

Section 12. Laboratory Considerations

12.1 Overview and General Guidance

This section contains information on the laboratory procedures performed in MTN 002.

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

Some laboratory procedures will be performed in the study site clinic or laboratory and others in the MTN Network Laboratory (NL). For samples going to any MTN NL (Pittsburgh or Johns Hopkins), please contact Pam Kunjara prior to the scheduled c-section for LDMS aliquot labels and after all samples are collected for final pick-up.

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Table 12-1 lists for each test the testing location, specimen type, specimen container and kit/method (if specified). Table 12-2 specifies blood collection by visit type and suggested volumes.

Regardless of whether tests are performed in clinic or laboratory settings, study staff that performs the tests must be trained in proper QC procedures prior to performing the tests for study purposes; training documentation should be available for inspection at any time.

Table 12-1
Overview of Laboratory Testing Locations, Specimens,
And Methods for MTN 002

Test	Testing Location	Specimen Type	Tube/Container	Kit/Method
Trichomonas Test	MTN Network Lab	Vaginal swab	InPouch TV	Network Lab Procedure
*Vaginal wet preparation	In Clinic	Vaginal fluid Swab	N/A	N/A
*Vaginal pH	In Clinic	Vaginal fluid Swab	N/A	Machary Nagel pH Strips
*Herpes culture	Local Lab	Ulcer Swab	Viral Transport Media (Must be appropriate for HSV-2)	Not specified
Urine SDA for Gonorrhea and Chlamydia	MTN Network Lab	Urine	Urine Preservation Tube (UPT)	BD Probetec/ GenProbe Aptima
Creatinine	Local Lab	Serum	Red or marble (serum separator) top tube	Not specified
AST and ALT	Local Lab	Serum	Red or marble top	Not specified
HIV antibody screen	Local Lab	Plasma	Purple (EDTA)	FDA approved rapid test
*Confirmatory Testing for HIV	Local Lab	Plasma or whole blood (<i>serum acceptable</i>)	Purple or red top tube	FDA approved Western blot test
*HBsAg	Local Lab	Serum or Plasma	Red or Purple top tube	Not specified
*RPR	Local Lab	Serum or Plasma	Red or Purple top tube	Not specified
*Confirmatory Test for Syphilis	Local Lab	Serum or Plasma	Red or Purple top tube	Not specified
Tenofovir Levels	MTN Network Lab	Serum	Red top tube	Network Lab Procedure
		Cord Blood	Red top tube	
		Amniotic Fluid	Cryovial	
		Placental Tissue	Cryovial	
		Endometrial Tissue	Cryovial	
Flow Cytometry	MTN Network Lab	Plasma	Purple top tube (EDTA)	Network Lab Procedure

*As clinically appropriate

Sites are responsible to ensure that specimen volumes do not exceed what is described in the informed consent process. The MTN NL may request details of collection containers and volumes for this purpose.

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

Visit Type	Total Blood Volume (ml)	Volume By Tube Type (ml)	Purpose
Screening and Enrollment Visit	13	Red Top:10	Creatinine, ALT, AST RPR, HBsAg
		Purple Top:3	HIV-1 Antibody Test,
Gel Administration Day	34	Red Top: 4 for each time point	Tenofovir blood levels (pre-gel, 1, 2, 4, 6, 8, 12 hour time points)
		Purple Top: 3 (x2)	Flow cytometry
24 Hour Evaluation	4	Red Top: 4	Tenofovir blood Level
Unscheduled Visit	17	Red Top: 10, 4	Creatinine, ALT, AST RPR, HBsAg, Tenofovir blood Level
		Purple Top: 3	HIV-1 Antibody Test,

Notes: Additional blood may be collected for any clinically indicated testing. Red top tubes contain no additive. Lavender top tubes contain EDTA.

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 implementing a change in methods. The MTN NL must be notified before implementing the change and the MTN NL can provide further guidance on validation requirements. Similarly, the MTN NL must be notified of changes to normal lab ranges.

Adherence to the specifications of this section is essential to ensure that primary and secondary endpoint data derived from laboratory testing will be considered acceptable to all regulatory authorities.

This section of the MTN 002 SSP manual gives basic guidance to the sites but is not an exhaustive procedure manual for all laboratory testing. This section must be supplemented with Standard Operating Procedures. The MTN NL is available to assist in the creation of any SOPs upon request. Essential SOPs include but are not limited to:

- SOPs created by the site
 - Specimen Collection and transport
 - Chain of Custody *
 - Hematology procedures
 - Chemistry (AST, ALT, Creatinine)
 - Syphilis Serology
 - Urine
 - SDA for GC/CT
 - Dipstick
 - HIV testing
 - HBsAG
 - Herpes Culture (for sites where standard of care)

*Must be approved by the MTN NL for study activation

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. 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, amniotic fluid, cord blood, placental tissue, and endometrial tissue.

12.3 Procedures for Specimens that can not be evaluated

When possible, specimens will be redrawn or recollected if it is found that they cannot be evaluated per site SOP's. The site will monitor specimen management problems as part of ongoing Quality Assurance. In cases where additional specimens need to be recollected due to a laboratory error (lost or broken specimen or clerical error) or a clinic error (clerical error), a protocol event form provided by the NL may be required.

12.4 Use of LDMS

The Laboratory Data and Management System (LDMS) is a program used for the storage and shipping of laboratory specimens. It is supported by the Frontier Science Foundation (FSTRF). LDMS must be used to track the collection, storage, and shipment of five types of specimens in MTN 002: Tenofovir blood specimens, amniotic fluid, cord blood, placental tissue, and endometrial tissue.

Detailed instructions for use of LDMS are provided at: <https://www.fstrf.org/ldms> (may require a password).

The site will be required to maintain the current version of LDMS and monitor updates relating to use of the LDMS. It is crucial to be aware of proper label formats to ensure that specimens are correctly labeled. 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 002 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 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 (address shown in table 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

If a response is not received within 15 minutes after emailing LDMS 1, try emailing LDMS 2, then finally, LDMS 3. The pagers also can be reached via telephone. When paging via telephone, after dialing you will hear a voice greeting followed by three quick beeps that indicate you are connected to the paging service. Please include the full telephone number at which you can be reached, including the area code. Please call LDMS pager 1 first (telephone number shown in table below). If you do not receive a response within 15 minutes after calling LDMS 1, please try LDMS 2, then finally, LDMS 3.

Table 12-3
LDMS User Support Paging Details

Pager	Email Address	Telephone Number
LDMS 1	ldmspager1@fstrf.org	716-556-0583
LDMS 2	ldmspager2@fstrf.org	716-556-0584
LDMS 3	ldmspager3@fstrf.org	716-556-0585

The site must export its LDMS data to Frontier Science (FSTRF) on a weekly basis. Exported data are used by the MTN SDMC to generate a monthly specimen repository report and to reconcile data entered in LDMS with data entered on study case report forms. Any discrepancies identified during the reconciliation are included in a monthly discrepancy report for the 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.

Table 12-4
LDMS Specimen Management Guide to Logging in 002 Specimens

The table below should be used as a guide when logging in 002 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 12-1 for a copy of the LDMS tracking sheet.

Test	Primary	Additive	Derivative	Sub Add/Derv	Primary Volume	Aliquot Volume	Units
*Blood for Tenofovir levels	BLD	NON	SER	N/A	4.0	1.5	ml
Amniotic Fluid	AMN	NON	AMN	N/A	2.0-4.0	1.0-2.0	ml
Cord Blood	CRD	NON	SER	N/A	4.0	1.5	ml
Placental tissue	PLC	NON	PLC	N/A	1-2	1-2	cm
Endometrial tissue	END	NON	END	N/A	1-2	1-2	cm

**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, 8, or 12 hour*

**Table 12-5
Specimen Shipping Summary**

Specimen	Use LDMS?	Ship to:	Shipping schedule
Vaginal Swab for Trichomonas Culture	No	MTN Network Lab - Pittsburgh	Within 48 hours of collection
Blood for PK	Yes	MTN Network Lab – Johns Hopkins	If already processed, may be batched
Amniotic Fluid	Yes	MTN Network Lab – Johns Hopkins	If already processed, may be batch
Cord Blood	Yes	MTN Network Lab – Johns Hopkins	If already processed, may be batched
Placental tissue	Yes	MTN Network Lab – Johns Hopkins	If already processed, may be batched
Endometrial tissue	Yes	MTN Network Lab – Johns Hopkins	If already processed, may be batched
Urine for GC/CT testing	No	MTN Network Lab - Pittsburgh	Batched with Trich pouch

Note: All samples going to the MTN Network Lab – Johns Hopkins should first be sent to the MTN NL – Pittsburgh for LDMS entry and shipping. Please contact the MTN NL for specimen pick-up.

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12.5 Urine Testing for Urinalysis, Chlamydia and Gonorrhea

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 aliquotted for each test when possible. When doing multiple tests from one specimen, the correct order is separation of urine for the Chlamydia and Gonorrhea first, then the urine dipstick last.

Note: Testing for Chlamydia and Gonorrhea is done at screening and when clinically indicated only.

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 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 Dipstick Urinalysis

Dip the urinalysis test strip into an aliquot of urine. Perform this test according to site SOPs and the package insert. Assess and record results for blood, glucose, protein, leukocytes and nitrites. If leukocytes or nitrites are positive, perform a urine microscopy and a urine culture according to local SOP. To avoid overgrowth of bacteria, refrigerate specimen before and during transport to laboratory.

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

12.5.3 Chlamydia and Gonorrhea Testing

Note: Testing for Chlamydia and Gonorrhea is done at screening and when clinically indicated only.

This testing will be done at the MTN NL using the BD Probe Tec Method. Sites will be required to send samples in the BD Urine Preservation Tubes (UPT). Following are collection and transport instructions:

Instructions for transferring urine into the UPT

- Collect urine as noted above.
- Open the UPT kit 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.

Transport instructions for urine samples to Magee-Women's Research Institute

- Fill out a shipping/tracking manifest with the information listed in the example located in appendix 12-2 (Do not use LDMS for urine specimens).
- Place the tubes in a biohazard zip-lock bag. Include manifest.
- Contact MTN NL by phone or pager for pick-up.

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12.6 Blood Testing for HIV, Syphilis, Liver and Renal Function, Blood Tenofovir Levels, and Flow Cytometry

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.

12.6.1 Specimen Collection and Initial Processing

Label all required primary tubes with a SCHARP-provided PTID label at the time of collection. If samples are to be processed and frozen, label aliquots with LDMS aliquot labels provided by the MTN Network Lab. Contact Pam Kunjara on the day of scheduled c-section in order to have LDMS labels ready.

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After collection:

- Allow red top tubes (no additive) or marble top (serum separator tubes) to clot, then centrifuge per site SOPs to yield serum for PK levels, syphilis, liver function, and renal function testing. For blood PK levels, the sample must be processed within eight hours from collection. Please contact MTN NL for pick-up and processing. If the lab is unavailable processing may be performed on site.
- Lavender top tubes (additive = EDTA) should be gently inverted at least eight times after specimen collection to prevent clotting. EDTA tubes are used for HIV testing, and flow cytometry.

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

12.6.2 HIV Testing

Plasma 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 002 HIV testing algorithm (see appendix II in the current version of the MTN 002 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.

All test results must be documented on local laboratory log sheets or other laboratory source documents. In addition to initialing or signing the testing logs to document review and verification of the results, the second lab staff member must also record the time at which the results were reviewed and verified.

12.6.3 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 002 Protocol Safety Review Team.

12.6.4 Liver and Renal Function Testing

The following tests will be performed to evaluate liver and renal function:

Liver Function

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

Renal Function

- Creatinine

These chemistry tests will be performed on serum.

12.6.5 Blood Tenofovir Levels

The specimens should be kept at room temperature and centrifuged as soon as possible after collection (\leq 8 hours). Contact NL for processing. If the lab is unavailable (i.e. late collection), processing can be done on site.

The whole blood will be centrifuged 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. The serum will be transferred into two approximately equal portions (approximately 1.5ml each) and placed in LDMS labeled cryovials and frozen at approximately -20°C or colder.

Please contact the MTN NL for pick-up and shipping.

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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 MTN NL - Pittsburgh until advised by the MTN leadership group.

The shipping address is:

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

12.6.6 Flow Cytometry for CD38 and HLA-DR

Two (2) EDTA purple top tubes will be collected. One will be sent along with a requisition (Appendix 12-3) via courier to the Flow Cytometry lab U Pitt, Parran Hall, Room 523 and the other will be sent to the Magee clinical lab. The Magee clinical lab will perform a CBC/Diff/platelet in order to obtain WBC and % lymphocyte values needed to calculate final CD38 and HLA-DR. EDTA whole blood is analyzed for CD38 and HLA-DR by methods defined in local SOP's.

To contact the courier for specimens going to the Flow Cytometry Lab at U Pitt, page 412-765-5075. If there is no response to the page within 10 minutes, call 412-647-8125 for further assistance.

Flow Cytometry results will be reported directly to the NL. The NL will obtain the CBC results needed to complete the calculations for Flow Cytometry and submit final results to the clinic on CRF. It is the clinic's responsibility to datafax the results to SCHARP once they have been completed and received.

12.7 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.

12.7.1 Vaginal pH

When clinically indicated 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, collect vaginal fluids using a swab. Dab the vaginal fluids from the swab onto the pH strip.
- 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.

12.7.2 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, trichomoniasis, and candidiasis, as summarized in Table 12-6.

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 appropriate case report form.

Prior to study initiation, the MTN NL will conduct on-site training and proficiency testing for clinic and laboratory staff designated to perform wet mounts. 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: rsilkr@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 12-6
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 (<i>Gardnerella vaginalis</i> and/or anaerobic GNR) to be counted as clue cells.	Not applicable (clue cells are lysed by KOH)
Trichomonads	Positive if at least one motile trichomonad is observed. Actively motile organisms are easily seen upon low power (10X). High power (40X) may be needed to detect less vigorously motile organisms when only the flagella may be moving.	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:

- 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 (*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 10X and 40X magnification for yeast and pseudohyphae.

12.7.3 *Trichomonas* Testing

The InPouch TV is a self-contained system for the detection of *Trichomonas vaginalis*. Prepare vaginal swab as follows:

- To avoid fluid leakage, squeeze the fluid from the top of the InPouch downward toward the bottom of the chamber.
- Tear off the plastic above the white closure.
- To admit the cotton swab, open the InPouch by pulling the closure tape's middle tabs apart.
- Milk the swab between the InPouch walls. Remove the swab and discard.
- Squeeze the top closed, roll top of upper chamber down one complete roll, pushing the medium into the bottom chamber, and fold the tabs over to prevent the InPouch from reopening.
- Fill in patient information and place patient label over the blue BioMed label – not on the viewing chamber.
- Store inoculated pouch upright for up to 48 hours at room temperature. **DO NOT REFRIGERATE!** If held longer than 48 hours, incubation at 37°C is required.
- Contact MTN NL by phone 412-641-6393 or pager 412-917-9343 for pick-up. MTN NL will process the inoculated pouches according to laboratory protocol.

Transport instructions for InPouch TV samples to Magee-Women's Research Institute

- Fill out a shipping/tracking manifest with the information listed in the example located in appendix 12-2 (Do not use LDMS for InPouch TV samples).
- Place the pouch in a biohazard zip-lock bag. Include manifest.
- Contact MTN NL by phone or pager for pick-up.

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12.7.4 *HSV-2 Culture*

When clinically indicated, HSV-2 culture will be performed. This testing should be done per local site standards. The specimens may be batched and tested at the end of the study unless results are needed for clinical management.

12.8 Testing of Specimens Collected during or after Cesarean Section (Cord Blood, Amniotic Fluid, Placental Tissue, and Endometrial Tissue)

Each of the following specimens will be tested for Tenofovir levels and should be collected, processed, and stored accordingly. Once processed, contact the MTN Network Lab for pick-up and shipment to the MTN Pharmacology Core.

12.8.1 PK levels in Cord Blood

Cord blood should be drawn in red top (no preservative) tubes and processed within one hour of collection. Processing can be performed on site if the lab is unavailable within one hour of collection.

The cord blood will be centrifuged 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. The serum will be transferred into two approximately equal portions (approximately 1.5ml each) and placed in LDMS labeled cryovials and frozen at approximately -20°C or colder.

12.8.2 PK Levels in Amniotic Fluid

Amniotic fluid will be collected according to surgical procedures and standards. Collect 2-4mls of amniotic fluid and dispense into two (2) cryovials at approximately 1-2mls each. Label each cryovial with the appropriate LDMS aliquot label and freeze at -20°C or lower.

12.8.3 Endometrial and Placental Tissue Biopsy for PK levels

Endometrial and placental tissue biopsies will be collected according to surgical procedures and standards. Two (2) biopsies measuring at least 1cm from each tissue must be collected. Place each 1cm biopsy specimen into a cryovial with no additive and label using the appropriate LDMS aliquot label. Place the cryovials on ice and freeze at -70°C.

12.8.4 Shipping of Specimens for Tenofovir Levels

Contact the MTN Network lab for pick-up and shipment to the MTN Pharmacology Core. Track the specimen in LDMS.

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Pager # 412-917-9343

One set of samples will be shipped to the MTN Network Lab in Baltimore, MD and assayed for Tenofovir levels. The other set will be retained at the MTN NL - Pittsburgh until advised by the MTN leadership group.

The shipping address is:

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

Appendix 12-1
Maternal PK- LDMS Specimen Tracking Sheet

Participant ID			Visit Code		
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
Site Number	Participant Number	Chk			
PK SPECIMEN TIME POINT	PRIMARY SPECIMEN TYPE	DATE COLLECTED dd- MMM -yy	TIME COLLECTED hh:mm <i>24-hr clock</i>	NUMBER OF TUBES COLLECTED	INSTRUCTIONS FOR PROCESSING LAB
Pre-Gel	Maternal Blood (BLD)			<input type="checkbox"/> Non (red top)	Process within eight hours of collection. Freeze immediately after centrifugation. Store with derivative SER
1 Hour	Maternal Blood (BLD)			<input type="checkbox"/> Non (red top)	Process within eight hours of collection. Freeze immediately after centrifugation. Store with derivative SER.
2 Hour	Maternal Blood (BLD)			<input type="checkbox"/> Non (red top)	Process within eight hours of collection. Freeze immediately after centrifugation. Store with derivative SER
4 Hour	Maternal Blood (BLD)			<input type="checkbox"/> Non (red top)	Process within eight hours of collection. Freeze immediately after centrifugation. Store with derivative SER
6 Hour	Maternal Blood (BLD)			<input type="checkbox"/> Non (red top)	Process within eight hours of collection. Freeze immediately after centrifugation. Store with derivative SER
8 Hour	Maternal Blood (BLD)			<input type="checkbox"/> Non (red top)	Process within eight hours of collection. Freeze immediately after centrifugation. Store with derivative SER
12 Hour	Maternal Blood (BLD)			<input type="checkbox"/> Non (red top)	Process within eight hours of collection. Freeze immediately after centrifugation. Store with derivative SER
24 Hour	Maternal Blood (BLD)			<input type="checkbox"/> Non (red top)	Process within eight hours of collection. Freeze immediately after centrifugation. Store with derivative SER Enter into LDMS with Visit Code 03.0

C-section- LDMS Specimen Tracking Sheet

Participant ID			Visit Code	
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
Site Number	Participant Number	Chk		
PRIMARY SPECIMEN TYPE	DATE COLLECTED dd- MMM -yy	TIME COLLECTED hh:mm <i>24-hr clock</i>	NUMBER OF TUBES/VIALS COLLECTED	INSTRUCTIONS FOR PROCESSING LAB
Amniotic Fluid (AMN)			<input type="checkbox"/> NON (no additive)	Freeze immediately after collection. Store with derivative AMN
Cord Blood (CRD)			<input type="checkbox"/> Non (red top)	Process within eight hours of collection. Freeze immediately after centrifugation. Store with derivative SER
Placental Tissue (PLC)			<input type="checkbox"/> NON (no additive)	Freeze immediately after collection.
Endometrial Tissue (END)			<input type="checkbox"/> NON (no additive)	Freeze immediately after collection.

Appendix 12-2: Sample Shipping/Tracking Manifest for GC/CT and Trich Pouch

MTN 002

Site:

**Contact person: (fill in)
(Fill in address)**

Phone number:

Fax number:

E-mail address:

Shipment/Transport Date _____

Specimen type: check appropriate column

PTID	Collection Date	Visit Code	Specimen Type	
			Urine (UPT) for GC/CT testing	Vaginal swab (InPouch) for Trich Test

Comments _____

<p>Contact for pick-up Pam Kunjara Magee-Womens Research Institute 204 Craft Ave. Room A540 Pittsburgh, Pa. 15213 Office 412-641-6393 Pager 412-917-9343 rsipk@mwri.magee.edu</p>

Appendix 12-3: Sample Shipping/Tracking Manifest for Flow Cytometry

<p><i>GSPH Specimen Routing Record</i></p> <p>TEST: _____ #TUBES/TYPE: _____</p> <p><input type="checkbox"/> Flow cytometry _____</p> <p><input type="checkbox"/> Other _____</p> <p>Special Handling :</p>	<p>MTN Studies</p> <p>Date: _____ Time: _____ <small>24 hour clock</small></p> <p>PTID: _____</p> <p>Study : _____ Week/Day: _____ _____ _____</p> <p>Study ID: _____ Step: _____ _____ _____</p>
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