MATRIX-002 Study-Specific Procedures (SSP) Manual
Section 9 – Laboratory Considerations

TABLE OF CONTENTS

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Version #	Revision date	Reason	Edit
2.0	2-20-2024	Modify EQA frequency of review to when requested	CTH-LC
		Modify Swab for microbiota handling procedures, increased time to freezer storage, up to 4 hours after collection	
		Clarified Gram stain shipping: one set shipped to CTH-LC	
		Minor corrections and clarifications	
		Added shipping address for CTH-LC	

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-002 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, the 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 (SOP)

- External Quality Assurance (EQA)
- 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.

The 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. CTH-LC may request to review any validation reports.

9.2.4 Standard Operating Procedures

These requirements waived for Laboratory testing done under CLIA.

Prior to activation, CRS will be required to verify that signed SOPs are current, i.e., reviewed within the past 2 years, for assays and laboratory processes performed at Local Labs. The CTH-LC may request to review any SOP.

Table 9-1: Overview of Local Laboratory Testing Specimens and Methods for MATRIX-002

Test	Specimen Type
Pregnancy	Urine
Dipstick, Microscopy and Culture	Urine
HIV Rapid Tests	Saliva (CLIA) or Blood
HIV Confirmatory test / RNA	Blood
AST,ALT,CREAT	Blood
CBC	Blood
Syphilis Serology	Blood
Plasma for Archive for HIV Confirmation, Viral Load	Blood
Pap Smear	Cervical Cells
NAAT for CT/GC, TV	Cervicovaginal Fluid
Vaginal Wet Mount/KOH	Cervicovaginal Fluid
Vaginal pH	Cervicovaginal Fluid

9.3 Specimen Labeling

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: Participant identification number (PTID) and collection date. Laboratory Data Management System (LDMS) labeling is required for sample aliquots as described in SSP Section 9.4.

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 9-3 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.*

9.4 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. 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 that must be used by all sites for the storage and shipping of sample types listed in Tables 9-2 and 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).

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

Specimen	Designated Test	Storage Instructions	Storage or Testing Lab
Vaginal	Nugent Score	Store in order of collection: slide -001 in primary box and Slide -002 in backup box	Batch shipping to CTH-LC at end of study for one set of slides. Second set will be stored on-site until requested
Vaginal	Microbiota	Store the two swabs adjacent in order of collection	Batch shipping to CTH-LC at end of study
Blood	Plasma Archive	Store in order of collection	Ship to CTH-LC upon request

Please use the LDMS codes listed in table 9-3 when logging in specimens for each specimen type listed.

Samples must be separated by sample type when storing. Label format must include barcode, study protocol, PTID, visit code, global specimen number (GUSPEC), Primary and aliquot derivative, and collection date. Use the MATRIX label format if available; contact the CTH-LC for guidance as needed.

Table 9-3: LDMS Specimen Management Guide to Logging in MATRIX-002 Specimens

Sample	Primary	Additive	Primary Volume	Primary Units	Primary Units	No. of Aliquots	Aliquot Units	Aliquot Derivative	Sub Add/ Derivative
Nugent Score	VAG	NON	1	EA	EA	2	EA	SLD	GRS
Microbiota	VAG	NON	1	EA	EA	2	EA	SWB	N/A
Plasma Archive	BLD	EDTA	~ 4 (whole blood)	mL	mL	~2 (plasma)	mL	PL1/ PL2	N/A

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.

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 Human chorionic gonadotropin (hCG) Pregnancy Test

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

9.6.3 Urine Dipstick and Culture

Perform urine dipstick 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. CTH-LC recommends that specimens not routinely be labeled in advance of collection but should occur immediately after collection. Participant Identification must be verified each time a specimen is collected.

Label all required tubes at the time of collection. After collection complete the following:

- Allow plain tubes (red, tiger top or gold top non-additive tubes or serum separator tubes [SST]) 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 Vacutainer EDTA (ethylenediaminetetraacetic acid) Blood Collection Tubes 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-002 study protocol.

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 or saliva sample (CLIA-waived Pittsburgh CRS). *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 will depend on whether testing is for screening/enrollment or follow up:

- **Screening/Enrollment**

  The participant is ineligible for the study. Continue testing per local standard of care 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 testing per local standard of care to ensure correct diagnosis; contact the CTH-LC as needed for assistance. If that result is positive, report as positive. If the result is negative or indeterminate, contact the protocol team for guidance.

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

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

9.7.3 Hematology Testing

Complete blood counts will be performed per local SOP. Each of the following must be analyzed and reported:

- Hemoglobin
- 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 Plasma Archive

Collect whole blood in EDTA tubes. Store a minimum of 2 mL of plasma in 1 ml aliquots.

- If the blood is held at room temperature, plasma must be processed and frozen within 4 hours of collection.
- If the blood is kept refrigerated or placed on ice, plasma must be processed and frozen within 24 hours of collection.

Use one of the following techniques to centrifuge blood at room temperature:

- Single spun: Spin blood at 1500×g for 10 minutes, remove plasma.
- Double spun: Spin blood at 800×g for 10 minutes, place plasma in a tube to spin again at 800×g for 10 minutes, remove plasma.

Prepare as many 1-mL aliquots.

- 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.

Plasma should be stored frozen on site ≤ -70°C until requested for shipping and/or testing by the CTH-LC.

9.8 Pelvic Specimens

Multiple pelvic samples may need to be collected at a single visit. Consult current visit checklists for collection order. The order stated in the checklist is to be followed when collecting multiple pelvic samples.

9.8.1 Papanicolaou (Pap) Test, if clinically indicated

Pap smears may be collected during screening to confirm eligibility as needed. For collection, ecto- and endocervical cells will be collected after all tissues have been visually inspected. Local laboratory specimen collection, testing, and QC procedures must be performed and documented in accordance with study site SOPs.

9.8.2 CT/GC/TV by NAAT

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

9.8.3 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.

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

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

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

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

MATRIX-002 sites will participate in the MATRIX Online Wet Mount EQA program unless other EQA is approved.

Vaginal wet mount is not a required protocol procedure for MATRIX-002, but may be performed for clinical indications.

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

- Potassium Hydroxide (KOH) prep
- Saline prep

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.

NOTE: If site SOP(s) differ from the described process, contact the CTH-LC for review and approval.

Prepare and examine wet prep slides according to the following process:

1. Label the two slides with PTID and specimen collection date. A pencil may be used on the frosted end of two microscope slides or affix a label onto each slide and write PTID and specimen collection date in indelible ink (e.g., Sharpie pen).

2. Using the same swab (polyester or cotton) 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) of sterile physiologic saline to allow for non-immediate slide preparation. In this case, vaginal fluid specimens should be inoculated onto the two slides upon receipt.
    - * If you cannot use the same swab as the pH (e.g., due to contamination or inadequate material), then collect a new swab from the lateral vaginal wall.

3. Apply one drop of 10% KOH to one slide, mix with the vaginal fluid specimen, and immediately perform whiff test for a "fishy" amine odor. Then apply cover slip.
    - Examine the KOH slide at both 100X and 400X magnification for yeast and pseudohyphae.

4. 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 must comprise at least 20 percent of the observed epithelial cells in order for the saline prep to be considered positive for clue cells.
        - Clue cells are irregularly bordered squamous epithelial cells that are completely covered with bacteria (Gardnerella vaginalis).

9.8.5 Vaginal Fluid for Gram Stain

Dried vaginal fluid smears will be prepared for Gram staining and Nugent score assessment for BV. Two slides (one designated as primary and the other as secondary) will be prepared using 1 swab. Both slides will be entered into LDMS as one primary specimen with two aliquots.

Both sets of slides will remain stored onsite until the CTH-LC sends guidance for shipping and/or Nugent score assessment. The one set of slides may be assessed locally depending on capacity or shipped to the CTH-LC to be scored.

- For sites that do Nugent assessment locally, the second slides will be shipped to the CTH-LC for QC to harmonize results across sites.
- When the assessment is done at the CTH-LC, the second set of slides will remain onsite unless requested.
- The CTH-LC will send guidance to destroy the slides once assessment and QC is completed.

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. Apply specimen to this side of the slide.
2. Collect vaginal sample from the lateral vaginal wall by rotating (polyester or cotton) swab at least three times. Collect the specimen from the opposite side of the vagina used for wet mount specimen collection, if applicable.
3. Immediately roll (do not drag) the swab across each slide. Do not place the swab in saline, transport medium, or any transport container prior to slide preparation.
4. Allow slides to air-dry. Do not heat-fix.
5. In the LDMS lab, log slides as aliquots of a primary sample.
6. Place the LDMS label on the frosted end of the slide on top of the pencil markings (same side as sample).
7. Store the primary slide in a separate slide storage box from the secondary slide. The secondary slide is a backup slide in case the first is lost, broken, or unevaluable.
8. Store the slide boxes at room temperature until requested by CTH-LC, generally at the end of study.

9.8.6 Vaginal Swabs for Microbiota

Supplies:

Two – Polyester swabs
- *Preferred*: Puritan polyester swab # 25-806 1PD
- If not available, site to provide brand and catalogue number; same lot number used through study duration is preferred.

Two – 2-mL cryovials, site choice – if possible, use same through study duration

Vaginal swabs are collected for detection of key microbiota using qPCR at visits 2, 6, and 9. The specimens are kept cold on wet ice or refrigerated, until stored at ≤-70°C and shipped to the CTH-LC at the end of the study.

1. Label 2 cryovials with PTID and collection date.
2. Collect the specimen by holding the polyester swab for 10 seconds over the lateral wall of the vagina, rotating at least 3 times in that time period. Do not collect swabs in the exact same area that another sample was collected. (The swabs may be collected together.)
3. Place each swab in separate 2-mL cryovials.
4. Break or cut shaft of swab lower than the cap of the vial, so not pressing against the cap.
5. Freeze within 4 hours of collection.
    a. If transport to LDMS laboratory is within 4 hours of collection, the specimens may be stored at room temperature or wet ice. If possible, select one storage option for the duration of the study. Contact the CTH-LC for guidance as needed.
    b. If transport to LDMS storage lab is delayed >4 hours of collection, the specimens may be placed on dry ice or stored ≤ -70ºC at the clinic. If this occurs, ensure that transport to LDMS laboratory is on dry ice.
6. Deliver the tubes and a tracking sheet for LDMS specimens to the local LDMS laboratory.
7. Log the cryovials into LDMS (Table 9-3) as two aliquots and label each vial with a LDMS label. If possible, avoid covering the entire PTID on the initial label.
8. Store at ≤ -70ºC.
9. Batch ship samples to the CTH-LC at the end of the study or upon request.

9.9 Shipping

9.9.1 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:

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Magee-Womens Research Institute
204 Craft Avenue
Pittsburgh, PA, USA 15213
+1 (412) 641-6041