Section 12. Laboratory Considerations

12.1 Overview and General Guidance

This section contains information on the laboratory procedures performed in MTN-008.

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

http://www.cdc.gov/ncidod/dhqp/bp_universal_precautions.html

Section Appendix 12-1 provides an overview of the laboratory testing locations, specimens and methods for MTN-008. Laboratory procedures may be performed in the study site clinics or laboratories, approved commercial laboratories and in the MTN Network Laboratory (NL), including the MTN Pharmacology Core (at Johns Hopkins University). Regardless of whether tests are performed in clinic or laboratory settings, study staff that performs the tests must be trained in proper testing methods and associated quality control (QC) procedures prior to performing the tests for study purposes; training documentation should be available for inspection at any time.

All site laboratories will be monitored by the MTN NL which will utilize information from DAIDS monitoring groups (PNL, IQA, VQA, etc) to monitor and certify laboratories for testing. US Laboratories that are certified by CLIA (Clinical Laboratory Improvement Amendment) will be able to substitute this for some of the documentation requirements required of other labs. Valid CLIA certificates must be provided in these cases. Please refer all questions related to laboratory testing to the MTN NL using the following e-mail address: MTNNetworkLab@MTNStopsHIV.org

In addition to the specimen guidelines provided in Section Appendix 12-1, laboratory processing guidelines are provided in Section Appendix 12-2. Although specimen collection volumes may vary somewhat across sites, all sites must ensure that volumes collected do not exceed the specifications of their study informed consent forms. The MTN NL may request details of specimen collection containers and volumes for purposes of assisting sites in meeting this requirement.

Ideally, one method, one type of test kit, and/or a 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 test 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 NL must be notified before the change and can provide further guidance on validation requirements. Similarly, the MTN NL must be notified of changes to normal lab ranges.

Provided in the remainder of this section is information intended to standardized laboratory procedures across sites. Adherence to the specifications of this section is essential to ensure that data derived from laboratory testing will be considered acceptable to all regulatory authorities across study sites. It should be noted however that this section is not intended to serve as an exhaustive procedures manual for all laboratory testing. This section must be supplemented with site standard operating procedures (SOPs) for specimen management, processing, and testing.

12.2 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. SCHARP will provide pre-printed labels or a template that can be used to generate labels. The date of specimen collection should also be included on the label. If the date is handwritten, it should be in indelible ink (such as a Sharpie pen).

Microscope slides used for evaluation of vaginal/cervical fluids also will be labeled with SCHARP provided PTID labels. PTIDs are pre-printed on these labels; however study staff must write the specimen collection date on each label. The visit code also may be written on the label.

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.

The following specimens will be entered into LDMS and labeled with LDMS-generated labels:

- Tenofovir blood specimens (serum and PBMCs)
- Cord blood (pregnancy cohort only)
- Breast milk (lactation cohort only)
- Vaginal microflora (pregnancy cohort only)
- Gram stain (pregnancy cohort only)
- Vaginal and cervical biomarkers

Specimens that are tested locally do not need to be logged into LDMS or labeled with LDMS-generated labels.

12.3 Procedures for Specimens that can not be evaluated

Specimen collection will 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.

12.4 Use of LDMS

The Laboratory Data and Management System (LDMS) is a program used to track storage and shipping of laboratory specimens. It is supported by the Frontier Science Foundation (FSTRF). LDMS must be used at all sites to track the collection, storage, and shipment of eight types of specimens in listed in 12.2 Detailed instructions for use of LDMS are provided at: https://www.fstrf.org/ldms (may require a password).

All sites are 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. The site will be responsible to back up their LDMS data (frequency determined by site) locally and to export their data to FSTRF at least weekly.

Questions related to use of LDMS in MTN-008 may be directed to Pam Kunjara or LDMS Technical (User) Support. Usual business hours for LDMS User Support are 7:30 am - 6:00 pm (ET) on Monday

and Fridays and 7:30 am - 8:00 pm (ET) on Tuesdays, Wednesdays, and Thursdays. During business hours, please contact LDMS User Support as follows:

Email: ldmshelp@fstrf.org
Phone: +716-834-0900, ext 7311
Fax: +716-898-7711

LDMS User Support can be paged via email during off business hours if you are locked out of LDMS or experience errors that prevent you from completing LDMS lab work. To page LDMS User Support, email LDMS pager 1, 2, or 3 (address shown in Table 12-1 below) and include the following information in the body of your email:

- LDMS lab number (this is a three-digit number that is different from your network assigned clinical site number)
- The full telephone number at which you can be reached, including the country code and city code if you are outside the United States
- A short description of the problem

FSTRF no longer supports the use of telephone paging.

Table 12-1
LDMS User Support Paging Details

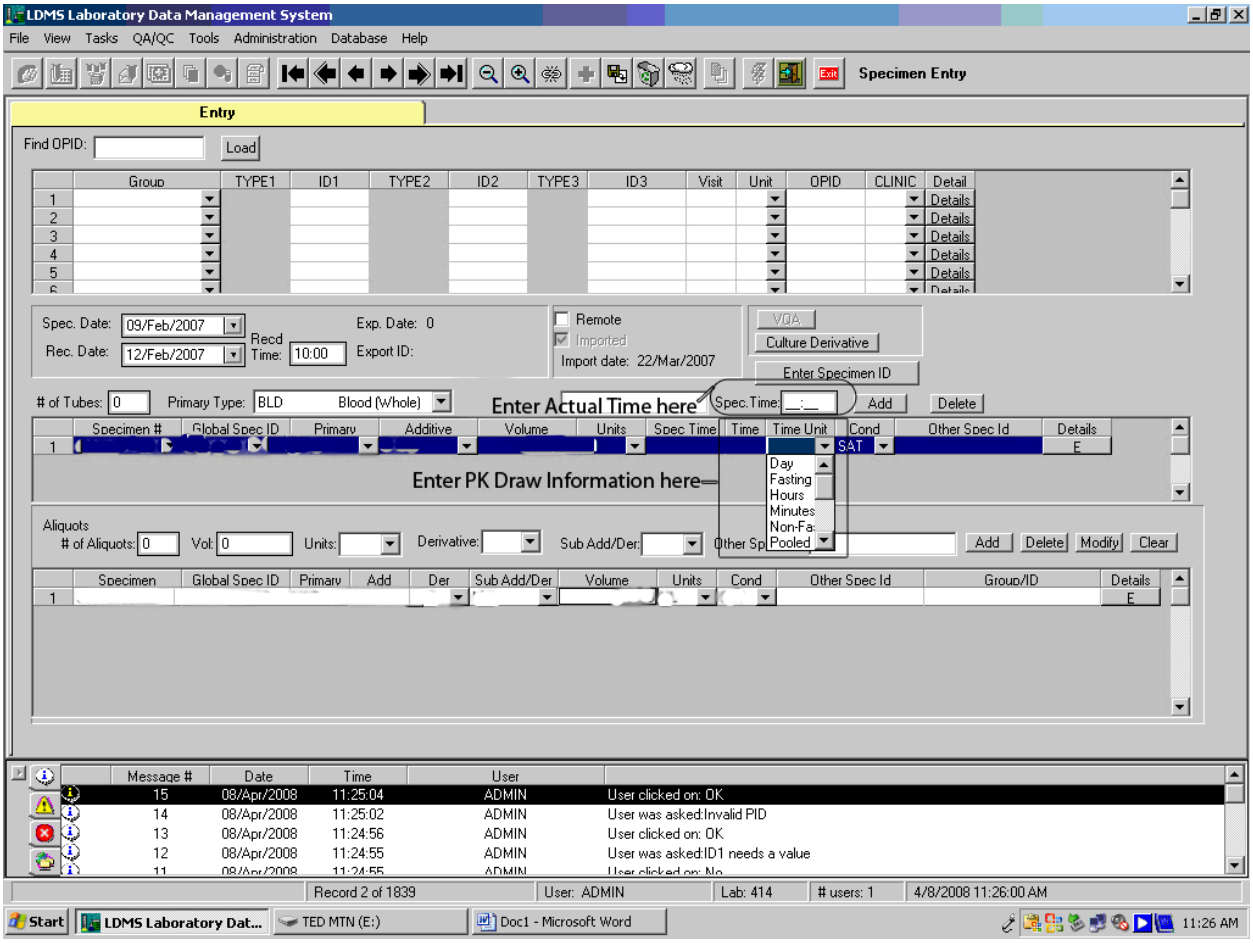
Pager	Email Address
LDMS 1	ldmspager1@fstrf.org
LDMS 2	ldmspager2@fstrf.org
LDMS 3	ldmspager3@fstrf.org

Each site must export its LDMS data to Frontier Science (FSTRF) on a weekly basis. Exported data are used by the MTN Statistical and Data Management Center (SDMC) 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 NL is responsible for reminding sites to adhere to the two week timeframe and for following up with sites that do not resolve discrepancies within two weeks. The MTN SDMC reviews the discrepancy reports for critical samples (e.g., blood needed for confirmatory HIV testing) that appear to be missing, and works with the NL and site staff to undertake appropriate corrective action. All corrective action should be documented in paper-based clinic and/or laboratory records as appropriate, and entered in the details section of LDMS. The NL and SDMC will discuss and document any items that, although resolved, appear 'irresolvable' in LDMS.

Logging in PK Samples

- Enter the actual time in the Specimen Time area (See Image 1)
- Enter the PK timepoint information in Time and Time Unit area (See Image 1)
    - For Pre dose samples
        - The time should be 0:00
        - Select "Pre-dose" from the drop menu for units
    - For Post dose samples
        - Enter the number that corresponds to the PK timepoint ("1" for 1-Hour, "2" for 2-Hour, etc…)
        - Select "Hours" from the drop menu for units

IMAGE 1: LDMS Entry Screen



12.5 Urine Testing

The urine tests performed at the study visit will depend on the time point of the visit and the clinical presentation of the participant. In general, at study visits when urine testing is required, a single specimen will be collected and then aliquoted for each test when possible. When doing multiple tests from one specimen, an aliquot of urine should first be obtained for pregnancy testing and/or dipstick urinalysis and the remaining specimen should be reserved for chlamydia and gonorrhea testing if indicated.
Collect urine specimens before collecting any pelvic specimens.

12.5.1 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 not to clean the labia prior to specimen collection.
- Collect the first 15-60 ml of voided urine in a sterile collection cup (not mid-stream).
- Instruct the participant to screw the lid tightly onto the cup after collection.

- At visits when pregnancy testing and/or dipstick urinalysis is required, aliquot 5-10 ml for these tests and store the remaining urine at 2-8° C or introduce the urine immediately into the UPT for subsequent chlamydia and gonorrhea testing.

12.5.2 Pregnancy Testing

At visits when pregnancy testing is required, aliquot approximately 5 to 10 mL of urine from the specimen collection cup and pipette from this aliquot for pregnancy testing. If the urine is too dark to read the pregnancy test, another urine sample will need to be collected. The Quidel QuickVue or Fisher Sure Vue One-Step hCG urine pregnancy test must be used at all sites. Perform the test according to site SOPs and the package insert. Do not perform any other urine pregnancy tests for confirmatory purposes.

12.5.3 Chlamydia and Gonorrhea Testing in Urine

This is done at follow-up visits when clinically indicated. Urine is preferred if the participant has been on product within the past 24 hours.

Instructions for transferring urine into the Urine Preservation Tube (UPT)

- Collect urine as noted above.
- Open the UPT kit (either BD ProbTec or GenProbe Aptima) and remove the UPT and transfer pipette. Label the UPT with the participants PTID number and date.
- Hold the UPT upright and firmly tap the bottom of the tube on a flat surface to dislodge any large drops from inside the cap.
- Uncap the UPT and use the transfer pipette to transfer enough urine to fill the tube to the level indicated on the tube between the black lines. Do not under fill or overfill the tube.
- Cap tightly and invert the tube 3-4 times to ensure that the specimen and reagent are mixed.
- The specimen can now remain at 2-30°C for 30 days.

12.6 Testing of Vaginal Specimens

Refer to the Screening and Follow-up Pelvic Exam checklists in other sections of this manual for further information of the required sequence of specimen collection and diagnostic procedures to be performed during study pelvic exams.

12.6.1 Vaginal pH

Vaginal pH will be assessed as part of on-site evaluations for bacterial vaginosis. Indicator Strips (pH range 3.6 to 6.1) must be used as follows:

- During pelvic examination, vaginal fluids should be collected via swab and then swabbed onto the pH strip. Avoid contact with cervical mucus, which has a higher pH. Do not insert the pH strip into the vagina to collect the vaginal fluid.
- Match the resulting color of the indicator strip to the color scale provided with the strips to determine the pH value.
- Record the pH value directly onto the appropriate case report form. It is not necessary to record pH values onto laboratory log sheets or other source documents prior to recording values onto case report forms.

12.6.2 Vaginal Fluid Wet Mount Testing

When clinically indicated wet mount will be performed. Wet mount procedures for this study consist of two different preparations —saline prep and potassium hydroxide (KOH) prep —for diagnosis of bacterial vaginosis and candidiasis, as summarized in Table 12-2.

If wet prep slides are read in-clinic by clinical staff, results may be recorded directly onto appropriate case report forms. If slides are read by lab staff (either in the local laboratory or a designated in-clinic lab area), results must be recorded onto laboratory log sheets or other laboratory source documents and then transcribed onto the appropriate case report form.

Prior to study initiation, clinicians should be proficient in reading wet mounts for clue cells and KOH preps for yeast. CLIA regulations require semi-annual proficiency testing; therefore the MTN NL will administer a web-based proficiency testing approximately every six months. The MTN NL will post wet mount slides on the MTN web pages for this purpose every 6 months; results will be entered directly on the website (contact: Lorna Rabe: lrabe@mwri.magee.edu). The MTN NL will report results back to the Laboratory Manager and also specify any corrective action that may be needed based on the results. Contact the MTN NL for additional information and guidance on performing and documenting the proficiency testing. Also contact the MTN NL when new applicable clinical or laboratory staff is hired, so that appropriate training can take place prior to such staff performing wet mounts for study purposes.

Table 12-2
Summary of Wet Prep Assessments and Diagnostic Criteria

Assessment	Saline Prep	KOH Prep
Whiff Test	Not applicable	Positive if fishy amine odor detected
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)
Trichomonads	Use OSOM Rapid Trichomonas test (see below)	Not applicable (organisms are lysed by KOH)
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.

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

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

Immediate examination of wet mount in clinic:

- Use a pencil to write the PTID and specimen collection date on one side of the frosted end of two microscope slides. Affix a SCHARP-provided PTID label to the other side of the slides (on the frosted end, under the pencil markings) and write the specimen collection date in indelible ink (e.g. Sharpie pen) on each label.
- Immediately following collection from the lateral vaginal wall via swab, 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.
- 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, emulsify with the vaginal fluid specimen, and then apply cover slip. Examine immediately at 10X magnification for epithelial cells, motile trichomonads, 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 (mainly *Gardnerella vaginalis*). Clue cells must comprise at least 20 percent of the observed epithelial cells for the saline prep to be considered positive for clue cells.
- Examine the KOH slide at both 10X and 40X magnifications for yeast and pseudohyphae.

Non-immediate wet mount examination in laboratory:

- Immediately following collection of vaginal fluid from the lateral vaginal wall via swab, place the swab in a glass or plastic tube with approximately 6 drops (100µl) sterile isotonic saline. Snap off the shaft of the swab and cap the tube.
- Deliver the tube to the laboratory for testing as described above for immediate examination. Testing must take place within 4 hours.

12.6.3 Rapid Test for Trichomoniasis

This testing will be done using the OSOM Rapid Trichomonas test with vaginal swabs per site SOPs approved by the MTN NL. The kit provides Dacron swabs for this test.

- Affix a SCHARP-provided PTID label to a clean glass or plastic tube with a cap.
- Collect specimen using kit-provided swab from the lateral vaginal wall (fluids also may be collected from the posterior fornix; avoid collecting specimens from the cervix).
- Immediately place the swab in the labeled tube, break off the shaft of the swab, and cap the tube.
- Testing is expected to be performed during the participant visit. However, specimens may be stored at room temperature for 24 hours or refrigerated for 36 hours before testing.

12.6.4 Vaginal Gram Stain

Dried vaginal fluid smears will be prepared for Gram staining and assessment for bacterial vaginosis at the MTN NL. Two slides will be prepared at each required time point and both will be entered into LDMS. One will be shipped to the MTN NL and the other will be archived on site until written notification is received from the MTN SDMC that the slide may be discarded. Instructions for slide preparation and shipping are provided below.

- Use a pencil to write the PTID and specimen collection date on one side of the frosted end of one microscope slide. Affix a SCHARP-provided PTID label to the other side of the slides (on the frosted end, under the pencil markings) and write the specimen collection date in indelible ink (e.g. Sharpie pen) on each label.

- Immediately following specimen collection from the lateral vaginal wall via swab, roll the swab across each of the slides. Do not place the swab in saline, transport medium, or any transport container prior to slide preparation.
- Allow the specimens to air-dry on the slides. Do not heat-fix.
- Deliver the slides and an LDMS Specimen Tracking Sheet to the local LDMS laboratory.
- Using the LDMS Specimen Tracking Sheet, log the slides into LDMS (specimen type = VAG) and label the slides with LDMS labels. Place the LDMS label on the frosted end of the slide, on the opposite side of the slide from the SCHARP provided label, on top of the pencil markings.
- Store both slides in the slide box locations assigned in LDMS at room temperature.
- The laboratory will be notified by the MTN NL to ship one of the two slides collected for each participant and visit.
- The duplicate slide will be archived on site until written notification is received from the MTN SDMC that the slide may be discarded.

Instructions for shipping slides to MTN NL
Prepare a LDMS shipping manifest.
Ship to:
Lorna Rabe
Magee-Womens Research Institute
204 Craft Ave, Room A530
Pittsburgh, PA, 15213

Phone: 412-641-6042
e-mail address: lrabe@mwri.magee.edu

12.6.5 Papanicolaou (Pap) Test

Pap smears will be performed when clinically indicated. At visits when Pap smears are needed, ecto- and endocervical cells will be collected after all tissues have been visually inspected and all other required specimens have been collected. Specimen collection, slide preparation, slide interpretation, and QC procedures must be performed and documented in accordance with study site SOPs.

- At some study sites, Pap smear results may include notations of findings associated with certain STIs (e.g., trichomoniasis). Because Pap smear methods are not adequately sensitive and specific for STIs (including HPV), Pap smear findings associated with STIs should not be used to diagnose any STIs during follow up.

12.6.6 HSV-2 Culture

When clinically indicated, HSV-2 culture will be performed. This testing should be done per local site standards.

12.6.7 Chlamydia and Gonorrhea Testing in Cervical swabs

Note: Testing for chlamydia and gonorrhea is done at screening and when clinically indicated only. If participant has used product within the past 24 hours, collect urine instead of cervical swab. (Section 12.5.3)

Following are collection and transport instructions:
Instructions for collection of endocervical swabs (Screening visit only)

- Remove excess mucus from the cervical os with the large-tipped cleaning swab provided in the collection kit (either BD ProbeTec or Gen-Probe Aptima). Discard swab.
- Insert the Endocervical specimen collection swab into the cervical canal and rotate for 15-30 seconds.
- Withdraw the swab carefully and immediately place the swab in the transport tube labeled with a SCHARP label. Make sure the cap is tightly secured to the tube.
- Transport at room temperature to the laboratory.

12.6.8 Vaginal Swab for Quantitative Vaginal Flora Culture

In addition to the wet mounts and Gram stains, vaginal swabs will be collected for Quantitative cultures and sent to the MTN NL. Shipping instructions follow.

- Collect the specimen for culture by rotating 2 Dacron swabs several times over the lateral wall of the vagina. Insert both swabs into 1 Port-A-Cul transport tube (labeled with a SCHARP label), submerging the swabs into the gel and breaking off the shafts of the swabs, and capping. (The Port-A-Cul transport tubes will be provided by MTN NL.)
- The specimen may be kept at controlled room temperature for up to 4 hours. After four hours, the specimen must be refrigerated and shipped with ice packs as detailed below.
- Deliver the Port-A-Cul and the LDMS specimen tracking sheet to the local LDMS laboratory.
- Using the LDMS Tracking Sheet, log the specimen into LDMS (specimen type = VAG) and label the Port-A-Cul tube with LDMS labels.
- Use LDMS to generate a shipping manifest for the cultures to be shipped.
- Ship the Port-A-Cul tube and the vaginal smear for Gram stain the same day of collection by overnight courier.
- Place the Port-A-Cul in a biohazard bag and secure in the leak-proof container with absorbent material. Place the container, ice packs, slides, and a copy of the manifest in a cardboard box lined with Styrofoam.
- Use diagnostics packing code 650, UN3373.
- Confirm the address is correct (see below). The Research Institute is not open for weekend deliveries. Therefore, specimens collected on Friday must be sent to the hospital address for delivery on Saturday.

If sending **Monday through Thursday**, send to:
Lorna Rabe
Magee-Womens Research Institute
204 Craft Ave, Room 530
Pittsburgh, Pa. 15213
Phone# 412-641-6042

If sending on **Friday** for Saturday delivery, send to:
Lorna Rabe, C/O Safety and Security
Magee-Womens Hospital of UPMC
300 Halket St.
Pittsburgh, Pa. 15213
Phone # 412-641-4191 (contact number for Safety and Security)
** Be sure to check Saturday delivery on the Fed Ex label

Notify the MTN NL via email (lrabe@mwri.magee.edu) when the shipment has been picked up from the site by the courier/shipping company. Attach an electronic copy of the shipping manifest to the email

notification, and include the following information in the notification: name of courier/shipping company, shipment tracking number, number of boxes shipped, date of shipment, and expected date of arrival.

12.6.9 Vaginal Swabs for Biomarker Analysis

At each pelvic exam, vaginal fluids are collected from the posterior fornix using a dacron swab with a plastic shaft for biomarker analysis at the MTN NL.

- Two separate dacron swabs will be taken.
- Collect vaginal fluid using a dacron swab from the posterior fornix.
- Place the swab in a SCHARP labeled cryovial containing 400 µL PBS (1X Concentration).
- Break shaft of swab at a minimum of 1cm beyond the swab and cap the vial.
- Repeat with the second dacron swab as described above.
- Store refrigerated or on ice for up to 8 hours prior to delivery to the lab.
- Deliver the tubes and an LDMS Specimen Tracking Sheet to the local LDMS laboratory within 8 hours.
- Using the LDMS Tracking Sheet, log the cryovial into LDMS (specimen type = VAG. See Section Appendix 13-3 for LDMS for additive codes) and label the vial with a LDMS label.
- Freeze at ≤ -70ºC.

12.6.10 Endocervical Swabs for Biomarker Analysis

At each pelvic exam, endocervical cells will be collected using a dacron swab with plastic shaft for biomarker analysis at the MTN NL.

- Remove cervical mucus with a large swab to expose the cell layer (discard swab).
- Two separate dacron swabs will be taken.
- Collect endocervical cells by inserting a dacron swab approximately 1 cm into the endocervical canal and rotating two full turns.
- Withdraw the swab, place it in a labeled cryovial containing 400 µL PBS (1X Concentration).
- Break shaft of swab at a minimum of 1cm beyond the swab and cap the vial.
- Repeat with the second dacron swab as described above.
- Store refrigerated or on ice for up to 8 hours prior to delivery to the lab.
- Deliver the tubes and an LDMS Specimen Tracking Sheet to the local LDMS laboratory within 8 hours.
- Using the LDMS Tracking Sheet, log the cryovial into LDMS (specimen type = CXS. See Section Appendix 13-3 for LDMS for additive and derivative codes) and label the vial with a LDMS label.
- Freeze at ≤ -70ºC.

12.7 Blood Testing

The blood tests performed depends 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 if applicable.

12.7.1 Specimen Collection and Initial Processing

The order of blood draw for multiple tube collections should be Citrate tube, SST and/or serum tube, Heparin tube, then EDTA tube.

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

- Allow serum tubes (i.e., no additive such as red top or serum separator tubes) to clot, then centrifuge per site SOPs to yield serum.
- Gently invert tubes with anticoagulant (i.e., EDTA, heparin) 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 aliquoted. If whole blood is to be used for multiple tests, ensure that the tube is well mixed before removing any specimen.

12.7.2 Plasma Archive

For plasma archive, use whole blood collected in EDTA tubes. 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 on ice, plasma must be processed and frozen within 24 hours of collection. Plasma should be stored frozen on site ≤ -70ºC until requested for shipping and/or testing by the MTN NL.

- Plasma samples may be used for confirmatory HIV testing at the MTN NL, testing for study drug levels at the MTN NL, and possible future research testing (if consent provided by the participant).
- For plasma archive, standard processing per site SOPs should be performed.
- Prepare as many 1 to 2 mL aliquots as possible. If the minimum volume specified is not obtained, notify the MTN NL.
- Use LDMS to label and track all aliquots.
- Store all aliquots frozen on site ≤ -70ºC.
- The MTN NL will send instructions when shipping and/or testing is required.

12.7.2 HIV Testing

Plasma, whole blood and/or serum will be tested for HIV using tests that have been validated at the study site per the Clinical Laboratory Improvement Amendment (CLIA) standards. All tests, and associated QC procedures, must be documented on local laboratory log sheets or other laboratory source documents.

HIV infection status at screening will be assessed using an FDA-approved rapid HIV test per the MTN-008 HIV testing algorithm (see appendix II in the current version of the MTN-008 protocol). If the rapid test is non-reactive, the participant will be considered HIV-uninfected. If the rapid test is reactive, an FDA-approved Western Blot (WB) will be performed; if additional blood must be drawn for the WB, this is still considered sample 1 per the algorithm. If the WB is negative, the participant will be considered HIV-uninfected; this situation is not anticipated-contact the MTN NL if this occurs. If the WB is positive, the participant will be considered HIV-infected. A second specimen will be drawn for confirmatory testing. If the WB is indeterminate, the site should contact the NL for further instructions.

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

12.7.3 Hepatitis B Surface Antigen

This testing will be done on serum per local SOPs. These tests may be done locally or, if approved by the MTN NL, shipped to a regional or network laboratory.

12.7.4 Syphilis Testing

Syphilis testing will be performed using a rapid plasma reagin (RPR) screening test followed by a confirmatory microhemagglutinin assay for *Treponema pallidum* (MHA-TP) or *Treponema pallidum* haemagglutination assay (TPHA). Any RPR, MHA-TP, and/TPHA test may be used; however titers must be obtained and reported for all positive RPR tests. RPR tests may be performed on either serum or plasma. MHA-TP and TPHA tests must be performed on serum. All testing and QC procedures must be performed and documented in accordance with study site SOPs.

For reactive RPR tests observed during screening, a confirmatory test result must be received and appropriate clinical management action taken, prior to enrollment in the study. Clinical management should include repeat RPR tests at quarterly intervals following syphilis diagnosis to confirm treatment effectiveness. If the RPR titer does not decrease fourfold or revert to sero-negative within three months after treatment, treatment should be repeated.

Please consult the MTN NL with any questions related to Syphilis testing to confirm treatment effectiveness and/or interpretation of unusual test results.

Questions related to result interpretation vis-à-vis eligibility and enrollment in the study should be directed to the MTN-008 Protocol Safety Review Team.

12.7.5 Blood Chemistries

The following chemistry tests will be performed per local SOPs:

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

12.7.6 Hematology Testing

Complete blood counts with five-part differentials will be performed on EDTA whole blood per local site SOPs at all sites. Each of the following must be analyzed and reported:

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

12.7.7 Flow Cytometry for CD38 and HLA-DR

- EDTA whole blood is analyzed for CD38 and HLA-DR by methods defined in local SOP's.
- WBC and % lymphocyte values needed to calculate final CD38 and HLA-DR will be obtained from the CBC with differential results.

Flow Cytometry results and CBC with differential results are needed to complete the calculations for final results reported on the CRF. Worksheets that may be used to calculate final flow cytometry results are

available in the Study Implementation Materials section of the MTN-008 web page. It is the clinic's responsibility to datafax the results to SCHARP once they have been completed and received.

12.8 Testing for Tenofovir levels

Collection of specimens for pharmacokinetic (PK) analysis should be performed within the collection time windows determined by the protocol. Once specimen time intervals have been set at the initial PK visit, each PK specimen collection at any following visit should be targeted at the same time interval. For example if at the initial PK visit the 1 hour specimen was collected 65 minutes after dosing then at each following visit, the 1 hour specimen should also be targeted to 65 minutes post-dose. The collection window should be adjusted accordingly. A PK collection timing tool is available on the MTN website to help set those collection time windows.

Each of the following specimens will be tested for Tenofovir levels and should be collected, processed, and stored accordingly. Once processed, these specimens can be batched and shipped to the MTN Pharmacology Core for testing.

12.8.1 Tenofovir Levels in blood

The specimens should be kept at room temperature and centrifuged as soon as possible after collection (≤ 8 hours).

Red Top tubes for serum Tenofovir

- Centrifuge whole blood at 800 RCF (Relative Centrifugal Force) for 10 minutes. If red blood cells are not sufficiently separated from serum, centrifugation for an additional 5 minutes may be required.
- Aliquot serum into two approximately equal portions (approximately 1.5ml each) and placed in LDMS labeled cryovials. For infant samples, serum may be pooled into one cryovial (approximately 0.5ml).
- Use LDMS to label and track all aliquots.
- Store all aliquots frozen on site at approximately -20°C or colder.
- The MTN NL will send instructions when shipping and/or testing is required.

Peripheral Blood Mononuclear Cells for Intracellular Tenofovir

- Draw two 8 mL Cell Preparation Tubes (CPT) with Sodium Citrate (BD Cat# 362761 is recommended) at each PK time point.
- Specimens must be kept upright at room temperature and processed within 8 hours. The following instructions were obtained from the Johns Hopkins SOP.
    1. Invert CPT tubes gently to mix anticoagulant thoroughly.
    2. Spin at 1700 RCF for 25 minutes using a refrigerated centrifuge set at 18-25°C. Centrifuge temperatures may rise when spinning for extended amounts of time.
    3. Gently invert tubes to resuspend PBMC in plasma.
    4. Pour off both tubes (same time point) into one 50mL conical tube.
    5. Add isotonic saline to bring volume up to 30mL.
    6. Remove 0.5mL for cell count. Perform cell count during step 6.
    7. Centrifuge conical tube for 15 minutes at 400 RCF.
    8. Aspirate/pour off supernatant without disrupting cell pellet. Invert tube onto absorbent tissue to remove as much supernatant as possible.
    9. Add 1.0mL of fresh ice cold 70% methanol (7 parts methanol and 3 parts distilled water). Vortex lightly.

10. Transfer all contents to cryovial ~ 1.0ml.
11. When logging into LDMS, although there were 2 CPT specimens collected, enter the primary specimen as 1 sample with a volume of 16ml since they are pulled at processing. Use LDMS to label and track all aliquots.
12. Place PBMC lysate directly into -70°C freezer. Record time frozen. This should be completed within 9.5 hours from collection.
13. The MTN NL will send instructions when shipping and/or testing is required.

12.8.2 Tenofovir Levels in Cord Blood

Cord blood should be drawn in red top (non-preservative) tubes and preferably processed within one hour of collection. Processing may be delayed for up to 8 hours if the whole blood specimen is kept upright at room temperature.

- Centrifuge the cord blood at 800 RCF (Relative Centrifugal Force) for 10 minutes. If red blood cells are not sufficiently separated from serum, centrifugation for a further 5 minutes may be required.
- Aliquot serum into two approximately equal portions (approximately 1.5ml each) and placed in LDMS labeled cryovials.
- Use LDMS to label and track all aliquots.
- Freeze aliquots at approximately -20°C or colder.

12.8.3 Tenofovir levels in Breast Milk

Breast milk collection may be obtained from one or both breasts. The participant should empty all the milk from at least one side. If milk is collected from both breasts, the milk can be combined in a single container.

- Gently swirl to mix.
- Label 2 cryovials provided with the participant's PID, collection date and time.
- Using a disposable pipette, transfer approximately 1.5 – 2.0 ml breast milk into each cryovial.
- Use LDMS to label and track all aliquots.
- Freeze aliquots immediately at approximately -20°C or colder.

12.8.4 Shipping of Specimens for Tenofovir Levels

One set of samples will be shipped to the MTN Network Lab in Baltimore, MD and assayed for PK levels. The other set will be retained at the site until advised by the MTN-008 leadership group. Do not ship samples until requested by the MTN NL or study team.

The shipping address is:

MTN Network Lab Pharmacology Core
Johns Hopkins University
527 Osler
600 N. Wolfe Street
Baltimore, MD 21237

Section Appendix 12-1
Overview of Laboratory Testing Locations, Specimens, and Methods for MTN-008

Test	Testing Location	Specimen Type	Tube/Container	Kit/Method
Urine hCG (lactation cohort)	In Clinic	Urine	Plastic screw top cup	Quidel Quick Vue or Fisher Sure Vue
Trichomonas test	Local Lab	Vaginal Swab	Sterile plastic tube	OSOM rapid test
*Vaginal wet preparation	In Clinic	Vaginal Swab	N/A	N/A
Vaginal pH	In Clinic	Vaginal Swab	N/A	Machary Nagel pH Strips
*Pap smear	Local Lab	Ecto- and Endo-cervical cells	Slides	Not specified
*Herpes culture	Local Lab	Ulcer Swab	Viral Transport Media (Must be appropriate for HSV-2)	Not specified
chlamydia and gonorrhea in cervical swabs (screening visit only)	Local Lab	Cervical Swab	Preservation tube	BD Probetec/ GenProbe Aptima
chlamydia and gonorrhea in urine (follow-up visits only)	Local Lab	Urine	Preservation tube	BD Probetec/ GenProbe Aptima
Vaginal microflora (pregnancy cohort)	MTN Network Lab	Vaginal Swab x 2	Port-a-Cul Tube	Network Lab Procedure
Gram stain (pregnancy cohort)	MTN Network Lab	Vaginal Swab	Slides x 2	Network Lab Procedure
Vaginal biomarkers	MTN Network Lab	Vaginal Swab	Cryovial x 2	Network Lab Procedure
Cervical biomarkers	MTN Network Lab	Cervical Swab	Cryovial x 2	Network Lab Procedure
Creatinine	Local Lab	Serum or plasma	Red or marble (serum separator) top tube Green (Sodium Heparin) top tube maybe used based on site capacity	Not specified
AST and ALT	Local Lab	Serum or plasma	Red or marble (serum separator) top tube Green (Sodium Heparin) top tube maybe used based on site capacity	Not specified
CBC with differential and Hemoglobin	Local Lab	Whole blood	Purple (EDTA) top tube	Not specified

HIV serology	Local Lab	Plasma or serum	Purple (EDTA) or Red top tube	FDA approved test
*Confirmatory Testing for HIV	Local Lab	Plasma or whole blood (*serum acceptable*)	Purple (EDTA) or red top tube	FDA approved Western blot test
*HBsAg	Local Lab	Serum or Plasma	Red or Purple (EDTA) top tube	Not specified
*RPR	Local Lab	Serum or Plasma	Red or Purple (EDTA) top tube	Not specified
*Confirmatory Test for Syphilis	Local Lab	Serum or Plasma	Red or Purple (EDTA) top tube	Not specified
Tenofovir Levels	MTN Network Lab	Serum	Red top tube	Network Lab Procedure
		Cord Blood (pregnancy cohort)	Red top tube	
		Expressed breast milk (lactation cohort)	cryovial	
		**PBMC	Blue tiger (CPT) top tube	
**Flow Cytometry	Local Lab	Plasma	Purple (EDTA) top tube	Not Specified
Plasma archive	On-site until notified by NL	Plasma	Purple (EDTA) top tube	N/A

*As clinically appropriate
** If site capacity allows

Section Appendix 12-2
Scheduled Blood Collection by Visit Type and Suggested Volumes

Visit Type	Total Blood Volume	Volume By Tube Type	Purpose
Screening Visit	12ml	Red or Green Top: 3ml	Creatinine, ALT, AST
		Red Top: 3ml	RPR, HBsAg
		Purple Top: 3ml (x2)	CBC w/ diff, Hb, HIV-1 Antibody Test
Enrollment Visit	3ml	Purple Top: 3ml	Plasma archive
	129ml	Red Top: 4ml for each time point	Tenofovir blood levels (pre-gel, 1, 2, 4, 6, 8 hour time points)
		CPT (Blue tiger): 8ml (x2) for each time point	Intracellular Tenofovir levels (pre-gel, 1, 2, 4, 6, 8 hour time points)
		Purple Top: 3ml (x3)	CBC w/ diff, Hb, Flow cytometry, HIV-1 Antibody Test
	1.2ml	Red Top: 0.6ml (x2)	Infant Tenofovir blood level 6 hour (lactation cohort)
Day 6 Visit	12ml	Red or Green Top: 3ml	Creatinine, ALT, AST
		Red Top: 3ml	RPR, HBsAg
		Purple Top: 3ml (x2)	CBC w/ diff, Hb, HIV-1 Antibody Test
	126ml	Red Top: 4ml for each time point	Tenofovir blood levels (pre-gel, 1, 2, 4, 6, 8 hour time points)
		CPT (Blue tiger): 8ml (x2) for each time point	Intracellular Tenofovir levels (pre-gel, 1, 2, 4, 6, 8 hour time points)
		Purple Top: 3ml (x2)	CBC w/ diff, Flow cytometry
	1.2ml	Red Top: 0.6ml (x2)	Infant Tenofovir blood level 6 hour (lactation cohort)
Interim Visit	12ml	Green Top: 3ml (x2) Red Top: 3ml	Creatinine, ALT, AST RPR, HBsAg
		Purple Top: 3ml	HIV-1 Antibody Test
Delivery Visit (Pregnancy Cohort)	4ml	Red Top: 4ml	Cord Blood Tenofovir level
	1.2ml	Red Top: 0.6ml (x2)	Infant Blood Tenofovir level
	10ml	Red Top: 4ml	Maternal Blood Tenofovir level
		Purple Top: 3ml (x2)	CBC w/ diff, Flow Cytometry

Notes: Additional blood may be collected for any clinically indicated testing. Red top tubes contain no additive. Lavender top tubes contain EDTA. Green top tubes contain Sodium Heparin. CPT tubes contain Na Citrate and Ficoll medium. The draw order for these tubes are Citrate (CPT), SST/serum (red), Heparin (green), then EDTA (purple) tubes.

Section Appendix 12-3
LDMS Specimen Management Guide to Logging in 008 Specimens

The table below should be used as a guide when logging in 008 specimens. Please use the LDMS codes listed below when logging in specimens for each test listed. Tests that are listed as local do not require that a sample be logged into the LDMS. See Appendix 12-5 for a copy of the LDMS tracking sheet.

Test	Primary	Additive	Derivative	Sub Add/Derv	Primary Volume	No. of Aliquots	Aliquot Volume	Units
*Serum for Tenofovir levels	BLD	NON	SER	N/A	4.0	1	1.5	ML
					1.0 (infant)	1 (infant)	0.5 (infant)	ML
PBMCs for Tenofovir levels	BLD	CPS	CEL	MET	16.0	1	Varies by cell count	CEL
Cord Blood	CRD	NON	SER	N/A	4.0	1	1.5-2.0	ML
Breast Milk	BMK	NON	BMK	N/A	3.0-4.0	2	1.5-2.0	ML
Vaginal microflora	VAG	PAC	SWB	N/A	2.0	1	2	EA
Gram stain	VAG	NON	SLD	GRS	2.0	2	1	EA
Vaginal biomarkers	VAG	PBS	VAG	N/A	0.80	2	0.40	ML
Cervical biomarkers	CXS	PBS	CXS	N/A	0.80	2	0.40	ML
Plasma archive	BLD	EDT	PLA	N/A	3.0	1	1.5	ML

**For blood Tenofovir levels, please enter time point of pre-dose using 0.00 pre-dose. All other time points use 1, 2, 4, 6, or 8 hour*

BLD: Whole Blood
CRD: Cord Blood
BMK: Breast Milk
VAG: Vaginal Swab
CXS: Cervical Swab

NON: None
CPS : Cell Preparation Tube
PAC : Port-a-cul Transport Tube
PBS: Phosphate Buffered Saline
EDT: EDTA

SER: Serum
CEL: Cell Pellet
SWB: Swab
SLD: Slide
PLA: Plasma
MET: Methanol
GRS: Gram Stain

Section Appendix 12-4
Specimen Shipping Summary

Specimen	Use LDMS?	Ship to:	Shipping schedule
Blood and PBMCs for PK	Yes	MTN Network Lab – Johns Hopkins	If already processed, may be batched
Cord Blood	Yes	MTN Network Lab – Johns Hopkins	If already processed, may be batched
Breast Milk	Yes	MTN Network Lab – Johns Hopkins	If already processed, may be batched
Vaginal microflora	Yes	MTN Network Lab – Pittsburgh	Ship same day overnight
Gram stain	Yes	MTN Network Lab – Pittsburgh	Ship same day overnight
Vaginal biomarkers	Yes	MTN Network Lab – Pittsburgh	Frozen and batched
Cervical biomarkers	Yes	MTN Network Lab – Pittsburgh	Frozen and batched
Plasma archive	Yes	On-site until requested by MTN Network Lab – Pittsburgh	If already processed, may be batched

Section Appendix 12-5
LDMS Tracking Sheet

MTN-008 Maternal PK- LDMS Specimen Tracking Sheet

For login of maternal MTN-008 stored specimens into LDMS

Participant ID ☐☐☐-☐☐☐☐-☐-0 (Site Number / Participant Number / Chk / Who) Visit Code ☐☐.☐ Specimen Collection Date ☐☐ dd ☐☐☐ MMM ☐☐ yy

MATERNAL PK BLOOD COLLECTION (PREGNANCY AND LACTATION COHORTS)

PK SPECIMEN TIME	PRIMARY SPECIMEN TYPE	TIME COLLECTED hh:mm 24-hr clock	NUMBER OF TUBES COLLECTED	INSTRUCTIONS FOR PROCESSING LAB
Pre-gel	Maternal Blood (BLD) **Tenofovir Level**		☐ Non (red top)	Process within eight hours of collection. Freeze immediately after centrifugation. Store with derivative SER
	Maternal Blood (BLD) **PBMC**		☐ CPS (CPT Tube)	The time from blood draw to centrifugation and lysis should be eight hours or less. Store with derivative CEL.
1 Hour	Maternal Blood (BLD) **Tenofovir Level**		☐ Non (red top)	Transport to lab and process within eight hours. Freeze immediately after centrifugation. Store with derivative SER.
	Maternal Blood (BLD) **PBMC**		☐ CPS (CPT Tube)	The time from blood draw to centrifugation and lysis should be eight hours or less. Store with derivative CEL.
2 Hour	Maternal Blood (BLD) **Tenofovir Level**		☐ Non (red top)	Transport to lab and process within eight hours. Freeze immediately after centrifugation. Store with derivative SER.
	Maternal Blood (BLD) **PBMC**		☐ CPS (CPT Tube)	The time from blood draw to centrifugation and lysis should be eight hours or less. Store with derivative CEL.
4 Hour	Maternal Blood (BLD) **Tenofovir Level**		☐ Non (red top)	Transport to lab and process within eight hours. Freeze immediately after centrifugation. Store with derivative SER.
	Maternal Blood (BLD) **PBMC**		☐ CPS (CPT Tube)	The time from blood draw to centrifugation and lysis should be eight hours or less. Store with derivative CEL.

MTN-008 Maternal PK- LDMS Specimen Tracking Sheet

For login of maternal MTN-008 stored specimens into LDMS

Participant ID ☐☐☐-☐☐☐☐-☐-0 (Site Number / Participant Number / Chk / Who) Visit Code ☐☐.☐ Specimen Collection Date ☐☐ dd ☐☐☐ MMM ☐☐ yy

6 Hour	Maternal Blood (BLD) **Tenofovir Level**		☐ Non (red top)	Transport to lab and process within eight hours. Freeze immediately after centrifugation. Store with derivative SER.
	Maternal Blood (BLD) **PBMC**		☐ CPS (CPT Tube)	The time from blood draw to centrifugation and lysis should be eight hours or less. Store with derivative CEL.
8 Hour	Maternal Blood (BLD) **Tenofovir Level**		☐ Non (red top)	Transport to lab and process within eight hours. Freeze immediately after centrifugation. Store with derivative SER.
	Maternal Blood (BLD) **PBMC**		☐ CPS (CPT Tube)	The time from blood draw to centrifugation and lysis should be eight hours or less. Store with derivative CEL.
Delivery visit	Maternal Blood (BLD) **Tenofovir Level**		☐ Non (red top)	Transport to lab and process within eight hours. Freeze immediately after centrifugation. Store with derivative SER.
	Maternal Blood (BLD) **PBMC**		☐ CPS (CPT Tube)	The time from blood draw to centrifugation and lysis should be eight hours or less. Store with derivative CEL.

MATERNAL PK BREAST MILK COLLECTION (LACTATION COHORT)

PK SPECIMEN TIME POINT	PRIMARY SPECIMEN TYPE	TIME COLLECTED hh:mm 24-hr clock	NUMBER OF TUBES COLLECTED	INSTRUCTIONS FOR PROCESSING LAB
Pre-gel	Breast milk (BMK)		☐ Non (cryovial)	Freeze immediately. Store with derivative BMK.
2 Hour	Breast milk (BMK)		☐ Non (cryovial)	Freeze immediately. Store with derivative BMK.
4 Hour	Breast milk (BMK)		☐ Non (cryovial)	Freeze immediately. Store with derivative BMK.
6 Hour	Breast milk (BMK)		☐ Non (cryovial)	Freeze immediately. Store with derivative BMK.

MTN-008 Maternal PK- LDMS Specimen Tracking Sheet

For login of maternal MTN-008 stored specimens into LDMS

Participant ID: ☐☐☐-☐☐☐☐-☐-0 (Site Number – Participant Number – Chk – Who)

Visit Code: ☐☐.☐

Specimen Collection Date: ☐☐ (dd) ☐☐☐ (MMM) ☐☐ (yy)

ALL OTHER MATERNAL SPECIMENS

# of TUBES or SPECIMENS	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALIQUOT DERIVATIVE	ALIQUOT SUB ADDITIVE/ DERIVATIVE	NOTES FOR LAB
☐	Blood (BLD) Plasma **Collection Time:** __ __:__ __ *hour : min*	EDT (purple top)	PLA	N/A	Store in aliquots of 1-2 ml. If held at room temperature, plasma must be frozen within 4 hours of collection. If refrigerated or on ice, plasma must be frozen within 8 hours of collection.
☐	Endo-cervical Swab (CXS) **Collection Time:** __ __:__ __ *hour : min*	PBS (Phosphate buffered saline)	CXS	N/A	Place swab in crovial with PBS. Freeze within 8 hours of collection.
☐	Vaginal Swab (VAG) **Collection Time:** __ __:__ __ *hour : min*	PBS (Phosphate buffered saline)	VAG	N/A	Place swab in crovial with PBS. Freeze within 8 hours of collection.
☐	Vaginal Swab (VAG) **Collection Time:** __ __:__ __ *hour : min*	PAC	SWB	N/A	Ship to NL on ice day of collection
☐	Vaginal Gram Stain Slide (VAG)	NON (no additive)	SLD	GRS	Re-label with LDMS label. Store duplicate slides (one for on-site storage, and one for shipping and testing at MTN Network Lab).

Comments: _____

Initials: _____ (Sending Staff) _____ (Receiving Staff) LDMS Data Entry Date: ☐☐ (dd) ☐☐☐ (MMM) ☐☐ (yy) / _____ (LDMS Staff)

Version 2.0, 11-APR-11

MTN-008 Maternal PK- LDMS Specimen Tracking Sheet

For login of maternal MTN-008 stored specimens into LDMS

Purpose: This non-DataFax form is used to document collection and entry of MTN-008 maternal PK blood and breast milk specimens into the Laboratory Data Management System (LDMS).

General Information/Instructions: A copy of this form accompanies maternal PK blood and breast milk specimens (in their original specimen collection containers) to the LDMS entry laboratory. Once the specimens have been entered into LDMS, this form is kept on file at the LDMS entry laboratory. If the site chooses, a copy of this completed form may be made once the specimens have been entered into LDMS and the copy kept in the participant's study notebook. This is not required, however. Because this form is a non-DataFax form, this form should NOT be faxed to SCHARP DataFax.

Item-specific Instructions:

- **Visit Code:** Record the visit code of the visit at which the LMDS specimens were collected.
- **NUMBER OF TUBES COLLECTED:** In the box to the left of each additive type, record the total number of tubes collected. If no LDMS specimens of the primary specimen type were collected, record "0."
- **Initials – Sending Staff:** The clinic staff person who completed the form and/or who is sending the LDMS form and specimens to the LDMS entry lab, records his/her initials here.
- **Initials - Receiving Staff:** The laboratory staff person who received this form (and the LDMS specimens accompanying the form), records his/her initials here.
- **LDMS Data Entry Date:** Record the date the LDMS specimens listed on this form were entered into LDMS.
- **LDMS Data Entry Date - LDMS Staff:** The LDMS laboratory staff person who entered the specimens into LDMS, records his/her initials here.

Version 1.0, 22-JUN-10