MATRIX-001 Study-Specific Procedures (SSP) Manual
Section 9 – Laboratory Considerations

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Version 1.0; 18OCT2023 by Edward Livant; reviewed by May Beamer, Terry Jacot, Natasha Samsunder

Revision date	Version #	Reason	Edit
19 Dec 2023	2.0	For ectocervical biopsy specimen for PD HIV (only affects EVMS), remove weighing procedure which is not necessary for data analysis	Terry Jacot (EVMS)
		Update PSA testing, clarification of specimen storage and time before testing	May Beamer (CTH-LC)
		Formatting updates, resulting in Tables 9-6 and 9-7 and Figure 2.	
		Update cryovial description to ”recommended, but not required” for: plasma PK, plasma archive, CVL aliquots, PD-HIV vaginal swabs	
		Updated EQA submision requirement from quarterly to ”as requested”	
		Format tables for consistency	
		Update visits for CVL in Table 9-8 (formerly 9-6)	
		Added Revision Table	
		Minor edits	

9.1 Introduction

This section provides information and instructions for site clinical and laboratory staff related to the processing, storing, shipping and testing of MATRIX-001 laboratory specimens.

"Local Laboratory" in this document refers to laboratory work done at Clinical Research Sites (CRS), at a CRS controlled laboratory or a contracted laboratory located near the CRS that will do initial specimen processing and testing.

The MATRIX Clinical Trials Hub Laboratory Center (CTH-LC) will provide guidance for the laboratory considerations.

Certain specimens will be shipped from Local Laboratories to External Laboratories for additional research testing.

9.2 Overview and General Guidance

9.2.1 Laboratory Readiness Approval

Prior to study activation, MATRIX CRS will be required to complete a Laboratory Requirements Documentation Checklist and submit requested documents. Requirements will vary between CRS laboratory activities operating under United States CLIA certification and international sites. The topics covered by the checklist may include:

- Standard Operating Procedures
- External Assurance
- Method Validation
- Normal Ranges
- Specimen Management
- Material Transfer Agreement Initiation
- Laboratory Supplies
- Staff Training

9.2.2 External Quality assurance

These requirements waived for Laboratory testing done under CLIA.

CRS will be required to submit EQA results for assays performed at Local Labs prior to activation and as requested while the study is active.

9.2.3 Method Validation

These requirements waived for Laboratory testing done under CLIA.

CRS will be required to verify signed validation reports for assays performed at Local Labs prior to activation. The CTH-LC may request to review any validation reports.

9.2.4 Standard Operating Procedures

These requirements waived for Laboratory testing done under CLIA.

CRS will be required to verify signed Standard Operating Procedures (SOP) are current (review within past 2 years) for assays and laboratory processes performed at Local Labs prior to activation. The CTH-LC may request to review any SOP.

9.3 Specimen Labeling

Sites should have processes in place to avoid specimen labeling errors. Participant identification (PTID) must be verified each time a specimen is collected.

All containers into which specimens are initially collected (e.g., urine collection cups, blood collection tubes) will be labeled with the following, at a minimum: PTID and collection date. Laboratory Data Management System (LDMS) labeling is required for sample aliquots as described in SSP Section 9.5.

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 (Table 9-1), any additional labeling required for on-site specimen management and chain of custody will be performed in accordance with site SOPs. Refer to Table 9-2 for tests that will be entered into LDMS and labeled with LDMS-generated labels. Note: Do not remove initial label prior to placing the LDMS label on the tube.

Table 9-1. Overview of Local Laboratory Testing Specimens for MATRIX-001

Test	Specimen Type
Pregnancy	Urine
Dipstick	Urine
Microscopy and Culture	Urine
HIV Rapid Tests	Blood
HIV Confirmatory test	Blood
HIV RNA	Blood
HBsAG	Blood
AST,ALT,CREAT	Blood
CBC	Blood
Syphilis Serology	Blood
HSV-2 (may be shipped)	Blood
NAAT for GC/CT/TV	Cervicovaginal Fluid
PSA	Cervicovaginal Fluid
Vaginal Gram stain Nugent scoring	Cervicovaginal Fluid
Vaginal pH	Cervicovaginal Fluid
Vaginal Wet Mount	Cervicovaginal Fluid
Pap Smear	Cervical Cells

9.4 Procedures for Specimens that cannot be Evaluated

Specimen collection may be repeated (whenever possible) if it is found that specimens 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. These may include issues such as expired lab commodities that were used in error,

broken tubes in centrifuges, and any situation where specimen integrity has been compromised for a particular assay or storage requirement.

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 is required.

9.5 Use of LDMS

LDMS is a program used for the storage and shipping of laboratory specimens and supported by the Frontier Science Foundation (FSTRF). Detailed instructions for use of LDMS are provided at: https://www.ldms.org/. LDMS must be used by all CRS to track the collection, storage, and shipment of the sample types described in Table 9-2.

It is crucial to be aware of proper label formats to ensure that specimens are correctly labeled. Samples must be separated by sample type when storing. Use Matrix Label format.

LDMS Help:
Questions related to use of LDMS in MATRIX-002 may be directed to CTH-LC or LDMS Technical (User) Support. LDMS User Support is available 24 hours a day, 7 days a week. Contact LDMS User Support at:

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

LDMS storage quality control:
Local laboratories will have internal QA and quality control (QC) processes to ensure accurate LDMS entry. LDMS data are used by the Clinical Trials Hub Data Management and Statistical Support team to generate a specimen repository reports and to reconcile data entered in LDMS with data entered on study case report forms (CRFs) and any other discrepancies noted.

Table 9-2. LDMS Specimen Management Guide to Logging in MATRIX-001 Specimens

Sample	Designated Test	Primary	Additive	Primary Volume	# Aliquots	Aliquot Units	Derivative	Sub Add/ Deriv	Other Spec ID*
Cervicovaginal Fluid	Microbiota	VAG	ZRD	1 EA	1	EA	SWB	N/A	VF-MB
Cervicovaginal Fluid	PK	CVF	NON	2 EA	2	EA	SWB	N/A	VF-PK1;VF-PK2
Cervicovaginal Fluid	Anti-HSV2	CVF	NON	2 EA	1	EA	SWB	N/A	PD-HSV
Cervicovaginal Fluid	Anti HIV	CVF	NON	1 EA	1	EA	SWB	N/A	PD-HIV
Cervicovaginal Fluid	PSA	VAG	NON	1 EA	1	EA	SWB	N/A	Not applicable
Cervicovaginal Fluid	Gram Stain Nugent Score	VAG	NON	1 EA	2	EA	SLD	GRS	Not applicable
Cervicovaginal Lavage	Secreted Soluble Markers	CVL	NSL	5 mL	4	mL	CVL	N/A	CVL-SM1, CVL-SM2, CVL-SM3; CVL-BU
Cervicovaginal Tissue	PK	VGL	NON	2 EA	2	mG	BPS	N/A	BXV-PK1; BXV-PK2
Cervicovaginal Tissue	IHC	VGL	FOR	1 EA	1	EA	BPS	N/A	BXV-IHC
Blood	Plasma PK	BLD	EDT	~4 mL	≥3**	mL	PL1 or PL2	N/A	PLS-PK1; PLS-PK2; PLS-BU
Blood	Plasma Archive	BLD	EDT	~ 5 mL	≥2**	mL	PL1 or PL2	N/A	PLS-ARC
Blood	Herpes Simplex Virus 2	BLD	NON	~6 mL	≥3**	mL	SER	N/A	Not applicable
Rectal Fluid	PK	REC	NON	2 EA	2	mG	SWB	N/A	RF-PK1; RF-PK2

***Note:** Additional specimen ID codes defined later in this document, see individual sections

****Note**: Refers to number of aliquots, see sections 9.7.7. 9.7.8, 9.7.9 for guidance on aliquot volume

Legend:

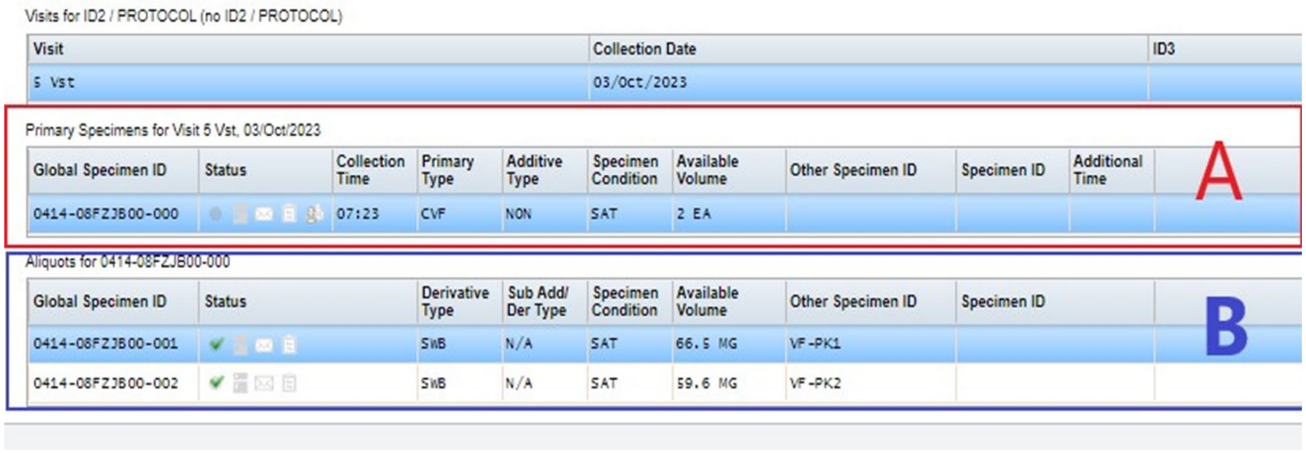
Code	Definition	Code	Definition	Code	Definition
VAG	Vaginal Swab	EA	Each	mL	milliliter

VGL	Vaginal Sample	NON	None	mG	milligram
IHC	Immunohistocompatibility	EDT	EDTA	BPS	Biopsy
PK	Pharmacokinetics	CVL	Cervicovaginal Lavage	SWB	Swab
REC	Rectal sample	ZRD	Zymo DNA Shield	PL1/PL2	Plasma, single or double spun
CVF	Cervicovaginal Fluid	NSL	Normal Saline	SER	Serum
BLD	Blood	FOR	Formalin	N/A	Not applicable

9.5.1 Documentation of specimen weights

All sites are required to maintain source documents with pre, post and net weights for CVF Swabs for PK, Vaginal biopsies for PK, and rectal swabs. These must be filed with study records and available for review as requested. Enter Net weights into LDMS as described in Figure 9-1.

Figure 9-1. LDMS Entry Screen



- In the primary sample area (image box A), use table 9-1 to enter correct code for the sample. Make sure to place the correct collection time under Spec Time field. Click the 'add' button to the right. This will add the sample to field. Under Units, enter EA (for each) and enter '2' for Volume (See Figure 9-1)
- To enter the actual weights, select the primary sample, and select add new aliquot(s) (image box B). For Volume, enter the net-weight and select 'MG' MG (milligrams) for UNITS. Enter the correct derivative and Sub-Add/Der codes, then click the add button (See Figure 9-1).
- In the example in figure 9-2:
    - 2 vaginal swabs are collected for PK. They are entered in LDMS as 1 primary sample with 2 aliquots.
    - Net weight of Swab 1 is 66.5 mg. Enter '66.5' under VOLUME and select 'MG' for Units, 'SWB' for Derivative, and 'N/A' for Sub-Add/Der, then press add.
    - Net weight of Swab 2 is 59.6mg. Enter 59.6' under VOLUME and select 'MG' for Units, 'SWB' for Derivative, and 'N/A' for Sub-Add/Der, then press add
- If net weight results in a negative value, investigate that the pre- and post-collection weights were not switched. Enter weights in the comment and storage comment for the aliquot(s). Please complete a protocol deviation form and include corrective action.

9.6 Urine Testing

9.6.1 Specimen Collection

Collect specimens for urine per package insert for test methods and/or local SOP.

9.6.2 Urine hCG

Perform rapid urine hCG per package insert for test methods and/or local SOP.

9.6.3 Urine Dipstick

Perform urine dipstick per package insert for test methods and/or local SOP. Protein, Blood and Glucose are reported for MATRIX-001.

9.6.4 Microscopy and Culture

Perform urine Microscopy and culture per local standard of care and package insert for test methods and/or local SOP.

9.7 Blood Testing

9.7.1 Specimen Collection and Initial Processing

Sites should have processes in place to avoid specimen labeling errors. Participant Identification must be verified each time a specimen is collected.

This manual listed minimum volumes in some places for serum and plasma storage; related recommended primary blood volumes are listed also. The amount of whole blood drawn to achieve minimum plasma and serum storage is determined by local research sites and labs. It is required for sites to verify minimum plasma and serum storage is occurring and to take appropriate corrective action when minimums are not met.

Label all required tubes at the time of collection as described in section 9.3. After collection complete the following:

- Allow plain tubes (red, tiger top or gold top SST non-additive tubes or serum separator tubes) to clot, then centrifuge per site SOPs to yield serum. Serum may be used for tests such as chemistry or syphilis serology as defined in local testing SOP.
- Gently invert EDTA at least eight times after specimen collection to prevent clotting. If whole blood and plasma are to be taken from the same tube, the whole blood testing must be completed before the tube is centrifuged and plasma aliquots are made. If whole blood is to be used for multiple tests, ensure that the tube is well mixed before removing any specimen.

9.7.2 HIV Testing

HIV status will be determined via Appendix II: Algorithm for HIV testing-Screening/Enrollment/Follow-up in the current version of Matrix-001.

HIV rapid tests need to be FDA approved or WHO prequalified in MATRIX Studies. Contact the CTH-LC as needed for guidance.

Testing will begin with 2 HIV rapid tests performed on a blood sample. *Note: 1 rapid test may be used if done under CLIA certification; Oral fluids may be used if done under CLIA certification.* If all results are negative, report as negative and testing is completed.

If the result is dual positive (+/+) discordant (+/-) or Indeterminate, the next steps depend on if the testing is for screening/enrollment or follow up:

Screening/Enrollment
The participant is ineligible for the study. Continue testing to ensure correct diagnosis; contact the CTH-LC as needed for assistance. If determined to be HIV infected, refer the patient for local counseling and treatment.

Follow up
Continue to a confirmatory assay. If that result is positive, report as positive. If the result is negative or indeterminate, contact the protocol team for guidance.

HIV-1 RNA may be requested in cases of ambiguous diagnosis. HIV-1 RNA is not approved for stand-alone diagnosis but may be used as part of testing process.

The confirmatory assay and HIV-1 RNA assay will be approved by the CTH-LC.

9.7.3 Hematology Testing

Complete blood counts with five-part differentials will be performed per local SOP. Each of the following must be analyzed and reported:

- Hemoglobin
- Hematocrit
- Mean Corpuscular Volume
- Platelets
- White blood cell count

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

9.7.4 Liver and Renal Function Testing

The following chemistry tests will be performed on serum per local SOPs:

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

9.7.5 Syphilis Testing

Syphilis testing will be performed on plasma or serum per local SOP using local screening and confirmatory algorithms.

9.7.6 Hepatitis B Surface Antigen

Hepatitis B Surface Antigen testing will be performed on plasma or serum per local SOP.

9.7.7 Herpes Simplex-2 Virus

Serum will be stored for batch HSV-2 testing at the end of the study. Store minimum **3 mL** of serum in 1 mL aliquots at ≤ -70°C until shipment or testing is requested. The laboratory performing the testing for the CRS will be determined at a later date.

Allow blood samples to clot at room temperature prior to centrifugation. Aliquot 1-mL aliquots of serum into 2-mL cryovials, store at ≤ -70˚C, and batch onsite until the CTH-LC study team requests shipping and/or local testing.

- LDMS will be used to label and track the specimens.
- If sample is collected and held at room temp, process and freeze within 4 hours.
- Centrifuge blood at 1300-1500×g for 10 minutes, aliquot serum.
- Prepare three cryovials of 1.0-mL aliquots, distribute the remainder or create additional cryovial aliquots.
- If samples are hemolyzed, enter comments in LDMS.

9.7.8 Blood for plasma PK

Collect whole blood in EDTA tubes. The minimum plasma to be stored is **1.5 mL**.

If the blood is held at room temperature, plasma must be processed and frozen within 3 hours of collection. If the blood is kept refrigerated or placed on ice, plasma must be processed and frozen within 24 hours of collection. Plasma should be stored frozen on site ≤ -70°C until requested for shipping and/or testing by the Study team.

<u>Blood Draw for TAF, TFV, & EVG Concentrations</u>

Supplies:

- *Thr*ee 2-mL cryovials (internally-threaded recommended, but not required)
- 4ml Vacutainer EDTA (purple-top tube)
- Refrigerated centrifuge
- Adjustable pipette and tips or graduated transfer pipette

Plasma PK Labeling

Endpoint	Sample code/Other Specimen Code
TAF/TFV	**PLS-PK1**
EVG	**PLS-PK2**
Backup	**PLS-BU**

Specimens should be collected and processed as follows:

1. Pre-label the three cryovials as indicated in Section 9.2 with Sample Code as shown above and set aside.
2. Invert the blood tube several times and store at room temperature. *Although samples can be processed within 3 hours of draw time, it is highly recommended that samples are processed as soon as possible following collection.*

3. Centrifuge the blood tube at 1200 x *g* for 10 minutes at 4°C.
4. Transfer minimum 0.5 mL of plasma into the PLS-PK1 and PLS-PK2 tubes first, then aliquot 0.5-mL in the PLS-BU tube. The remainder, if any, will be distributed between the three tubes.
5. Screw the caps tightly and freeze at ≤ -70°C.
6. Storage: Store PLS-PK1 and PLS-PK2 next to each other in series of cryovial storage box PLASMA PK Primary BOX 1. The "PLS-BU" cryovial will be stored at the site as PLASMA PK BACKUP BOX 1 and will be shipped when requested by CTH-LC.
7. Document relevant information in LDMS, i.e., hemolyzed or < 0.5-mL
8. The specimens will be stored at the site until requested for shipment.

9.7.9 Plasma Archive

Collect whole blood in EDTA tubes. The minimum plasma to be stored is **3 mL**.

Supplies:

- 2-mL cryovials (internally-threaded recommended but not required)
- 4-6 ml Vacutainer EDTA (purple-top tube)
- Centrifuge with swinging buckets
- Adjustable pipette and tips or graduated transfer pipette

Plasma Archive Labeling

Endpoint	Sample code/Other Specimen Code
Plasma Archive	**PLS-ARC**

1. Centrifuge blood at 1200×g for 10 minutes, remove plasma.
2. Prepare at least two cryovials of 1.5-mL aliquots. Prepare additional aliquots for remaining plasma.
3. Store ≤-70°C in Plasma Archive storage box

NOTES:

- If sample is collected and held at room temp, process and freeze plasma within 3 hours. If refrigerated or placed on ice after collection, process and freeze plasma within 24 hours.
- 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 CTH LC for instruction.
- If samples are hemolyzed, store the aliquots as per normal and enter comments in LDMS.
- The CTH-LC will send instructions to the site when shipping and/or testing is required.

9.8 Cervicovaginal Specimens

9.8.1 Specimen Collection

Multiple samples may need to be collected at a single visit. In this scenario, samples should be collected in the order listed in table 9-3.

Table 9-3: Pelvic and Rectal Specimen Collection Order

Order of collection	Test (# Specimens)
1	GC/CT/TV NAAT (1)
2	Wet Mount (1), if symptomatic
3	CVF for PK (2)
4	Vaginal pH/Gram stain (1)
5	CVF for PD Anti-HIV (1)
6	CVF for PD Anti- HSV2 (2)
7	PSA (1)
8	Vaginal swab for microbiota (1)
9	PAP
10	CVL (5-mL)
11	BXV for IHC (1)
12	BXV for PK(2)
13	BXC for HIV (EVMS Only)
14*	Rectal swabs (2)

Notes:

- Note that Gram stain and pH can be collected from the same swab when possible.
- *Rectal samples can be taken first before pelvic sample collections or last after all pelvic sample collections are completed. Rectal samples should not be collected in between different pelvic sample collections.

9.8.2 Vaginal pH

pH Indicator Strips (pH range 3.6 to 6.1) from Machery-Nagel (92130), Baker (4394-01), or SP/Cardinal Health (P1119-22) must be used unless other strips are approved by the CTH-LC.

- Vaginal fluids are collected from the lateral vaginal wall via swab and then swabbed onto the pH strip.
  - Avoid contact with cervical mucus, which has a higher pH.
  - Do not touch pH paper directly to the study participant.
- Match the resulting color of the indicator strip to the color scale provided with the strips to determine the pH value.

9.8.3 Cervicovaginal Fluid for Gram Stain

Dried vaginal fluid smears will be Gram stained and assessed for bacterial vaginosis (BV). Two slides (one designated as primary and the other as secondary) will be prepared using one swab. Both slides will be entered into LDMS. The primary slide will be evaluated locally, and the secondary will be stored as a backup.

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 each 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, same side as frosted end. Do not place the swab in saline, transport medium, or any transport container prior to slide preparation.

3. Allow the specimens to air-dry. Do not heat-fix.

4. Place the specimen 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 until evaluated. Batch testing may be done at end of study.

7. Store the secondary slide in assigned location in the Gram stain backup slide box. Slides are stored at room temperature. The secondary slide will be shipped to CTH-LC at end of study.

NOTE: When Gram staining, check if the staining method will interfere with label. If so, use transparent tape to tape over the label to prevent information from disappearing due to ethanol and acetone.in Gram stains.

9.8.4 Vaginal Wet Mount

Vaginal wet mount is not a required protocol assay for MATRIX-001 but may be performed as part of mucosal assessment for enrollment or due to clinical indications. MATRIX-001 sites will participate in the MATRIX Online Wet Mount EQA program unless other EQA is approved.

Table 9-4. Summary of Wet Prep Assessments and Diagnostic

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 (*Gardnerella vaginalis* and/or anaerobic GNR) to be counted as clue cells.	Not applicable (clue cells are lysed by KOH)

Wet mount procedures for this study consists of two different preparations:

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

These procedures are for diagnosis of BV and candidiasis as summarized in Table 9-4.

Note: BV 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 the frosted end of two microscope slides.
- *Using the same swab as for 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.

9.8.5 Cervicovaginal Testing for *Neisseria gonorrheae* (GC)/*Chlamydia trachomatis* (CT) and *Trichomonas vaginalis (TV)* by Nucieic Acid Amplification Testing (NAAT)

Perform GC/CT and TV NAAT per manufacturer guidance and local SOP.

9.8.6 Pap Smear

Perform pap smear per local standard of care.

9.8.7 Cervicovaginal Fluid for Prostate Specific Antigen

Prostate Specific Antigen (PSA) testing will be done in real time, batched. Results will be entered onto case report forms. The leftover extracted sample will be stored, LDMS tracking is optional.

Supplies:

- One - packaged sterile single polyester swab (Preferred: Puritan 25-806 1PD)
- One 2-mL cryovial
- Wet ice
- Refrigerator, 2 – 8°C
- ABAcard p30 Detection Kit: card, dropper, extraction buffer
- Adjustable pipette and tips, if not using dropper
- Timer
- Source document

Perform PSA per manufacturer insert for ABAcard p30 Detection Test:

1. Samples tested same day of collection or within 5 days, continue with procedure.
    - If testing will be done 5 days after collection, store at ≤-70°C within 8 hours of collection until ready to test.
2. If using frozen samples, when ready to test: place participant swab on wet ice, allow the sample to acclimate to 2 – 8°C refrigerator.
3. Add 750 µl of extraction buffer to a cryovial or mini-centrifuge tube and keep on ice.

4. Add the swab into the cryovial or mini-centrifuge tube and gently vortex or shake the swab in the buffer.
5. Incubate for 2 hours at 2-8°C. If ready to test, continue with procedure.
    - It may be stored at this temperature or frozen until ready to test.
6. When ready to test, allow sample to warm to room temperature.
7. Remove testing device and dropper from the sealed pouch.
8. Label the testing device with the PTID, visit, collection date.
9. Vortex the sample and add 80 µl (or 2 drops) of the sample to the sample well (S).
10. Set timer for and read the results at 10 minutes.
11. Take a picture, if questionable, and record results on source document.
12. Store remaining extractions, ≤ -70°C, in storage boxes of PSA Extractions.

9.8.8 Vaginal Fluid for TAF, TFV, & EVG Concentration (PK)

Supplies:

- *Two* - packaged sterile single polyester swab (*Preferred:* Puritan 25-806 1PD)
- *Two* – Nalgene cryovials (Thermo-Fisher Scientific #5000-0020)
- Permanent marker
- Ziplock biohazard sample bags
- Gloves
- Analytical balance (at least 4 decimal places in grams; accurate to 0.1 milligrams)
- If applicable, lightweight container to tare that will hold items to weigh
- Rack for cryovial
- Sterile scissors to cut swab shaft
- Speculum
- *For Pre-Cut Swab Collection Method:* Hemostat, Ring Forceps, or Transfer Pipet (recommend 8 inches or longer)

PK Vaginal Fluid Labeling

Endpoint	Sample code/Other Specimen ID
TAF/TFV	**VF-PK1**
EVG	**VF-PK2**

Notes:

- Each day of collection that requires weighing of rectal or vaginal swabs and vaginal biopsies for PK, perform QC of the analytical scale to accurately weigh to a weight of at least 0.1 milligrams.
- Do not turn off balance until weighing for the day is completed.
- Handle items to be weighed with gloves, especially after the pre-weight has been completed.
- Two specimens must be collected
- Same scissors may be used for other swabs from same body site from same participant.
- CRS will select one of the two methods for the collection of CVF for PK and weighing of swabs:
    - Pre-cut Swab Collection Method
    - Post-cut Swab Collection Method

POST-CUT SWAB COLLECTION METHOD

1. Place an identical label on items from each set of one cryovial, one packaged swab, and a biohazard sample ziplock bag.
   a. For the second set, use distinguishing information to label the set of cryovial, packaged swab, and bag
2. Perform pre-weight of cryovial and packaged swab.
   a. Zero the balance with urine cup or similar type of container on it
   b. Place the labeled 2-mL cryovial in the urine cup and the packaged sterile swab upright in the urine cup. (Make sure it is not leaning on a part of the scale.)
   c. Record this pre-weight on the Tracking Sheet.
   d. Place the cryovial and the packaged swab in a biohazard sample ziplock bag with corresponding label
3. Sample collection

   NOTE: All of the items in the bag should return to the bag. Nothing will be thrown into the garbage.
   a. Remove swab from packaging. Do NOT discard the packaging. Place all of the packaging back into the bag.
   b. Insert the swab and hold against the mucosa for 10 seconds, 3 rotations during that time period.
   c. Place the swab in the cryovial and cut the swab shaft using scissors at the pivot point. Be sure to hold onto the shaft to avoid losing it. Do NOT discard the shaft!
   d. Place the cut shaft into the swab packaging that was placed in the specimen bag in step a.
   e. Screw the lid back on the cryovial and place sample in the bag with the swab packaging and the swab shaft.
   f. Document the collection time on to the tracking sheet or source document.
4. Perform Post Weight:
   a. Zero the balance with urine cup or similar lightweight container on it .
   b. Weigh the capped cryovial containing the absorbed swab tip, the swab packaging and the remainder of the swab shaft
   c. Record post-weight on the Tracking sheet or source document and record NET weight.
5. Affix the LDMS label and store ≤-70˚C within 2 hours of collection.
6. Record freezing time.

PRE-CUT SWAB COLLECTION METHOD

1. Use sterile technique to unpackage sterile swab, place in cryovial, cut swab, and cap.
2. Affix label to the cryovial containing the pre-cut swab.
   a. Distinguish labels of two identical specimens
3. Perform pre-weight measurement by weighing the labeled capped cryovial with pre-cut swab and record on the Tracking Sheet.
4. Sample collection
   a. Uncap the pre-weighed cryovial. Use a clean hemostat, forceps, or transfer pipette to extend the length of the swab shaft.

b. Insert the swab and hold against the mucosa for 10 seconds, 3 rotations during that time period.

b. Immediately place swab into the cryovial after sampling and recap.

c. Document collection time on the tracking sheet or source document.

5. Perform post-weight measurement by weighing the capped cryovial containing the absorbed swab tip and record on the Tracking Sheet or source document.
6. Calculate and record the NET weight on the Tracking Sheet or source document.
7. Within 2 hours, place the sample tubes in the freezer at ≤-70°C.

- The specimens will be stored at the site until notified by CTH-LC for shipment.

9.8.9 Vaginal Fluid for Anti-HIV Activity

Supplies:

- One 2.0-mL cryovial (Nalgene, recommended but not required)
- One sterile polyester-tipped swab in sleeve (Puritan #25-806 1PD)
- Speculum

Vaginal Fluid PD for HIV Labeling

Endpoint	Sample code	#Swabs
Anti-HIV Activity	PD-HIV	1

Specimens should be collected and processed by following these steps:

1. Pre-label the cryovial and set it aside.
2. Have the participant lay in a dorsal lithotomy position.
3. Place speculum (lubricated with water) in the vagina to visualize the cervix and vaginal walls.
4. Using the swab, collect fluid by placing the swab against the vaginal side wall.
5. Hold the swab in place for at least 10 seconds but roll it at least 3 times during that time period, to ensure that the entire swab surface contacts the sampling site.
6. Place swab in cryovial, cut swab shaft with scissors or snap it, and cap it.
7. Place it on ice and transport to the lab.
8. Freeze at ≤ -70°C or place in dry ice within 2 hours of collection. If placing in dry ice first, maintain specimen in dry ice until frozen at ≤ -70°C.

9.8.10 Vaginal Fluid for Anti-HSV Activity

Supplies:

- Double-swab, dry, rayon Starplex swab in transport tube (Reference S09D)
- Speculum
- Ziplock bags

Vaginal Fluid PD for HSV2 Labeling

Endpoint	Sample code/Other Specimen ID	#Swabs
Anti-HSV Activity	PD-HSV	2 in one transport tube

1. Retain the transport tube that contains the Starplex swabs.
2. Have the participant lay in a dorsal lithotomy position.

3. The two swabs may be collected at the same time. Collect fluid by placing the swabs against the vaginal wall. Hold the swabs in place for at least 10 seconds, rolling at least 3 times during the time period to ensure that the entire swab surfaces contact the sampling site.
4. Place the swab back in the original transport tube.
5. Close and label the transport tube.
6. Storage: ≤ -70°C within 2 hours of collection. Store in large specimen ziplock bag, CVF for Anti-HSV2 Bag 1.

9.8.11 Cervicovaginal Fluid for Microflora

Supplies:

- ZYMO DNA/RNA Shield
- Copan FLOQ swabs
- 1.9 ml FLuidX tubes polyester-tipped swab in sleeve (Puritan #25-806 1PD)

VF for Microbiota Labeling

Endpoint	Sample code/Other Specimen ID	#Swabs
Vaginal Microbiome	VF-MB	1

1. 1 ml of ZYMO DNA/RNA Shield should be aliquoted into a 1.9 ml FLuidX tubes
2. FluidX tubes may come with barcode labels.
3. Remove the cap from the orange sample tube, but keep it nearby
4. Stand the tube upright in the tray
5. Remove the swab from the package.
6. Insert the swab about 2 inches in the vagina
    a. Swab the vaginal mucosa with the Copan swab.
    b. Hold the swab in place for at least 10 seconds but rotate the swab three times within that time period.
    c. A speculum does not need to be used for this sample.
7. After collecting the specimen, Insert the swab into the sample tube.
8. Stir the swab for 30 seconds.
9. Remove and discard the swab.
10. Tightly screw the cap onto the tube.
11. Shake the tube for 30 seconds
12. Affix an LDMS label to the tube; do not cover the bar code label if possible.
13. Store upright at ≤ -70°C in VAG MICRO boxes. Freeze within 4 hours of collection.
14. Keep all tubes frozen until requested for shipment.

9.8.12 Cervicovaginal Lavage for Soluble Markers and Cytokines

Supplies:

- Two 15-mL BD polypropylene Falcon tubes
- Four 2.0-mL cryovials (internally-threaded, recommended but not required)
- Normal saline
- Speculum
- 10mL syringe

- Wet ice
- Adjustable pipette and tips or graduated transfer pipettes
- Refrigerated centrifuge

CVL Aliquot labeling

Endpoint	Sample Code/Other Specimen ID	# Aliquots
Soluble Marker	CVL-SM1, CVL-SM2, and CVL-SM3	3
Back-up	CVL-BU	1

Preparation of CVL Supernatant

1. Pre-label the following tubes and set them aside:
    a. Two 15 mL BD Falcon tube: PTID #1; PTID #2
    b. Four 2.0 mL cryovials: CVL-SM1, CVL-SM2, CVL-SM3, and CVL-BU
2. Before lavage: the participant should be in supine position with the pelvis/lower portion of the body slightly elevated to prevent leaking of the lavage.
3. Insert speculum lubricated with water. Gently inject 5-mL normal saline using a 10-mL syringe, lavaging the cervical fornices, and vaginal walls using the transfer pipet. Do not aim directly at the cervical os. Repeat this procedure approximately 4 times with the same fluid.
4. Aspirate back all the lavage fluid carefully from the vagina and place it in Falcon #1. Place immediately on ice.
5. Within 30 minutes of collection, centrifuge for 10 min at 500 × g at 4°C.
6. While not disturbing the cell pellet, use a pipet to transfer the entire CVL supernatant to Falcon #2.
    a. Discard cell pellet.
7. Vortex the Falcon well before aliquoting.
8. Transfer 1 mL of the supernatant from the Falcon #2 in order of the labeling. Distribute additional volume into each of the cryovials. Note volume in CVL-BU, if < 1 mL.
9. Make sure the caps are tightly sealed.
10. Freeze at ≤ -70ºC or dry ice. If on dry ice, maintain specimen in dry ice until stored at ≤ -70°C.
11. Note any findings of mucus or blood in the CVL specimen in comments in sample database.
12. The specimens will be stored at the site until requested for shipment.

9.8.13 Cervicovaginal Biopsies Overview

Depending upon the site and the visit, approximately 2 to 5 cervicovaginal biopsies may be taken. Biopsies may be taken from the cervix and 2/3 of the distance from the posterior fornix to the introitus understanding this may not always be possible. Biopsies should be done, so that bleeding from initial biopsies will not obscure the area of the subsequent biopsies. Biopsy locations should alternate sides per visit. For example, biopsies can be taken from 4-5 and 7-8 o'clock. The transformation zone should be avoided when possible. Sites should make every effort to obtain biopsies weighing at least 20 mg.

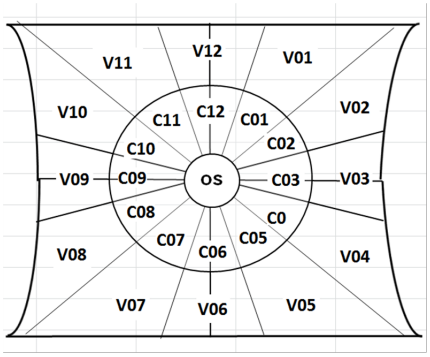


Figure 2.
Biopsy locations should be charted, and alternate sides chosen at subsequent visits.

1. Using a speculum, the areas of the planned biopsy should be gently cleaned with saline. Avoid vigorous wiping that could inadvertently remove epithelial cells. Benzocaine gel* (not spray) or lidocaine may be applied using a swab to the areas of the planned biopsy and left on for about 2 minutes for pain control.

   Women can opt to have the biopsy without benzocaine by preference or if they are allergic to the benzocaine, can be given acetaminophen prior to the procedure for pain relief. If adequate pain control is not achieved, injection of local anesthetics may be administered by the clinician, as appropriate.

   ***Important: Tissue to be used to assess HIV (BXC-HIV1 & BXC-HIV2) and IHC (BXV-IHC) should not be treated with benzocaine or any other anesthetic prior to biopsy.**

2. Tension on the speculum should be relaxed to allow natural rugal folding. Each biopsy should be taken across the rugal fold with 3 × 5 forceps.

3. Hemostasis should be attempted via adequate pressure, whenever possible. The biopsy site may be treated with silver nitrate (preferred) or Monsel's solution to control bleeding. Sites should use just enough Monsel's to ensure adequate hemostasis. If adequate hemostasis is not obtained with these measures, infrared, electrocautery, or suturing may be used.

Table 9-5. Biopsy Collections Overview

Sample Code	Endpoint	Total # of samples per PTID	Visit Collected	Cryovial Preparation	Lab
		Vaginal			
BXV-PK1 BXV-PK2	PK: TAF, TFV, & EVG	4	4, 7*	Empty	CAVP
BXV-IHC^	IHC	2	2, 7*	Formalin	EVMS
		Cervical			
BXC-HIV1^ BXC-HIV2^	HIV**	5-6	2, 4, 7*	Explant Media	EVMS

^Tissue to be used to assess HIV (BXC-HIV1 & BXC-HIV2) and IHC (BXV-IHC) should not be treated with benzocaine or any other anesthetic prior to biopsy.
* Only one third of the participants will have their biopsies at one visit 7 timepoint, either 24, 48, or 72hrs, depending on sampling timepoint randomization
**EVMS site only

9.8.14 Cervicovaginal Biopsies for PK

Supplies

- Two 2.0-mL Nalgene cryovials
- Analytical Balance
- Wet ice
- Permanent marker, if needed
- Chemical-resistant marker, if needed and using dry ice/ethanol

Labeling for Vaginal Biopsy for PK

Endpoint	Sample Code/Other Specimen ID	Location	#Biopsies
TAF/TFV	BXV-PK1	Vagina	1
EVG	BXV-PK2	Vagina	1

1. Pre-label two cryovials. Make sure the tube body and cap have matching numbers for each cryovial, and set them aside.
2. Pre-weigh each cryovial.
3. Place each vaginal biopsy in a separate, empty pre-labeled and pre-weighed BXV-PK cryovial.
4. Weigh each capped cryovial and biopsy after obtaining the sample using the same scale that was used to collect the pre-weight.
5. Document the weight on source document.
6. Within 15 minutes of collection, snap-freeze the samples in liquid nitrogen or place in dry ice/ethanol slurry for one minute.
7. Transport the samples on dry ice to the lab for storage.
8. Storage: ≤ -70°C in VAG BX for PK boxes

9. The specimens will be stored at the site until notified by CTH-LC regarding shipment.

Note: the clinical team may require additional supplies such as Tischler forceps or silver nitrate in order to collect pelvic biopsies.

9.8.15 Vaginal Biopsies for Immunohistocompatibility (IHC)

Table 9-6. Description of Vaginal Biopsy Specimen, Collection and Processing

Category	Description
Sample Type	Vaginal biopsy
Sample Size	As large as can accommodate. Most important to ensure intact epithelium is included in biopsy
Collection Tube and Temp	10% buffered formalin in tube at room temperature
Other requirements	Final sample to be shipped is a paraffin block. See further important instructions of sample processing *

Supplies

- Gloves
- 10 % buffered formalin
- Phosphate buffered saline
- Cryovial, any kind
- Cassettes
- 100% ethanol
- Xylene
- Paraffin

Vaginal Biopsy IHC Labeling

Endpoint	Sample Code/Other Specimen ID	Location	# Biopsies
IHC	BXV-IHC	Vagina	1

*** Protocol for paraffin embedding:**

1. Have tubes with 1ml formalin ready to accept the biopsy at point of collection. Fix tissue immediately in 10% Buffered Formalin for 24 hrs (minimum) – 30 hrs(maximum). Do not leave the tissue in the fixative for more than 30 hrs. After the specified time, remove the formalin from the tube and add 1 ml of cold Phosphate buffered saline (PBS) and keep the tube in cold (refrigerator) for a minimum of 24 hours. After 24 hours, tissues in PBS can be processed any time up to and no later than 5 days.
2. Send tissue to facility for processing and embedding. If tissue will be processed at the onsite laboratory, follow these steps:

a. Place cassettes in the automated tissue processor and start the selected processing program for these tissues. This program takes the tissues (under vacuum) through a series of increasing grades of alcohol, to xylene, and finally into paraffin.
b. At the end of the programmed processing cycle, the cassettes are removed from the processor and the paraffin-infiltrated tissues are embedded in paraffin.
c. While embedding, place the tissue in such a way that the epithelium and connective tissue faces up, so that when sections are cut you get both epithelium and connective tissue below it on slide

3. The paraffin blocks will be stored at the site until notified by requested for shipment.

9.8.16 Ectocervical Biopsies for HIV Infectivity PD EVMS ONLY

Table 9-7. Description of Ectocervical Biopsy Specimen for PD, Collection and Processing

Category	Description
Sample Type	Ectocervical biopsy
Sample Size	5x5 mm
Collection Tube and Temp	Explant medium in tube or cryovial on ice
Other requirements	Needs to be received by testing lab within 30 minutes of collection

Supplies:

- Two microcentrifuge tubes
- Small Tissue Forceps
- Speculum
- Normal saline
- 3 × 5mm Tischler forceps
- Permanent marker, if needed
- Explant media

Cervical Biopsies PD Labeling

Sample Code/Other Specimen ID	Location	# Biopsies
BXC-HIV1	Ectocervix	1
BXC-HIV2	Ectocervix	1

1. The laboratory will pre-fill two microcentrifuge tubes with 1-mL of refrigerated explant media.
2. The clinic will pre-label the two microcentrifuge tubes with Sample Code as shown above and set them aside.
3. The clinic will collect the biopsies using the Tischler forceps.
4. Once the biopsies are collected, transfer it/them into the labeled tubes. NOTE: Small tissue forceps may be used to loosen each biopsy from the forceps.
5. Transport the sample(s) to the lab on ice as soon as possible.

Note: the clinical team may require additional supplies such as Tischler forceps or silver nitrate in order to collect pelvic biopsies.

9.9 Rectal Fluids

9.9.1 Rectal Fluid for TAF, TFV, & EVG Concentration (PK)

Supplies:

- *Two* - packaged sterile single polyester swab (*Preferred:* Puritan 25-806 1PD)
- *Two* – Nalgene cryovials (ThermoFisher Scientific #5000-0020)
- Permanent marker
- *Two* Ziplock biohazard sample bags (to separate two sets and protect pre-weighed items)
- Analytical balance (at least 4 decimal places in grams; accurate to 0.1 milligrams)
- If applicable, lightweight container to tare that will hold items to weigh.
- Rack for cryovial
- Sterile scissors to cut swab shaft.
- Speculum
- *For Pre-Cut Swab Collection Method:* Hemostat, Ring Forceps, or Transfer Pipet (recommend 8 inches or longer)

PK Rectal Fluid Labeling

Endpoint	Sample Code/Other Specimen ID
TAF/TFV	RF-PK1
EVG	RF-PK2

Notes:

- Each day of collection that requires weighing of rectal or vaginal swabs and vaginal biopsies for PK, perform QC of the analytical scale to accurately weigh to a weight of at least 0.1 milligrams.
- Do not turn off balance until weighing for the day is completed.
- Handle items to be weighed with gloves, especially after the pre-weight has been completed.
- Two specimens must be collected.
- Same scissors may be used for other swabs from same body site from same participant.
- Two Methods for Collection of CVF for PK assessment and weighing swab, selected by CRS.
  - Pre-cut Swab Collection Method
  - Post-cut Swab Collection Method

POST-CUT SWAB COLLECTION METHOD

1. Place an identical label on items from each set of one cryovial, one packaged swab, and a biohazard sample ziplock bag.
   a. For the second set, use distinguishing information to label the set of cryovial, packaged swab, and bag.
2. Perform pre-weight of cryovial and packaged swab

a. Zero the balance with urine cup or similar container on it.
b. Place the labeled 2-mL cryovial and the packaged sterile swab upright in the urine cup. (Make sure items are not leaning on a part of the scale.)
c. Record this pre-weight on the Tracking Sheet.
d. Place the cryovial and the packaged swab in a biohazard sample ziplock bag with corresponding label.

3. Sample collection

   NOTE: All of the items in the bag should return to the bag. Nothing will be thrown into the garbage.

   a. Remove swab from packaging. Do NOT discard the packaging. Place all of the packaging back into the bag.
   b. Insert the swab and hold against the mucosa for 10 seconds, 3 rotations during that time period.
   c. Place the swab in the cryovial and cut the swab shaft using scissors at the pivot point. Be sure to hold onto the shaft to avoid losing it. Do NOT discard the shaft!
   d. Place the cut shaft into the swab packaging that was placed in the specimen bag in step a.
   e. Screw the lid back on the cryovial and place sample in the bag with the swab packaging and the swab shaft.
   f. Document the collection time on to the tracking sheet or source document.

4. Perform Post Weight:
   a. Zero the balance with urine cup or similar lightweight container on it
   b. Weigh the capped cryovial containing the absorbed swab tip, the swab packaging and the remainder of the swab shaft (Suggestion: Place the swab shaft into the packaging and have it upright during weighing.)
   c. Record post-weight on the Tracking sheet or source document and record NET weight.

5. Affix the freezer label and store ≤-70˚C within 2 hours of collection.
6. Record freezing time.

PRE-CUT SWAB COLLECTION METHOD

1. Use sterile technique to unpackage sterile swab, place in cryovial, cut swab, and cap.
2. Affix label to the cryovial containing the pre-cut swab.
   a. Distinguish labels of two identical specimens
3. Perform pre-weight measurement by weighing the labeled capped cryovial with pre-cut swab and record on the Tracking Sheet.
4. Sample collection
   a. Uncap the pre-weighed cryovial. Use a clean hemostat, forceps, or transfer pipette to extend the length of the swab shaft.
   b. Insert the swab and hold against the mucosa for 10 seconds, 3 rotations during that time period.
   b. Immediately place swab into the cryovial after sampling and recap.
   c. Document collection time on the tracking sheet or source document.
5. Perform post-weight measurement by weighing the capped cryovial containing the absorbed swab tip and record on the Tracking Sheet or source document.
6. Calculate and record the NET weight on the Tracking Sheet or source document.

7. Within 2 hours, place the sample tubes in the freezer at ≤-70°C.

The specimens will be stored at the site until notified by CTH-LC for shipment.

9.10 Shipping

9.10.1 Shipping from Africa to USA

CTH-LC will coordinate shipments of samples from sites in Africa to USA. The delivery address for the CTH-LC is the following:

May Beamer
Magee-Womens Research Institute
204 Craft Avenue
Pittsburgh, PA 15213
(412) 641-6041

9.10.2 Shipping to testing labs

The testing labs in USA will receive samples from EVMS and CTH-LC. CTH-LC will contact EVMS to coordinate shipments of samples to the testing labs.

Table 9-8. Summary of Collections and Timepoints

Collection	Sample Type	Sample Code	Endpoint	# samples per time point	Visit Number	Lab
Blood	Plasma	PLS-ARC	Plasma for Archive	1	2	CTH-LC
		PLS-PK1	PK: TAF/TVF	1	4, 5, 6, 7*, 8	CAVP
		PLS-PK2	PK: EVG	1		
		PLS-BU	PK: Backup	1		
CVF	Swab	CVF-PK1	PK: TAF/TVF	1	3, 4, 5, 6, 7*, 8	
		CVF-PK2	PK: EVG	1		
RF (if participant consents)	Swab	RF-PK1	PK: TAF/TVF	1	3, 4, 5, 6, 7*, 8	
		RF-PK2	PK: EVG	1		
VGL tissue	BXV1/2	BXV-PK1	PK: TAF/TVF	1	4, 7 *	
		BXV-PK2	PK: EVG	1		
CVF	Swab	VF-MB	CVF-microbiota	1	3, 4, 5, 6, 7 *, 8	Ravel
		PD-HIV	CVF: Anti-HIV	1	2, 3, 4, 5, 6, 7 *, 8	EVMS
		PD-HSV	CVF: Anti-HSV2	1 (2 swabs in primary container)		
CVL	Supernatant	CVL-SM1, -SM2, -SM3	CVL: soluble markers and cytokines	1	2, 3, 4, 5, 6, 7 *, 8	Fichorova
		CVL-BU	CVL Backup	1	2, 3, 4, 5, 6, 7 *, 8	
Tissue	BXV IHC	BXV-IHC	IHC (all sites)	1	2, 7 *	EVMS
	BXC1/2	BXC-HIV1	HIV (EVMS site only)	2	2, 4, 7 *	
		BXC-HIV2				

* Only one third of the participants will have their biopsies at one visit 7 timepoint, either 24, 48, or 72 hrs, depending on sampling timepoint randomization