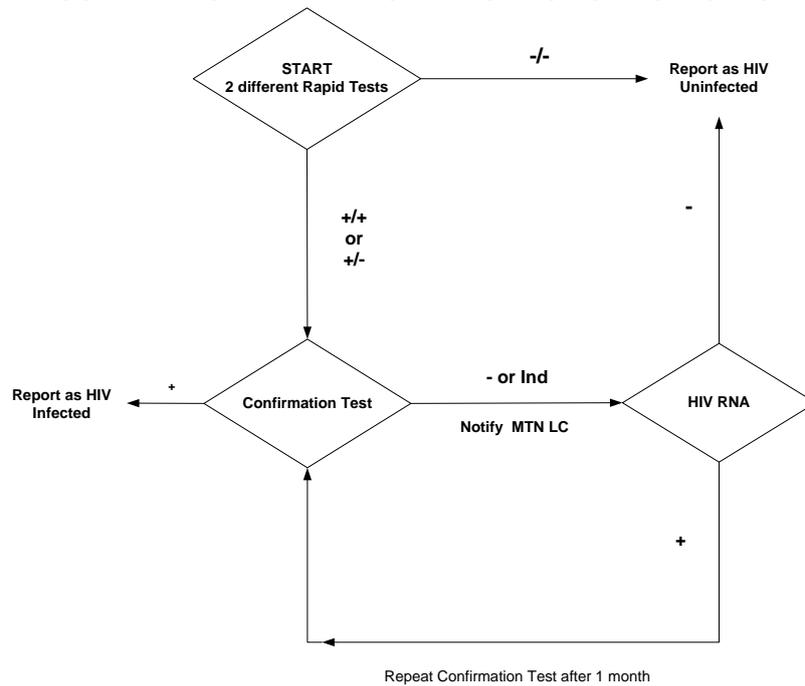
- Processing should occur within 2 hours from obtaining specimen.
- Follow site SOP for processing cytobrush and performing flow cytometry.

Appendix 9-1 MTN-034 HIV Antibody Testing Algorithms

ALGORITHM FOR HIV ANTIBODY TESTING- SCREENING/ENROLLMENT



ALGORITHM FOR HIV ANTIBODY TESTING FOR FOLLOW-UP



Ind: Indeterminate test results
LC: Laboratory Center

Appendix 9-2 MTN Network Lab HIV Testing Query Form

MTN Laboratory Center HIV Testing Notification & Query Form

Study	MTN-034
PTID	
Site/Contact Person	
Last Update	Click here to enter a date.

Form Closed

Please check one:

- Notification (LC response not required)
- Query (Waiting for LC Response)

VISIT CODE:

VISIT DATE: Click here to enter a date.

SITE COMMENTS/QUERY:

	Rapid Test 1	Rapid Test 2	Confirmatory Assay	HIV RNA	Other Test
Testing Date					
Kit Name			Geenius		
Result					

LC RESPONSE:

Participant Final Outcome

- HIV NEGATIVE
- HIV POSITIVE
- OTHER (Please describe further)