Section 12. Laboratory Considerations

12.1 Overview and General Guidance

This section contains information on the laboratory procedures performed in MTN 004.

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 websites:

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

Some laboratory procedures will be performed in study site clinics or laboratories and others in the MTN Network Laboratory (NL) in Pittsburgh, PA. Serum SPL7013 levels will be done at Starpharma Ltd Bioanalytical Laboratory in Melbourne, Australia. 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 associated QC procedures prior to performing the tests for study purposes; training documentation should be available for inspection at any time.

The Pittsburgh site was added in June of 2009; some modifications will be noted in the rest of this section for this site because shipping is not required to get specimens to the MTN Network Laboratory.

Table 12-1
Overview of Laboratory Testing Locations, Specimens, and Methods for MTN 004

Test	Testing Location	Specimen Type	Tube/Container	Kit/Method
Urinalysis* (dipstick)	In clinic	Urine	Plastic screw top cup	Bayer Multistix 9 or Uristix 4
Urine pregnancy test	In clinic	Urine	Plastic screw top cup	Quidel Quick Vue
Urine SDA for	MTN Network	Urine	Plastic screw	BD Probetec/

gonorrhea and Chlamydia	Lab		top cup	GenProbe Aptima
HIV antibody screen	Clinic/Local Lab	Plasma or whole blood (*serum acceptable*)	Lavender (EDTA) or red (no additive) top tube	FDA approved test
Complete blood count	Local Lab	Whole Blood	Lavender top tube	Not specified
Liver function panel	Local Lab	Serum	Red or marble (serum separator) top	Not specified
Creatinine	Local Lab	Serum	Red or marble top	Not specified
Coagulation panel	Local Lab	Plasma	Blue (sodium citrate) top tube	Not specified
Pap Smear	Local Lab	Ecto- and Endocervical cells	Slides	Not specified
Vaginal pH	In clinic	N/A	N/A	S/P pH Indicator Strips
Vaginal wet preparation	In clinic	Vaginal fluid swab	N/A	N/A
Gram-stained vaginal smear	MTN Network Lab	Vaginal fluid Swab	Slides	Network Lab procedure
Quantitative vaginal culture	MTN Network Lab	Vaginal swab	Port-a-Cul transport tubes by BD	Network Lab procedure
Cervical cytokine panel	MTN Network Lab	Cervical Swab	2 Swabs → cryovials w PBS	Luminex 100™
RPR	Local Lab	Serum or Plasma	Red or lavender tube	Not specified
Herpes culture	Local Lab	Ulcer Swab	Viral Transport Media (Must be appropriate for HSV-2)	Not specified
Serum SPL7013 level	Starpharma	Plasma	Green (Lithium Heparin) tubes	Capillary Electrophoresis

*Perform Urine Culture and Sensitivity as clinically indicated per local SOP

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 1 Visit	15	Red Top:5	Liver and Kidney Tests, Syphilis Test
		Purple Top:5	Hematology, HIV-1 Antibody Test
		Blue Top:5	Coagulation
Enrollment	25	Red Top:5	Liver and Kidney Tests
		Purple Top:5	Hematology, Plasma Archive
		Blue Top:5	Coagulation
		Green Top:10	SPL7013 Level
One Week Visit	15	Red Top:5	Liver and Kidney Tests
		Purple Top:5	Hematology
		Blue Top:5	Coagulation
Two Week Visit	25	Red Top:5	Liver and Kidney Tests
		Purple Top:5	Hematology, Plasma Archive
		Blue Top:5	Coagulation
		Green Top:10	SPL7013 Level

Notes: Additional blood may be collected for any clinically indicated testing. Red top tubes contain no additive. Lavender top tubes contain EDTA. Blue top tubes contain sodium citrate. Green tops contain Lithium Heparin.

Ideally, one method, type of test kit, and/or combination of test kits will be used for each protocol specified test throughout the duration of the study. If for any reason a new or alternative method or kit must be used after study initiation, site laboratory staff must perform a validation study of the new method or test prior to changing methods. The MTN NL must be notified before the change and can provide further guidance on validation requirements. Similarly, contact the MTN NL in cases of changes to normal ranges.

Provided in the remainder of this section is information intended to standardize laboratory procedures across sites. Adherence to the specifications of this section is essential to ensure that primary and secondary endpoint data derived from laboratory testing will be considered acceptable to all regulatory authorities across study sites.

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 the specimens are collected 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. (The Pittsburgh site may label the slides with Pencil and place SCHARP label on the slide holder).

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: stored plasma specimens, cervical swabs for cytokines, vaginal fluid slides prepared for Gram stain evaluation, vaginal cultures and SLP 7013 levels. These specimens will be shipped to the MTN NL or Starpharma for testing. (The Pittsburgh site will not use LDMS for gram stains, vaginal cultures and cervical cytokines.)

12.3 Procedures for Specimens that can not be evaluated

Specimens will be redrawn or recollected if it is found that they can not be evaluated per site SOP's. Sites will monitor specimen management problems as part of ongoing Quality Assurance. In cases where additional specimens need to be recollected either due to a laboratory error (lost or broken specimen or clerical error) or clinic error (clerical error), a protocol event form will 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 at all sites to track the collection, storage, and shipment of five types of specimens in MTN 004: plasma archive, cervical swabs for cytokines, vaginal cultures, vaginal fluids (air-dried on microscope slides for Gram stain evaluation), plasma SLP 7013 levels and returned applicators. (The Pittsburgh site will not use LDMS for gram stains, vaginal cultures and cervical cytokines.)

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

All sites will be required to maintain the current version of LDMS and monitor updates relating to use of the LDMS. It is crucial to be aware of proper label formats to ensure that specimens are correctly labeled. Sites will be responsible to back up their LDMS

data (frequency determined by site) locally and to export their data to FSTRF (at least weekly).

Questions related to use of LDMS in MTN 004 may be directed to Edward Livant 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 (addresses shown in Table 3 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 pagers. The email addresses in Table 12-3 can still be used as needed.

Table 12-3
LDMS User Support Email 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 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 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., plasma 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 004 Specimens

The table below should be used as a guide when logging in 004 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
Vaginal Swab for Culture to NL	VAG	PAC	SWB	N/A	1	1	N/A
Cervical Swab for Cytokines	CXS	PBS	CXS	N/A	1	1	N/A
Vaginal Swab for Gram stain to NL	VAG	NON	SLD	GRS	1	1	N/A
Plasma for storage	BLD	EDT	PL 1/2	N/A	2 to10	0.5	ml
SPL7013 level to Starpharma	BLD	HEP	PL 1/2	N/A	10	2.5	ml

Table 12-5
Specimen Shipping Summary

Specimen	Use LDMS?	Ship to:	Shipping schedule
Vaginal Swab for Culture to NL	Yes*	MTN Network Lab	Must be shipped overnight
Cervical Swab for Cytokines	Yes*	MTN Network Lab	Batched until end of study
Vaginal Swab for Gram stain to NL	Yes*	MTN Network Lab	Ship at the same time as cultures
Plasma for storage	Yes	MTN Network Lab	Store at site until notified by MTN
SPL7013 level	Yes	**Starpharma**	Batched until end of study
Urine for GC/CT testing	**No**	MTN Network Lab	1-2 times per week

*Except Pittsburgh

12.5 Urine Testing for Pregnancy, Urinary Tract Infection, Chlamydia, and Gonorrhea

The urine tests performed at each 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, pregnancy test next, then the urine dipstick last.

Note: Testing for Chlamydia and Gonorrhea is done at screening and when clinically indicated only. Viva gel interferes with the BD Probetec so post enrollment GC/CT testing will be performed on the Gen Probe Aptima. See below for details.

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 5-10 ml of urine from the specimen collection cup and pipette from this aliquot for pregnancy testing. If the supernatant is too dark to read the pregnancy test, another urine sample will need to be collected.

Note: Protocol-specified pregnancy testing is not discontinued during pregnancy.

The Quidel QuickVue One-Step hCG urine pregnancy test must be used at all sites. This test was selected for use in MTN 004 because of its ease of use and the validity of test results in the presence of the study gels. Perform the test according to site SOPs and the package insert. Do not perform any other urine pregnancy tests for confirmatory purposes.

Pregnancy status is a critical participant safety consideration in MTN 004. All sites must maintain an adequate inventory of the QuickVue One-Step test kits at all times. Inventory should be monitored closely and re-supply orders placed at least 8-12 weeks in advance of actual need (or longer if needed per site procurement policies and procedures). The date and time of pregnancy testing must be documented.

12.5.3 Dipstick Urinalysis

Dip the urinalysis test strip into an aliquot of urine. At visits when both pregnancy testing and dipstick urinalysis are required, the same aliquot should be used for both tests, but the urinalysis should be performed after urine has been pipetted from the aliquot for the pregnancy test.

Bayer/Siemens urine test strips must be used at all sites. Perform this test according to site SOPs and the package insert. Assess and record results for 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.

Inventory should be monitored closely and re-supply orders placed at least 8-12 weeks in advance of actual need (or longer if needed per site procurement policies and procedures). Notify the NL immediately if any kit inventory or quality control problems are identified, so that appropriate action can be taken.

12.5.4 Chlamydia and Gonorrhea Testing

Note: Testing for Chlamydia and Gonorrhea is done at screening and when clinically indicated only. Viva gel interferes with the BD Probetec so post enrollment GC/CT testing will be performed on the Gen Probe Aptima. The only change for collection and transport procedures will be the transport tube used. In cases where GC/CT testing is indicated at follow up, follow these instructions but use the alternate transport tube.

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

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

(If vaginal cultures are being shipped on the same day combine the specimens in one shipping container. See shipping instructions for vaginal specimens section 12.7.5)

- Urine specimens are stable for 30 days therefore specimens can be batched and sent once a week if your turn around time is 8-9 days.
- Fill out a shipping manifest with the information listed in the example located in appendix 12-2 (Do not use LDMS for urine specimens).
- Package the specimens according to the IATA packing instructions 650 for non-refrigerated specimens.
  - Place the tubes in a biohazard zip-lock bag.
  - Enclose the tubes in the small Styrofoam container without ice packs.
  - Place the Styrofoam container inside the cardboard box.
  - Insert the box and the shipping manifest in a FedEx Diagnostic envelope.
  - Check 2 day delivery on the FedEx air bill when shipping only urines (2 day delivery will save shipping costs)
- The day of shipment, send Lorna Rabe an e-mail at rsilkr@mwri.magee.edu with the FedEx tracking number.

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, do not check Saturday delivery)

12.6 Blood Testing for HIV, Syphilis, Hematology, Liver, Renal Function, and Plasma Archive and Plasma SPL7013 Levels

The blood tests performed at each study visit vary depending on the time point of the visit and potentially the clinical presentation of the participant. Perform all tests according to site SOPs and package inserts.

12.6.1 Specimen Collection and Initial Processing

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

- Allow red top tubes (no additive) or marble top (serum separator tubes) to clot, then centrifuge per site SOPs to yield serum for syphilis, liver function, and renal function testing.
- Lavender top tubes (additive = EDTA) should be gently inverted at least eight times after specimen collection to prevent clotting. EDTA tubes are used for hematology, HIV testing and plasma archive. If whole blood for hematology testing and plasma are to be taken from the same tube, the hematology must be completed before the tube is centrifuged and aliquotted. If whole blood is to be used for multiple tests, ensure that the tube is well mixed before removing any specimen.

- Blue top tubes (additive=sodium citrate) should be gently inverted at least eight times after specimen collection to prevent clotting. They are then centrifuged per site SOP's for coagulation testing. NCCLS recommends using 3.2% sodium citrate.
- Green top tubes (additive=lithium heparin) should be inverted at least eight times after collection to prevent clotting. These are used for SPL7013 levels. The specimens are placed immediately after collection into an ice water bath (slurry) and centrifuged as soon as possible after collection (≤ 1hour).

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 (whole blood and serum are also acceptable) 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.

At all sites, HIV infection status at screening will be assessed using an FDA-approved enzyme immunoassay (EIA) per the MTN 004 HIV testing algorithm (see appendix III in the current version of the MTN 004 protocol). If the EIA is non-reactive, the participant will be considered HIV-uninfected. If the EIA 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 participant will be asked to present to the study site in approximately one month for re-testing. At that time, the EIA will be repeated and the above-described algorithm will be followed. A WB will only be performed if the EIA is reactive.

Kit inventories should be monitored closely and re-supply orders placed at least 8-12 weeks in advance of actual need (or longer if needed per site procurement policies and procedures). Notify the NL immediately if any kit inventory or quality control problems are identified, so that appropriate action can be taken.

At all sites, 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 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 at each study site; 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 004 Protocol Safety Review Team.

12.6.4 Hematology Testing

Complete blood counts with five-part differentials will be performed at all sites. Each of the following must be analyzed and reported:

- Hemoglobin
- Hematocrit
- Platelets
- White blood cell count with differential
- Red blood cell count

These tests will be performed on EDTA whole blood.

12.6.5 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.

12.6.6 Coagulation Panel

The following tests will be performed to evaluate coagulation function:

- Activated Partial Thromboplastin Time
- International Normalized Ratio (calculated from Prothrombin Time)

Coagulation tests will be performed on Sodium Citrate Plasma.

12.6.7 SPL7013 Plasma Levels

The lithium heparin specimens are placed immediately after collection into an ice water bath (slurry) and centrifuged as soon as possible after collection (≤ 1hour). The whole blood will be centrifuged at 3000 rpm (approximately 1000×g) for 10 minutes. If red blood cells are not sufficiently separated from plasma, centrifugation for a further 5 minutes may be required. The plasma will be transferred into two approximately equal portions (approximately 2.5ml each) and placed in labeled 5ml polypropylene tubes and frozen at approximately -20°C.

Samples will be assayed for SPL7013 levels using a validated capillary electrophoresis bioanalytical method at the Starpharma Pty Ltd bioanalytical laboratory, Melbourne, Australia. The samples will be stored on site during the study and then shipped directly from the sites to Starpharma in Australia. At the end of the study, one set of samples should be shipped and the other retained until advised by the MTN leadership group. The MTN NL will communicate with the sites to help coordinate the shipment with Starpharma.

The shipping address is:
Starpharma Pty Ltd Bioanalytical Laboratory
Baker Building
75 Commercial Road
Melbourne, 3004, VIC Australia.

Samples will be shipped in a sufficient amount of dry-ice to keep the plasma frozen for transport. A separate SOP from Starpharma will be provided to the sites with more detailed instructions.

12.6.8 Plasma Archive

EDTA plasma will be archived from enrollment and week 2 visits. These will be stored at -70°C and batched until the end of the study.

- LDMS will be used to label and track the specimens.
- Within 24 hours of collection, process the blood for plasma according to site SOP's.
- Prepare as many 0.5 ml aliquots as available to store. If less than one 0.5 ml aliquot is available, store that plasma and inform the MTN NL for instruction.
- At the end of the study, the MTN NL will contact the sites with instructions for shipping.

- Note: plasma archive is only applicable if participant consents to long term storage.

12.7 Testing of Vaginal and Cervical 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

Vaginal pH will be assessed as part of on-site evaluations for bacterial vaginosis. S/P pH Indicator Strips (pH range 3.6 to 6.1) provided by MTN NL must be used at all sites, as follows:

- During pelvic examination vaginal fluids are collected via swab on the vaginal walls and then swabbed onto the pH strip. Avoid collecting the swab from the cervix and the pooled secretions in the fornix which have a higher pH.
- 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

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 12-4.

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 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 (*Gardnerella vaginalis* 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 coverslip. 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 Vaginal Fluid Dried Smears for Gram Staining

In addition to the wet mounts described above, 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 SDMC that the slide may be discarded. Instructions for slide preparation and shipping are provided below.

12.7.4 Slide Preparation and Storage

- 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. Also write "V" for vaginal on each label.
- Immediately following specimen collection from the lateral vaginal wall via swab, roll the swab across each of the slide. (Be sure to collect the specimen from opposite the vaginal wall used for the wet mount specimen collection.) Do not place the swab in saline, transport medium, or any transport container prior to slide preparation.
- 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 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.

- Place one slide in a plastic slide holder and send to the MTN NL at Magee with the vaginal swab for culture. (See shipping instructions below).
- The slide from the screening visit should be sent with the enrollment visit specimens.
- Store the second slide in the slide box location assigned in LDMS at room temperature. (This is a backup slide incase the first is lost or unreadable).
- Pittsburgh only: label the gram stain slide using pencil on the frosted site with the PTID, Date and Visit Code. Place a SCHARP sticker on the outside of the slide holder.

Note: The MTN 004 protocol requires that dried smears be prepared for all potential study participants at Screening, however all slides will not have Gram stains done at the MTN NL. Slides will only be assessed for participants who enroll in the study and, for enrolled participants who undergo more than one screening pelvic exam, only slides from the exam that confirmed eligibility will be assessed.

12.7.5 Vaginal Swab for Quantitative 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. It must be refrigerated after that and shipped with ice packs.
- 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). Because the Research Institute is not open for delivery on the weekend the specimens taken on Friday must be sent to the hospital address in order for delivery on Saturday.
- Pittsburgh only: label the port-a-cul with the SCHARP label. LDMS not required.

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
300 Halket St.
Pittsburgh, Pa. 15213
Phone # 412 641-4191 (this is the Safety and Security #)
** Be sure to check Saturday delivery on the Fed Ex label

Notify the MTN NL via email 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.

12.7.6 Cervical Sample for Cytokines Collection

Cervical swab for Cytokines

- Two Dacron swabs will be taken.
- Gently insert one Dacron swabs 1 cm into the cervical os and rotate 360 degrees to absorb the fluid.
- Place the swab in a cryovial with 400ul of PBS. Break off the end of the swab to allow closure of the cryovial and securely attach the cap. Attach a SCHARP-provided label to the vial.
- Repeat with the second Dacron swab as described above.
- Samples must be placed on dry ice and frozen at -70°C as soon as possible after collection.
- Deliver both cryovials and an LDMS Specimen Tracking Sheet to the local LDMS laboratory.
- Using the LDMS Tracking Sheet, log the cryovials into LDMS (specimen type=CXS) and generate an LDMS cryovial label for each tube. Affix the LDMS label to the cryovial (over the SCHARP-provided PTID label).
- Store the cryovial(s) in the freezer locations assigned in LDMS at -70°C.
- Specimens will be batched and shipped to the NL on dry ice at the end of the study.
- Pittsburgh only:
    - Label the cryovials with the SCHARP label. LDMS not required.
    - Dry Ice not required if the specimen is received at the lab within 60 minutes of collection.

12.7.7 Papanicolaou (Pap) Test

Pap smears will be performed at sites. At visits when Pap smears are required, 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, Pap smear findings associated with STIs should not be considered diagnostic of any infections. Rather, such findings should be handled as follows:

- Do not consider STI-related notations on Pap smear result reports when assessing participant eligibility for the study. Use only the results of protocol specified STI tests for purposes of eligibility determination.
- If protocol-specified STI testing was performed on other specimens (i.e., blood, urine, vaginal fluids) collected on the same day as specimen collection for Pap smear, the results of the protocol-specified testing overrule STI-related findings noted on the Pap smear result report.
- Provide treatment as needed based on the results of the protocol-specified tests.
- If protocol-specified testing was not performed on other specimens (i.e., blood, urine, vaginal fluids) collected on the same day as specimen collection for the Pap smear, collect specimens for indicated protocol-specified STI testing at the participant's next study visit that takes place after receipt of the Pap test result report. Provide treatment as needed based on the results of the protocol-specified tests.

12.7.8 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.

Appendix 12-1 LDMS Tracking Sheet

Participant ID ☐☐☐-☐☐-☐☐☐-☐
Site Number Protocol Participant Number Chk

Visit Code (Vst) ☐☐.☐

Specimen Collection Date ☐☐ ☐☐☐ ☐☐
dd *MMM* *yy*

# of TUBES (or Specimens)	PRIMARY SPECIMEN TYPE	ADDITIVE	INSTRUCTIONS FOR LAB *Unless otherwise noted, enter sub add/derivative code as N/A*
☐	Blood **(BLD)**	**EDT** (purple top)	At the Enrollment and 2-Week Visits, lab to divide plasma into as many 0.5 mL aliquots as available to store for plasma archive. Store with derivative PL 1/2.
☐	Blood **(BLD)**	**HEP** (green top)	At the Enrollment and 2-Week Visits, lab to divide plasma into (2) aliquots of approximately 2.5 mL each for SPL7013 level testing. Store with derivative PL 1/2.
☐	Vaginal Gram Stain Slide **(VAG)**	**NON** (no additive)	Re-label with LDMS label. Store duplicate slides (one for on-site storage, and one for shipping and testing at MTN Central Lab). Store with derivative SLD and sub add/derivative GMS.
☐	Cervical Swab **(CXS)**	**PBS**	Re-label cryovial with LDMS label. Store with derivative CXS.
☐	Vaginal Swab (**VAG**)	**PAC**	Re-label cryovial with LDMS label. Store with derivative SWB.

Comments: _____

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

Appendix 12-2: Sample Shipping Manifest

MTN 004
Site:
Contact person: (fill in)
(Fill in address)

Phone number:
Fax number:
E-mail address:

Shipment Date _____

Specimen type: Urine for GC/CT testing

PTID	Collection Date	Visit Code

*Comments*_____
_____

Ship to:
Lorna Rabe Magee-Womens Research Institute 204Craft Ave. Room 530 Pittsburgh, Pa. 15213 412-641-6041

On the day of shipment E-mail the FedEx tracking # to rabelk@upmc.edu and cosentinola@upmc.edu