

Section 9. Laboratory Considerations

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9.1. Overview and General Guidance

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/>.

The tests to be performed at each visit during the MTN-030 study are listed in Tables 9-1A and 9-1B. Sites are responsible to ensure that specimen volumes do not exceed what is described in the informed consent process. The total blood volumes calculated in Table 9-1A include additional blood that may be collected for any clinically indicated testing. The MTN LC may request details of collection containers and volumes for this purpose, as shown in Table 9-2.

Table 9-1A: Overview of Laboratory Tests by visit for MTN-030

		Visit 1 SCR	Visit 2 ENR (Day 0)	Visit 3-5 (Days 1, 2, 3)	Visit 6 (Day 7)	Visit 7 (Day 14)	Visit 8 & 9 (Days 15 and 16)
LABORATORY							
URINE	hCG	X	X	*	*	*	* Day 16 Mandatory Only
	Urine dipstick/culture	*	*				
BLOOD	HIV-1 testing	X	X	*	*	X	*
	Plasma archive		X				
	Serum creatinine	X	X	*	*	X	*
	CBC with platelets and differential	X	X	*	*	X	*
	AST/ALT	X	X			X	*
	Syphilis serology	X		*	*	*	*
	DPV levels		X [∞]	X [∞]	X [∞]	X [∞]	X [∞]
	LNG levels		X [∞]	X [∞]	X [∞]	X [∞]	X [∞]
	Sex hormone-binding globulin (SHBG) and albumin		X			X	
	Serum progesterone and estradiol		X				
PELVIC	NAAT for GC/CT	X	*	*	*	*	*
	Trichomonas test	X	*	*	*	*	*
	Herpes lesion testing	*	*	*	*	*	*
	Pap test	^					
	Saline/ KOH wet mount with pH for candidiasis and/or BV	*	*	*	*	*	*
	Vaginal Gram stain		X	* Day 3 mandatory	*	X	*
	CVF DPV levels		X [∞]	X [∞]	X [∞]	X [∞]	X [∞]
CVF LNG levels		X [∞]	X [∞]	X [∞]	X [∞]	X [∞]	
STUDY PRODUCT	Collect IVR					X	
Blood volume	Approximate, check local laboratory requirements ♪	22 mL	82 mL	10 mL (max 28-mL)	10 mL (max 28-mL)	39 mL (max 43mL)	10 mL (max 32-mL)

* = if indicated; ^ = if indicated (if participant [over age 21] is unable to provide documentation of a satisfactory Pap test within 3 years prior to Enrollment); ∞ = See Table 9-1B below for additional details on sample collection; ▲ = Modified
 ♪ Maximum volume needed for study requirement, if all specimens are collected including "if clinically indicated".

Table 9-1B:

Visit	Specimens Collected for PK (Blood and CVF)
Visit 2: Enrollment (Day 0)	<ul style="list-style-type: none"> • Blood for DPV level (Baseline & Hrs 1, 2, 4, 6) • Blood for LNG level (Baseline & Hrs 1, 2, 4, 6) • CVF for DPV level (Baseline & Hrs 1, 2, 4, 6) • CVF for LNG level (Baseline & Hrs 1, 2, 4, 6)
Visits 3-5: Day 1, Day 2, Day 3 Study Visits	<ul style="list-style-type: none"> • Blood for DPV level • Blood for LNG level • CVF for DPV level • CVF for LNG level
Visit 6: Day 7	<ul style="list-style-type: none"> • Blood for DPV level • Blood for LNG level • CVF for DPV level • CVF for LNG level
Visit 7: Day 14	<ul style="list-style-type: none"> • Blood for DPV level (Prior to ring removal and at Hr 6) • Blood for LNG level (Prior to ring removal and at Hr 6) • CVF for DPV level (Prior to ring removal and at Hr 6) • CVF for LNG level (Prior to ring removal and at Hr 6)
Visit 8: Day 15 Visit 9: Day 16	<ul style="list-style-type: none"> • Blood for DPV level • Blood for LNG level • CVF for DPV level • CVF for LNG level

Table 9-2 also shows where laboratory procedures may be performed: study site clinics or laboratories, approved commercial laboratories, and laboratories within the MTN Laboratory Center (MTN LC), including the MTN Pharmacology Core at Johns Hopkins University Clinical Pharmacology Analytical Laboratory (JHU CPAL). Regardless of whether tests are performed in clinic or laboratory settings, study staff that performs the tests must be trained in properly associated QC procedures prior to performing the tests for study purposes (i.e. training documentation should be available for inspection at any time).

Table 9-2: Overview of Laboratory Testing Locations, Specimens, and Methods for MTN-030

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	Quidel Quickvue or SureVue
Urine Dipstick and Culture*	Local lab	Urine	Plastic screw top cup	Siemens Multistix® 10 SG or Uristix 4 or other MTN LC approved methodology
Complete Blood Count with Differential and Platelets	Local Lab	Consult Local Lab Requirements		Local methodology
Chemistries (AST, ALT, Albumin, Creatinine)	Local Lab	Consult Local Lab Requirements		Local methodology
Progesterone & estradiol	Local Lab	Consult Local Lab Requirements		Local methodology
Sex hormone-binding globulin	Local Lab	Consult Local Lab Requirements		Local methodology
Syphilis Serology	Local Lab	Consult Local Lab Requirements		Local methodology
HIV serology	Clinic/Local Lab	Plasma, serum, or whole blood	EDTA or plain, 4-mL	FDA approved tests
Plasma for Archive or Confirmation of Viral Load and HIV Resistance Testing	Clinic/Local Lab	Plasma	EDTA 10-mL tube	MTN LC procedure MTN LC Virology
Plasma for Blood PK (dapivirine & LNG)	CPAL	Plasma	EDTA 10-mL tube	CPAL collection procedure
Vaginal pH*	In clinic	Vaginal swab	N/A	S/P pH Indicator Strips
Vaginal Saline Wet Preparation (for BV and/or KOH wet mount)*	In clinic	Vaginal swab	tube with 6 drops of saline	MTN LC procedure
Vaginal NAAT for GC/CT	Local lab	Urine or vaginal swab	Kit specific Transport tube	BD Probetec, Cepheid GeneXpert or Gen-Probe Aptima
Trichomonas Test	Local lab or in clinic	Vaginal swab (supplied with kit)	NAAT collection tube	NAAT
Vaginal Swab for PK (dapivirine and LNG)	CPAL	Swab	2.0-mL Cryovial	CPAL collection procedure
Vaginal Smear for Gram-stain	MTN LC	Vaginal Swab	2 Slides	MTN LC procedure
Herpes Lesion Testing*	Local lab	Local method	Local method	Local methodology
Pap Test**	Local Lab	Consult Local Lab Requirements		Local methodology
Used Intravaginal Ring for PK residual assessment	IPM designated lab	Used IVR	Biohazard labeled 3"×5" amber Zippit pouch	MTN LC /IPM procedure

*Perform only if clinically indicated per local SOP.

**Perform if participant is over the age of 21 and does not have a documented satisfactory Pap within 3 years prior to Enrollment.

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.

Specimens that will be stored and shipped to the MTN LC or CPAL are highlighted in Table 9-3. These samples will be entered into LDMS (section 9.4).

Table 9-3: Overview of Specimens for Storage and Shipment

Specimen	Processing	Ship to	Shipping schedule
Plasma for Archive (at enrollment) or for Confirmation of Viral Load and HIV Resistance (at f/u)	Prepare as many 1.5-mL aliquots as possible. If sample is collected and held at room temp, freeze $\leq -70^{\circ}\text{C}$ within 4 hours. If refrigerated after collection, freeze $\leq -70^{\circ}\text{C}$ within 24 hours.	MTN LC	Store frozen at site until notified by MTN LC. However, if plasma for HIV confirmation, ship immediately to MTN LC Virology Core.
Plasma for Blood PK (dapivirine & LNG)	Centrifuge and aliquot into two or more cryovials with a minimum of 1.0-mL in each. Freeze within 8 hrs of blood collection.	CPAL:DPV MTN LC:LNG	Store frozen at site until conclusion of study or notified by MTN LC.
Vaginal Swab for PK (dapivirine & LNG)	Record Pre- and Post-collection weight of swab. Freeze at $\leq -70^{\circ}\text{C}$ within 2 hours of collection	CPAL:DPV MTN LC:LNG	Store frozen at site until conclusion of study or notified by MTN LC.
Vaginal smear for Gram-stain	Make 2 slides. Room temp. Label with LDMS label.	MTN LC	Store one set of slides that will be batch-shipped at conclusion of the study. Store 2nd set of slides (as backup) at site until all slides from first set are confirmed as received.
Used Intravaginal Ring for PK Residual Assessment	Place IVR in amber pouch	MTN LC	-20°C storage at site until conclusion of study.

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.

9.2. Specimen Labeling

All containers into which specimens are initially collected (e.g., urine collection cups, blood collection tubes) will be labeled with SCHARP-provided Participant ID (PTID) labels. Although PTIDs are pre-printed on these labels, study staff must write the specimen collection date on each label. The visit code also may be written on the label. Use an indelible ink pen (e.g., Sharpie) if information is handwritten such as the date or collection time point.

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 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 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.

If additional specimens need to be collected for the same test due to either laboratory error (lost, broken tube, clerical, etc.) or clinical error, a protocol deviation form may be required. The MTN 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 specimens have been mishandled, possibly compromising specimen integrity
- Any situation that may indicate a protocol deviation

If site staff has any questions regarding time windows or collection processes, call MTN LC staff as soon as possible for guidance.

9.4. Use of LDMS

The Laboratory Data and Management System (LDMS) is a program that must be used by all sites for the storage and shipping of sample types listed in Table 9-3. LDMS is supported by the Frontier Science Foundation (FSTRF). 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 locally (frequency determined by site) and to export their data to FSTRF (at least weekly).

LDMS Help: Questions related to use of LDMS in MTN-030 may be directed to MTN LC or LDMS Technical (User) Support. Usual business hours for LDMS User Support are 12:00 am - 6:00 pm (ET) from Monday through Friday. Contact LDMS User Support at:

Email: ldmshelp@fstrf.org
Phone: +716-834-0900, ext 7311
Fax: +716-834-8432

All other hours and weekends, an on-call user support specialist will be available if you are locked out of your LDMS or are experiencing errors that prevent you from completing your LDMS lab work. Use the LDMS Web Pager utility to page LDMS User Support. 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.

Discrepancy Reports: Each site must export its LDMS data to Frontier Science (FSTRF) on a weekly basis. The MTN Statistical and Data Management Center (SDMC) uses exported data to generate a monthly specimen repository report and to reconcile data entered in LDMS with data entered on study case report forms (CRFs). Any discrepancies identified during the reconciliation are included in a monthly discrepancy report for each 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 LC reviews the discrepancy reports for critical samples (e.g., plasma needed for confirmatory HIV testing) that appear to be missing and works with site staff, in consultation with the SDMC when needed, to undertake appropriate corrective action. All corrective action should be documented in clinic and/or laboratory records, including CRFs, as appropriate, and entered in the details section of LDMS. The MTN LC and SDMC will discuss and document any items that, although resolved, appear 'irresolvable' in LDMS.

9.4.1. LDMS Codes for Specimen Log In

Table 9-4 should be used as a guide when logging in MTN-030 specimens for storage or shipping. Please use the LDMS codes listed below when logging in specimens for each test listed. LDMS tracking sheets for Enrollment/Day 0 and follow-up visits can be found in the Study Implementation Materials section on the MTN-030 webpage.

Table 9-4 LDMS Specimen Management Guide to Logging in MTN-030 Specimens*

Sample	# tubes, Primary specimen	Primary Specimen	Primary Additive	Primary volume	Primary unit	Time [♦]	Time Unit [♦]	# of Aliquots	Aliquot volume	Aliquot unit	Aliquot Derivative	Aliquot Sub additive/ derivative	Other Spec ID
Plasma for Archive or Confirmatory Test	1	BLD	EDT	3	ML			2	> 1.0	ML	PL1	N/A	CON (for sample 2 at follow-up visit)
Plasma for PK (LNG and DPV)	1	BLD	EDT	6	ML	0.0, 1.0, 2.0, 4.0, 6.0	HRS	4	> 1.0	ML	PL1	N/A	
Vaginal PK Swabs (LNG and DPV)	2	VAG	NON	1	EA	0.0, 1.0, 2.0, 4.0, 6.0	HRS	1	Net weight	MG	SWB	N/A	LNG = Heavier swab DPV = Other Swab
			NON	1	EA	0.0, 1.0, 2.0, 4.0, 6.0	HRS	1	Net weight	MG	SWB	N/A	
Vaginal Smear for Gram Stain	1	VAG	NON	1	EA			2	1	EA	SLD	GRS	
Used Vaginal Ring for PK residual assessment	1	IVR	NON	1	EA			1	1	EA	IVR	N/A	

♦For visits with multiple PK time points

*List of Codes and their definitions:

BLD: Whole Blood

IVR: Used Intravaginal Ring

PL1/2: Single or double spun plasma

GRS: Gram stain slide

VAG: Vaginal Swab

EDT: EDTA

SLD: Slide

N/A: Not Applicable

NON: No Additive

SWB: Swab

9.4.2. Logging in VAG for PK Samples and Time Point for All PK Samples

Prior to logging vaginal swabs for PK in LDMS, calculate net weights (post-weight minus pre-weight) in order to designate the heavier swab for LNG testing. In Figure 9-1, the vaginal swabs for PK are entered in the primary area (see yellow rectangle A) as primary VAG sample with 2 tubes, and each tube has an aliquot of weight entered in the lower section for the derivative (see blue rectangle B). The collection time, using the 24-hour clock notation, is entered in the Specimen Time area (see red rectangle C). For this example, it is 15:55.

After the primary sample is added, two lines corresponding to the # of TUBES appear having the same SPECIMEN # (see green rectangles, E). A unique specimen # and global spec ID will appear after the aliquot information is entered. In this study, there will be multiple PK time-point visits, and in LDMS, in addition to time of collection, the TIME and TIME UNIT fields are used to note the specific time point on

the aliquot labels. During multiple PK time-point visits, the PK time point information is entered in Time and Time Unit area (see brown rectangle D). Figure 9-1 shows entry of the 1.0 HRS time point. Time points in this study will include 0.0, 1.0, 2.0, 4.0, and 6.0 HRS for enrollment visit and 0.0 and 6.0 HRS for day 14 visit.

In Figure 9-1, the aliquot information for the first tube has already been added, and the information in the orange rectangle F corresponds to the second vaginal sample selected (orange arrow F). For MTN-030, the OTHER SPEC ID for the aliquot is utilized. The heavier swab will be marked for LNG, and the other swab will be marked as DPV (see Figure 9-2, purple box).

Figure 9-1: LDMS Screen for VAG PK Entry.

Spec. Date: 31/Jan/2017 Exp. Date: Rec. Date: 31/Jan/2017 Recd Time: 16:40 Export ID: VAG Culture Derivative Enter Specimen ID

# of Tubes	Primary Type	Other Spec ID	Spec Time
1	VAG		15:55
2	VAG		15:55

Specimen #	Global Spec ID	Primary	Additive	Volume	Units	Spec Time	Time	Time Unit
1	414/1700438	VAG	NON	1.00	EA	15:55	1.00	Hours
2	414/1700438	VAG	NON	1.00	EA	15:55	1.00	Hours

of Aliquots: 1 Vol: 102 Units: MG Derivative: SwB Sub Add/Der: N/A Other Spec ID: DPV

Specimen	Global Spec ID	Primary	Add	Der	Sub Add/Der	Volume	Units	Cond	Other Spec Id
1	414/1700438	VAG	NON	SwB	N/A	123.40	MG	SAT	

Figure 9-2: LDMS Screen after vaginal swab for PK entered, global specs assigned

Spec. Date: 31/Jan/2017 Exp. Date: Rec. Date: 31/Jan/2017 Recd Time: 16:40 Export ID: VAG Culture Derivative Enter Specimen ID

# of Tubes	Primary Type	Other Spec ID	Spec Time
1	VAG		15:55
2	VAG		15:55

Specimen #	Global Spec ID	Primary	Additive	Volume	Units	Spec Time	Time	Time Unit
1	414/1700438	VAG	NON	1.00	EA	15:55	1.00	Hours
2	414/1700438	VAG	NON	1.00	EA	15:55	1.00	Hours

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
1	414/1700438	VAG	NON	SwB	N/A	123.40	MG	SAT	LNG
2	414/1700440	VAG	NON	SwB	N/A	102.00	MG	SAT	DPV

9.4.3. LDMS Entry for Vaginal Smear for Gram Stain

For Vaginal Smear for Gram Stain, the one swab that was used to inoculate the two slides is the primary sample. After the primary sample information is entered, then added, the two slides are entered as aliquots. An example is shown in figure 9-3. Note that after the 2 aliquots are added, a pop-up message will warn the user that the total aliquot volume exceeds the primary volume. Ignore the message and continue.

Figure 9-3 LDMS Entry for Vaginal Smear for Gram Stain

	Group	TYPE1	ID1	TYPE2	ID2	TYPE3	ID3	Visit	Unit
1	MTN	PID	335000159	PROTOCOL	024.0				5.00 Vol
2									
3									
4									
5									

Spec. Date:	26/Jun/2014	Exp. Date:	0	<input type="checkbox"/> Remove	VGA
Rec. Date:	26/Jun/2014	Recd Time:	15:30	<input type="checkbox"/> Imported	Culture Derivative
Export ID:		Import date:			

# of Tubes:	1	Primary Type:	VGL	Other Spec ID:		Spec. Time:	
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Specimen #	Global Spec ID	Primary	Additive	Volume	Units	Spec Time	Time	Time Unit	Cond
1	414/14006370	DC60756Y-00	VAG	NON	1.00	EA			SAT

# of Aliquots:	2	Vol:	1	Units:	EA	Derivative:	SLD	Sub Add/Der:	GRS	Other Spec ID:	
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Specimen #	Global Spec ID	Primary	Additive	Volume	Units	Spec Time	Time	Time Unit	Cond	Other Spec Id
1	414/14006371	DC60756Y-01	VAG	NON	SLD	GRS	1.00	EA	SAT	
2	414/14006371	DC60756Y-02	VAG	NON	SLD	GRS	1.00	EA	SAT	

9.5. Urine Testing for Pregnancy, Urinary Tract Infection, and Urinalysis

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.
- Instruct the participant to collect the portion of the urine flow that is required by the test
- If the urine is to be used for culture, instruct the participant to clean the labia prior to specimen collection and to collect a midstream urine sample.
- Instruct the participant to screw the lid tightly onto the cup after collection.

9.5.2. Pregnancy Testing

Pregnancy status is a critical participant safety consideration in MTN-030. The Quidel QuickVue One-Step hCG urine, Quidel QuickVue Combo hCG urine/serum pregnancy, or Fisher HealthCare Sure-Vue Urine hCG test must be used at all sites. All sites must maintain an adequate inventory of the pregnancy test kits at all times. Inventory 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). The date and time of pregnancy testing must be documented.

The pregnancy test is performed according to site SOPs and the package insert (i.e. a negative result is based on the recommended total time for test to be considered complete.) Do not perform any other urine pregnancy tests for confirmatory purposes. If the urine pregnancy test cannot adequately be interpreted because of interfering factors (e.g. 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.

In the rare event in which a participant becomes pregnant, study product use will be permanently discontinued. The participant will be terminated from the study.

9.5.3. Urinary Tract Infection

Urine Dipstick and/or Culture: Perform the tests according to the package insert for the dipstick and your local SOP for culture.

For initial diagnosis and treatment of a UTI, follow the local standard of care. Refer to sections 7.6.2 and 7.6.3 of the SSP for MTN guidance for UTI diagnosis and treatment. Refer to section 8.3 of the SSP for additional information.

9.6. Blood Specimens for Chemistry, Hematology, HIV testing, Syphilis, Plasma Archive, Dapivirine and Levonorgestrel Blood Levels

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.

9.6.1. Specimen Collection and Initial Processing

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

- Allow serum tubes (no additive or serum separator tubes) 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 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. EDTA tubes will be used for plasma dapivirine and levonorgestrel PK levels, plasma archive at enrollment, and if applicable, plasma for confirmation of viral load and HIV resistance testing.
- Light blue top tubes (additive = Na Citrate) are used for coagulation determinations. These tubes should be gently inverted at least 4 times after specimen collection to prevent clotting.

Note: If locally available tube top colors do not correspond with the tube additives specified above, use appropriate tubes based on the additives, not the listed tube top colors.

9.6.2. Chemistry (Alanine transaminase, Aspartate aminotransferase, Albumin and Creatinine), Hematology (CBC with Diff and Platelets), Sex hormone-binding globulin (SHBG), Progesterone, Estradiol

Testing will be performed per local standard of care.

- Tests performed for Chemistry
 - Albumin
 - Liver Function:
 - Alanine transaminase (ALT),
 - Aspartate aminotransferase (AST).
 - Renal Function:
 - Creatinine
- Hematology tests (Complete blood counts (CBC) with five-part differentials)
 - Hemoglobin,
 - Hematocrit,
 - Platelets,
 - White Blood Cell Count and differential
 - Red Blood Cell Count
- Sex hormone-binding globulin (SHBG)
- Progesterone
- Estradiol

9.6.3. HIV Testing

EDTA plasma, whole blood (fingerstick or venipuncture) and serum can be used to test for HIV using tests that have been validated at the study site. All HIV testing in laboratories must be done under Clinical Laboratory Improvement Amendment (CLIA) certification. All tests and associated QC procedures must be documented on local laboratory log sheets or other laboratory source documents.

HIV infection status will be assessed using an FDA-approved HIV immunoassay per the HIV testing algorithm (see section appendix 9-1). Rapid tests, such as Oraquick, are considered immunoassays and can be used with whole blood (fingerstick or venipuncture). The first specimen drawn for immunoassay and confirmatory testing is considered Sample 1. If Sample 1 is HIV positive by the confirmatory test, a second specimen (Sample 2) is drawn to confirm the first set of results.

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

9.6.3.1 HIV Test Result Interpretation

- If SAMPLE 1 immunoassay result is negative, the participant will be considered HIV-seronegative.
- If the SAMPLE 1 immunoassay result is positive or indeterminate, an FDA-approved confirmatory test should be performed on SAMPLE 1.
 - Go to 9.6.3.2 if SAMPLE 1 is Screening or Enrollment sample
 - Go to 9.6.3.3 if SAMPLE 1 is Follow-up Visit sample
- If there is insufficient sample to perform the confirmatory test, then additional blood must be drawn. This re-draw will still be regarded as Sample 1 per the protocol testing algorithm.

9.6.3.2 HIV Confirmatory Test for Screening or Enrollment Visit

- Until a participant is randomized/enrolled, treat enrollment testing as part of the screening process to determine participant eligibility.
- If the confirmatory test for SAMPLE 1 is negative, indeterminate or invalid, contact the MTN Virology Core: mtnvirology@mtnstopshiv.org for guidance.
 - It is not recommended for participants with discrepant HIV testing results to continue with the Enrollment Visit.
- If the confirmatory test is positive for the screening visit, the participant is considered seropositive and is not eligible for enrollment.

9.6.3.3 HIV Confirmatory Test for Follow-Up Visits

- If, at a follow-up visit, the confirmatory test result on SAMPLE 1 is negative, indeterminate or invalid, contact the MTN Virology Core for guidance:
 - 412-383-8138
 - mtnvirology@mtnstopshiv.org
- If the confirmatory test is positive at a follow-up visit, a second sample of blood (SAMPLE 2) will be drawn for additional confirmatory testing, HIV RNA resistance testing and plasma storage at the MTN Virology Core.
 - Draw enough whole blood to store a total of 5 mL of plasma to send to the virology core. The virology core can work with less but 5 mL is the desired amount to complete all testing.

NOTE: Draw extra blood with Sample 2, if required for local standard of care or at discretion of clinician. This blood is sent directly to a local lab following their procedures.

- Processing of SAMPLE 2 is similar to Plasma for Archive:
 - Log aliquots into LDMS using Other Spec ID = CON.
 - Centrifuge at least 1300xg and aliquot 1.5 mL plasma into 2-mL cryovials and freeze at <-70°C.
- Alert the MTN Virology Core, 412-383-8138, about shipment.
- Package and ship 3 aliquots immediately on dry ice to:

Dr. Urvi Parikh
University of Pittsburgh
3550 Terrace St.
Scaife Hall S804
Pittsburgh, PA 15261

- MTN Virology Core will provide test results to the site.
 - If positive, the participant is HIV positive.
 - If negative, indeterminate or invalid, the MTN Virology Core will supply guidance.

9.6.4. Syphilis Testing

Serum is the specimen of choice for treponemal assays (EIA, MHA-TP, TPHA, TPPA, or FTA-ABS) and the non-treponemal VDRL assay. RPR tests may be performed on either serum or plasma. All testing must be done with FDA approved assays and by a CLIA certified laboratory.

Syphilis testing for MTN 030 will be performed using the reverse sequence syphilis screening algorithm :

- At screening, syphilis assessment is done using a specific FDA approved treponemal test (such as EIA, MHA-TP, TPHA, TPPA, or FTA-ABS).
 - If negative, the participant is eligible for enrollment.
 - If positive, confirm with a non-treponemal assay (RPR or VDRL).
 - If the confirmatory non-treponemal assay is reactive at screening or enrollment visit, the participant is not eligible for the study.
 - If the confirmatory non-treponemal assay is negative, follow up with a second treponemal assay that has different antigens than the original treponemal assay. (See note below).
 - If the second assay is negative, the participant is considered eligible for the study.
 - If the second assay is positive, the participant is not eligible and appropriate clinical management should be taken. This scenario indicates that the participant has had prior exposure to syphilis and, depending on the clinical scenario, may or may not require treatment. If needed, consult with the Protocol Safety Physicians.
- For enrolled participants that are being tested “as indicated” at a follow up visit:
 - Follow the same testing algorithm as for screening but if any test is positive consult with the Protocol Safety Physicians for guidance on clinical care and product hold.

NOTE: 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).

Questions related to result interpretation concerning eligibility and enrollment in the study should be directed to the MTN-030 Protocol Safety Physicians (mtn030safetymd@mtnstopshiv.org).

9.6.5. Plasma Archive

For plasma archive, use collection tubes with EDTA anticoagulant. Aliquot plasma into 2-mL cryovials, store at $\leq -70^{\circ}\text{C}$, and batch onsite until the MTN LC study team requests shipping and/or testing.

- LDMS will be used to label and track the specimens.
- If sample is collected and held at room temp, freeze within 4 hours. If refrigerated or placed on ice after collection, freeze within 24 hours.
- Spin blood at room temperature in a centrifuge according to one of these techniques:
 - Single spun: Spin blood at 1300xg for 10 minutes, 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.
- Prepare as many 1.5-mL aliquots as possible, at least 3-mL total volume.
- If total volume is less than 0.5 mL, redraw as soon as possible.
- If less than 1 mL of plasma is available, store that plasma and inform the MTN LC for instruction.
- If samples are hemolyzed, store the aliquots as per normal and enter comments in LDMS.
- The MTN LC will send instructions to the site when shipping and/or testing is required.

9.6.6. Blood for PK of Dapivirine and Levonorgestrel

On single time-point days (visit days 1, 2, 3, 7, 15 and 16), the clinician will collect the vaginal PK swabs as close as possible to when the blood is drawn for PK (within 15 minutes). On multiple time-

point days (visit days ENR and 14), it is even more critical to collect PK samples as closely as possible to each other (collect vaginal swabs for PK within 15 minutes of blood collection time point). Sites should consider clinic flow when planning for PK draws to ensure sample collection occurs in close succession. See section 9.7.6 for details.

Collect blood into a labeled 10-mL EDTA Vacutainer tube using either an indwelling venous catheter or direct venipuncture.

1. Mix blood sample with the anticoagulant using gentle inversions (8 to 10 times).
2. Centrifuge the sample at approximately 1500xg for 10 minutes. The centrifugation must be completed and sample placed in the freezer within 8 hours of blood collection.
3. Use a pipette to aliquot at least 1.0 mL of the resulting plasma into two cryovials (one for DPV and the other for LNG); these will serve as the primary sample. Aliquot the remaining plasma equally into two cryovials; these will serve as a back-up in case the primary samples are accidentally destroyed during shipment.
4. Prepare four storage boxes and label one as “plasma DPV primary samples”, one “plasma LNG primary samples” and the other two boxes “plasma DPV back-up samples” and “plasma LNG back-up samples”. Transfer the tubes from each participant in chronological order into the storage boxes. All samples will be tracked in LDMS.
5. Store the boxes with samples at $\leq -70^{\circ}\text{C}$ until shipped.

SHIPPING:

- MTN LC will coordinate sample shipments throughout course of study if necessary and at its conclusion.
- All shipments will be on dry ice that will be sufficient for a 24-hour period and can be initiated Monday through Wednesday to insure that samples arrive in the lab during the work week.
- The back-up samples will be retained at the site until advised by the MTN LC or MTN-030 leadership team. One purpose of the extra aliquots is to be available in case the shipment is not received in the proper condition (e.g. thawing of samples).

9.7. Vaginal Specimens for Herpes Lesion Testing, Gram Stain, Vaginal Fluid pH, Vaginal Wet Mount, GC/CT NAAT, Trichomonas, Vaginal Fluid for PK, and IVR for Remnant Drug Content Analysis

Refer to the Pelvic Exam checklist located on the MTN-030 Study Implementation Materials webpage for further information on the required sequence of specimen collection and diagnostic procedures to be performed during study pelvic exams.

9.7.1. Herpes Lesion Testing

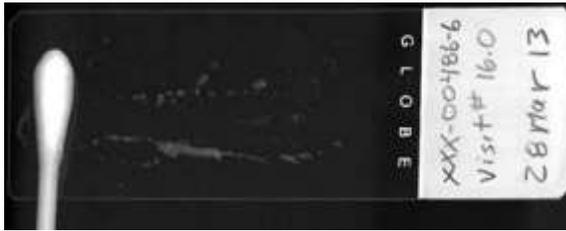
Testing will be performed per the local standard of care.

9.7.2. Gram Stains of 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 at each required time point and both 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 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 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 swab (Dacron or cotton), roll the swab across each of the slide. (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., Sharpie 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 positioned in a plastic slide holder and sent to the MTN LC. If there is no culture on the visit for which a gram stain is collected, then hold the gram stain slides until other samples are to be sent to the Magee-Womens Research Institute. (See shipping instructions below).
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).

9.7.3. Vaginal pH and Wet Preps, if indicated for Bacterial Vaginosis (BV) and/or Yeast

BV will be diagnosed based on the presence of any three of the four Amsel's criteria:

- Homogenous vaginal discharge
- Vaginal pH greater than 4.5
- Positive whiff test
- At least 20% clue cells.

Wet prep assessments used to diagnose BV and candidiasis are discussed in section 9.7.3.2 and summarized in Table 9-5.

CLIA regulations require semi-annual **wet mount proficiency testing**. The MTN LC administers a web-based proficiency test approximately every six months. Wet mount slides on the MTN web pages are posted for this purpose every 6 months.

- Contact Lorna Rabe (lrabe@mwri.magee.edu) and May Beamer (mbeamer@mwri.magee.edu) of the MTN LC to register names of clinicians who need to take the test.
- The registrants take the test and enter their answers directly on the website.
- The MTN LC sends a report of the results, including any necessary corrective action, to the Laboratory Manager.

Contact the MTN LC for additional information and guidance on performing and documenting the proficiency testing. Also contact 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.

9.7.3.1 Vaginal Fluid pH, if indicated for BV

Vaginal fluid pH will be assessed if clinically indicated 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 (Dacron or cotton) may be collected in one of 2 ways depending on if a speculum is used at that particular visit:

- Obtained by the clinician during the pelvic examination
 - 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 appropriate case report form (CRF). It is not necessary to record pH values onto laboratory log sheets or other source documents prior to recording values onto CRFs.

9.7.3.2 Vaginal Fluid Wet Mount Testing, if indicated for BV and Yeast (KOH)

Table 9-5 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 not observed in saline prep may be observed in 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)

Wet mount procedures for this study are only performed if indicated, and consists of two different preparations: Potassium Hydroxide (KOH) and Saline. These procedures are for diagnosis of BV and candidiasis as summarized in Table 9-5.

Preparation and Examination of Wet Prep Slides

Materials:

- Pencil
- 2 SCHARP labels, 3 if using optional tube
- 2 frosted end slides
- Glass or plastic tube, optional
- Sterile physiologic saline
- 10% KOH
- Dacron Swab
- 2 cover slips
- Microscope, 10x and 40X magnification

1. 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)
2. Immediately following collection from the lateral vaginal wall via swab (Dacron or cotton), smear vaginal fluid specimens 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.
3. Apply one drop of 10% KOH to one slide and immediately perform whiff test for a “fishy” amine odor. Then apply cover slip.
4. Apply one drop of sterile physiologic saline to the second slide, emulsify with the vaginal fluid specimen, and then apply cover-slip. Examine immediately at 10X magnification for epithelial cells, budding yeast, and pseudohyphae. Examine at 40X 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.
5. Examine the KOH slide at both 10X and 40X magnification for yeast and pseudohyphae.

RESULTS:

- If wet prep slides are read in-clinic by clinical staff, results may be recorded directly on to appropriate case report forms (CRFs).
- 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 appropriate CRF.

9.7.4. Testing for GC/CT (*Neisseria gonorrhoeae* and *Chlamydia trachomatis*) and *Trichomonas vaginalis* by NAAT

Testing for chlamydia, gonorrhea and *Trichomonas* is performed at screening and when clinically indicated. Sites can choose to use the Cepheid GeneXpert, or Gen-Probe Aptima. If the site does not have access to these tests, they can send the samples to the MTN LC for testing. Contact the MTN LC prior to sending specimens for GC/CT testing.

- If using GenProbe Aptima, both GC/CT and *Trichomonas* tests can be performed from one swab. Use only one collection kit.
- If using Cepheid GeneXpert you must use two collection kits, one for GC/CT and the other for *Trichomonas*.
- Use the manufacturer’s vaginal collection swab and transport tube
- Affix a SCHARP-provided PTID label onto the transport tube.
- Swab the lateral wall of the vagina.
- Immediately place the swab in the transport tube, break off the shaft of the swab, and cap the tube.
- Transport the specimen at ambient temperature to the local laboratory

9.7.5. Vaginal Swabs for PK of Dapivirine and Levonorgestrel

PK collection times need to be recorded on the LDMS Specimen Tracking Sheet. In addition to sample collection, this section discusses acceptable 'windows' on collection time points and action to be taken if collection falls outside these windows.

Collection Timing and Target Times for Vaginal Swabs and Blood for PK

Visits with multiple PK collection time-points:

- When to start the timer
 - At Enrollment,
 - 0 HR is collected before ring is inserted.
 - The 1, 2, 4, and 6 HR collection times are determined by starting a timer upon ring insertion.
 - On Day 14,
 - 0 HR is collected before ring removal.
 - The 6 HR time point is determined by starting a timer at time of ring removal.
 - If the ring is removed by the participant prior to the clinic visit, collect only one time point of blood and swabs for PK.
- When each time-point is due:
 - Blood will be drawn first.
 - Ideally, the clinician will collect the PK swab within 15 minutes of the blood draw. The 15 minute guideline is not applicable for the 0 HR timepoint at enrollment (to accommodate logistics of sample collection prior to ring insertion).
- Make sure that specimen times are accurate, in case there are delays in sample collection. Correct recording will allow the interval of time to be correctly gauged.
- Missed or delayed blood draw time point:
 - There will be no bearing on the next time point.
 - Example: Although the 1 HR time point draw was 15 minutes late (drawn at 75 minutes), the 2 HR PK blood would still be drawn at the 2 hour (120 minutes) mark.
 - If a collection is missed entirely, notify the MTN-030 management team.

Follow-up Visits with single PK collection time point

The blood is drawn, and then the clinician should collect the swab as close as possible to the blood draw (must be within 15 minutes).

In the case that the ring is removed and/or reinserted prior to a visit:

- Vaginal swab for PK should still be collected even if the ring has been out of the vagina for up to 7 days.
- This ring removal / re-insertion should be noted on LDMS tracking sheet, as well as the Ring Adherence CRF (for participant ring removals that occur between study visits) and/or the Ring Insertion and Removal CRF (for ring removals and insertions that occur at study visits).

Procedure for Vaginal Fluid Sampling for PK assessment and weighing swab

1. Each day of collection of vaginal swabs for PK, perform QC that is required for the analytical scale to accurately weigh samples to a weight of at least 0.1 milligrams. Do not turn off balance until weighing for the day is completed.
2. Materials
 - a. For each swab, need TWO sets at each time point (Suggestion: Prior to weighing, use Scharpie to label ziplock bag, swab packaging, and tube with a "1" or "2" to keep track of items in each set):
 - Two SCHARP labels with PTID, visit number, visit date, time point, and swab order #.
 - One 2-mL Nalgene cryovial
 - One Pre-packaged Polyester-Tipped (Dacron) Swab (Puritan brand)

- One Ziplock biohazard sample bag
 - b. Urine cup (without lid) or similar lightweight container, placed on middle of scale, to contain items to be weighed. (Some balances have an optional basket.)
 - c. A rack that will hold the cryovial
 - d. For clinical staff, scissors to cut swab shaft
 - e. Calculator
3. Handle items to be weighed with gloves.
 4. Place identically-labeled SCHARP label on the cryovial and the biohazard sample ziplock bag.
 5. Perform pre-weight.
 - a. Zero the urine cup or similar container
 - b. Place the labeled 2-mL cryovial in the urine cup.
 - c. Place the packaged sterile Dacron swab upright in the urine cup. (Make sure it is not leaning on a part of the scale.)
 - d. Record this pre-weight on the LDMS Tracking Sheet.
 - e. Place the cryovial and the packaged Dacron swab in the biohazard sample ziplock bag with the matching label to the tube.
 - f. If multiple time points or multiple participants on that day, pre-weights for all time points may be obtained with careful observation of time-point labels.
 6. Make sure you have the correct participant time-point.
 - a. In the exam room none of the items in the bag should be thrown into the garbage – only into the ziplock bag.
 - b. Prep for the clinician:
 - i. Have the rack ready.
 - ii. Unscrew the lid of the 2-mL cryovial and place the tube in the rack, the lid in the ziplock bag.
 - iii. Start the peel of the packaging of the swab. (Sometimes not a sufficient separation)
 - iv. Partially peel the packaging
 - v. The clinician will use the Dacron swab to collect vaginal fluid (slow count to 10).
 - vi. Place the swab in the tube and the swab packaging into the ziplock bag.
 - c. Cutting or bending to break the swab shaft. (!!!Potential to lose swab shaft!!!)
 - i. For the lid to close properly, raise the swab from the bottom of the tube, then cut or bend.
 - ii. If cutting the shaft, a suggestion, for leverage, is to not use tip of blades to cut, but make sure shaft of swab is at the pivot point of the scissors, then cut.
 - iii. If clinical staff will perform a repeated bend to break the shaft with dominant hand, while doing so, it may be easiest to hold the top of the tube with the forefinger and thumb of the other hand.
 - d. Place the cut shaft in the ziplock bag (Suggestion: place the cut shaft in the packaging).
 - e. Screw the lid on the cryovial and place sample in the bag with the swab packaging and the swab shaft.
 7. Perform Post Weight:
 - a. Zero the urine cup or similar lightweight container.
 - b. Weigh the capped cryovial containing the absorbed swab tip, the swab packaging and the remainder of the swab shaft (Suggestion: Place the swab shaft into the packaging and have it upright during weighing.)
 - c. Make sure that the post-weight is larger than the pre-weight.
 - d. Record post-weight on the LDMS Tracking sheet.
 8. Within 2 hours, place the sample tubes in the freezer at $\leq -70^{\circ}\text{C}$.

Shipping of PK swab samples

- LC will coordinate sample shipments throughout course of study if necessary and at its conclusion.
- The back-up samples will be retained at the site until advised by the LC or MTN-030 leadership team.
- All shipments will be on dry ice that will be sufficient for a 24 hour period and can be initiated Monday through Wednesday to insure that samples arrive in the lab during the work week.

9.7.6. Testing of Intravaginal Ring (IVR) for Remnant Content Analysis

Used rings will be analyzed for residual levels of Dapivirine and Levonorgestrel, and will be collected at day 14 or early termination visit. The used rings may contain vaginal secretions, and therefore should be treated as a biohazard. The rings will remain in the amber pouch and stored at -20°C until further notice from the MTN LC. Rings that are defective and have been inserted briefly should be photographed and then destroyed at the site via biohazard procedures. Rings that are defective but not inserted should be given to the pharmacist.

Important notes:

- On Day 14, Blood and Vaginal swabs for PK should first be collected immediately before ring removal.
- If the ring is removed by the participant prior to the clinic visit and will not be reinserted, instruct the participant to blot it dry with tissue and place it in a ziplock container and store at room temperature. At the clinic, follow the directions below:

Materials:

- 3"X5" amber Zippit pouch with affixed biohazard label
- SCHARP label for amber pouch

Preparation of used ring for storage on-site:

1. Site staff will place the ring into a 3"X5" amber Zippit pouch (see figure 9-4) that was provided by LC to store the rings.
2. Label the pouch with the participant ID number and visit number.
3. Add a biohazard sticker if one is not already attached to the pouch, making sure not to cover the identifier information.
4. Store the used ring within the biohazard labeled amber pouch at -20°C.
5. The use of LDMS is required to log in all used rings.
6. At the end of the study, LC will contact site to coordinate shipment.

Figure 9-4: 3"×5" amber Zippit pouch



9.8. Cervical Specimens: Pap Test

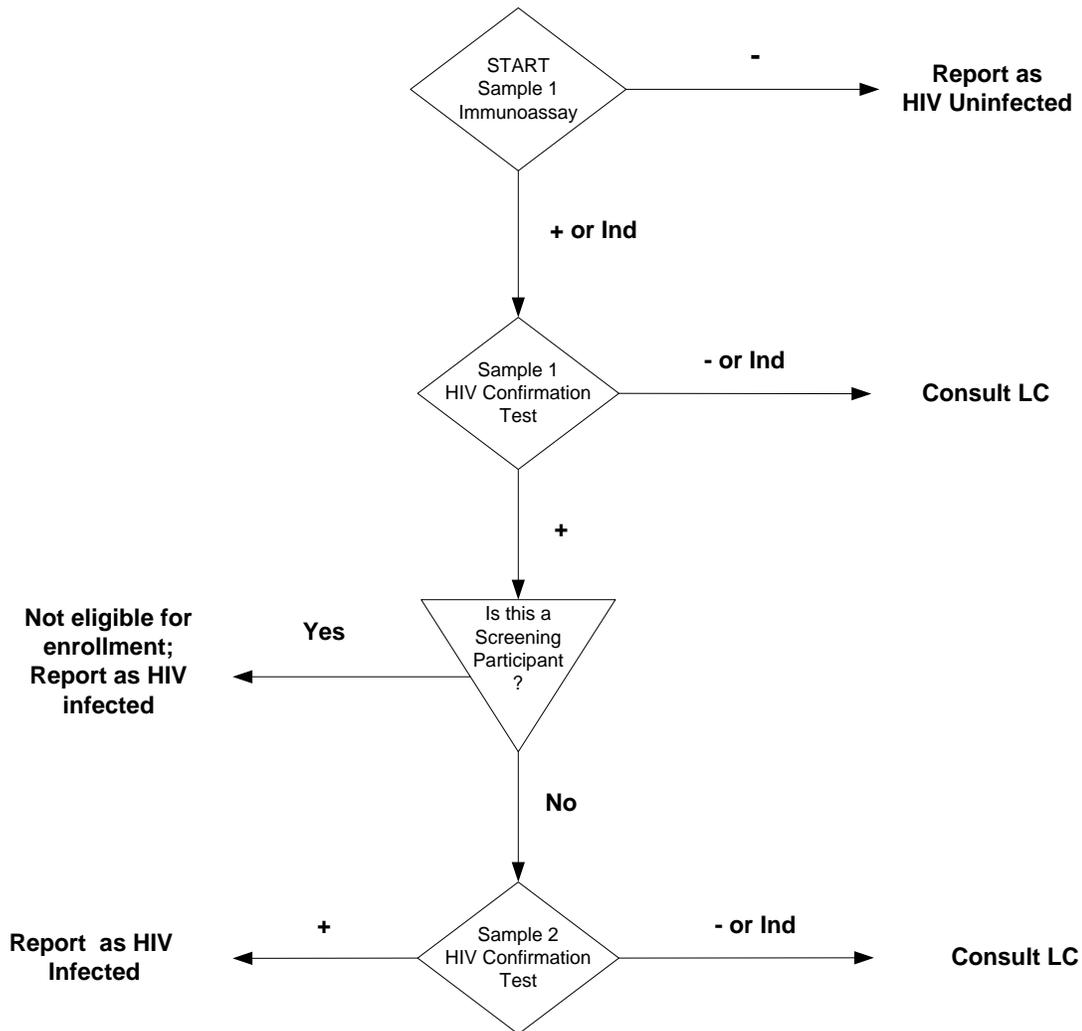
Pap smears are only required if clinically indicated or if a participant is over the age of 21 and has not had a documented normal test within 3 years prior to Enrollment.

9.8.1. Papanicolaou (Pap) Test (*only if indicated)

If a Pap is required, ecto- and endocervical cells will be collected after all tissues have been visually inspected, and all other required specimens have been collected. The testing will be done at the site's

local laboratory. Specimen collection, testing and QC procedures must be performed and documented in accordance with study site SOPs.

Appendix 9-1: HIV ANTIBODY TESTING ALGORITHM



Ind: Indeterminate test results
LC: Laboratory Center