

Section 12. Laboratory Considerations

12.1 Overview and General Guidance

This section contains information on the laboratory procedures performed in MTN 005.

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 and the World Health Organization 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. Table 12-1 lists for each test the testing location, specimen type, specimen container and kit/method (if specified). Table 12-2 lists the tests to be performed at each visit.

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.

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.

Notes: Additional blood may be collected for any clinically indicated testing.

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.

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.

**Table 12-1
Overview of Laboratory Testing Locations, Specimens, and Methods for
MTN 005**

Test	Testing Location	Specimen Type	Tube/Container	Kit/Method
Urinalysis ¹ (dipstick)	In clinic	Urine	Plastic screw top cup	Siemens Multistix or Uristix
Urine pregnancy test	In clinic	Urine	Plastic screw top cup	Quidel Quick Vue
HIV antibody testing	Clinic/Local Lab	Plasma/Serum	Lavender (EDTA) / Red or tiger top (no additive)	FDA approved test
Syphilis testing	Local Lab	Serum or Plasma	Red or lavender tube	Not specified
Cervical NAAT for gonorrhea and Chlamydia	Local lab	Cervical swab	Kit specific Transport tube	BD Probetec/ Gen-Probe Aptima/Roche Amplicor
Pap Smear ²	Local Lab	Ecto- and Endocervical cells	Slides	Not specified
Vaginal pH	In clinic	Vaginal swab	N/A	S/P pH Indicator Strips
Trichomonas rapid test	Local lab or in clinic	Vaginal swab	Sterile tube with no additives	OSOM
Vaginal wet preparation	In clinic	Vaginal swab	N/A	N/A
Gram-stained vaginal smear	MTN Network Lab/NARI lab	Vaginal Swab	Slides	Nugent scoring criteria
Vaginal swab for microflora (India site only)	NARI lab	Vaginal flocked swab	Sterile cryovial	Not specified
Innate factors (US sites only)	MTN network Lab	Cervical swab	Cryovial with 400 µL PBS	Network Lab procedure
Quantitative vaginal culture (US sites only)	MTN Network Lab	Vaginal swab	Port-a-Cul transport tubes by BD	Network Lab procedure
Quantitative culture of IVR (US sites only)	MTN Network Lab	Swab of IVR	Port-a-Cul transport tubes by BD	Network Lab procedure
FISH and SEM of IVR (US sites only)	MTN Network Lab	Section of IVR	Tube with 2.5% paraformaldehyde then transfer to tube with PBS	Network Lab procedure
Herpes culture ³	Local Lab	Ulcer Swab	Viral Transport Media (Must be appropriate for HSV-2)	Not specified

1. Perform Urine Culture and Sensitivity as clinically indicated per local SOP

2. Perform only if clinically indicated or if participant does not have a documented normal Pap within the last 2 months.

3. Perform only if clinically indicated per local SOP or per local standards

**Table 12-2
Overview of Laboratory Tests by visit for MTN 005**

	SCR	ENR	4W	8W	12W	16W/Study Term	Interim
	Up to and incl. 45 days prior to ENR	Day 0	Must occur within ±7 days of scheduled visit				
Qualitative hCG	x	x	▲	▲	▲	x	▲
Dipstick UA	x	▲	▲	▲	▲	▲	▲
Urine Culture	▲	▲	▲	▲	▲	▲	▲
Plasma Archive		x					
Syphilis Serology	x	▲	▲	▲	▲	▲ ¹	▲
HIV-1 Test	x	▲	▲	▲	▲	x	▲
Pelvic Exam	x	x	x	x	x	x	▲
Vaginal pH	†▲	†▲	†▲	†▲	†▲	†▲	†▲
Cerv. NAAT for GC/CT	x	▲	▲	▲	▲	▲ ¹	▲
Test for Trichomonas	x	†▲	†▲	†▲	†▲	†▲ ¹	†▲
Wet Mount for Vulvovag. Candidiasis	†▲	†▲	†▲	†▲	†▲	†▲	†▲
Wet Mount for BV	†▲	†▲	†▲	†▲	†▲	†▲	†▲
Innate Factors (US sites only)		x				x	
Gram Stain	x	x	x	x	x	x	
Pap Smear	▲						▲
Vaginal Flora Assessments		x	x	x	x	x	▲
Biofilm Assessment (at US sites only)			■	■	∞		■
Herpes Culture	†¥▲	¥▲	¥▲	¥▲	¥▲	¥▲	¥▲
Collect Used IVR			+	+	●	+	+

X collect specimen

▲ if clinically indicated

● For group A (randomized to Study IVR)

■ For group A (if permanently discontinued and removed by study clinician)

∞ For group A (randomized to Study IVR and removed by study clinician) †

¥ Per local standards

+ For Group A if indicated, + if applicable

1. India sites only: testing required at this visit.

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 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: plasma for archive, vaginal fluid slides prepared for Gram stain evaluation, vaginal cultures, vaginal swab

for PCR, vaginal swab for innate factors, IVR culture, IVR staining. (See Table 12.4 for detailed description of LDMS codes).

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 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 at all sites to track the collection, storage, and shipment of 8 types of specimens (only 3 for Pune site) in MTN 005: plasma archive, vaginal swab for PCR, vaginal smears for Gram stain evaluation, vaginal cultures, vaginal swab for innate factors, IVR cultures, IVR for SEM, and IVR for FISH.

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 005 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

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 12-3
LDMS Specimen Management Guide to Logging in 005 Specimens

The table below should be used as a guide when logging in 005 specimens. Please use the LDMS codes listed below when logging in specimens for each test listed. See Appendix 12-2 for a copy of the LDMS tracking sheet.

QUANTITY	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALIQUOT DERIVATIVE	ALIQUOT SUB ADD/DER	INSTRUCTIONS FOR PROCESSING LAB
10 mL of whole blood (4 mL of plasma)	Blood (BLD)	EDT (purple top)	PL1/2	N/A	At Enrollment (Visit Code 2.0): lab to make 1.5 mL aliquots. Notify NL is storing < 4.0 mL plasma.
2 slides	Vaginal Smear for Gram Stain (VAG)	NON (no additive)	SLD	GRS	Re-label with LDMS label. Store duplicate slides (one for on-site storage and one for shipping and reading at the NL Pune will send 10% to NL for QA).
1 flocked swab	India Only: Vaginal Swab for PCR (VAG)	NON (no additive)	SWB	N/A	Store swab at ≤-70°C locally. Freeze within 4 hours of collection
2 Dacron swabs	US Only: Vaginal Swab for Culture (VAG)	PAC	SWB	N/A	Ship on ice packs to NL on the day of collection.
1 Dacron swab	US Only: Cervical Swab for innate factors (CXS)	PBS	SWB	N/A	Store swab at ≤-70°C locally.
1 Dacron swab	US Only: Swab of used IVR for Culture (IVR)	NON (no additive)	SWB	N/A	Ship on ice packs to NL on the day of collection.
½ of IVR	US Only: Used IVR for FISH and SEM (IVR)	NON (no additive)	IVR	PFM	Ship on ice packs to NL on the day of collection.

BLD: Whole Blood
EDT: EDTA
PL1: Single spun Plasma
PBS: Phosphate buffered saline
GRS: Gram stain

NON: No Additive
IVR: Intravaginal ring
PFM: Paraformaldehyde
SEM: Scanning electron microscopy
FISH: Fluorescence *in situ* hybridization

SWB: Swab
PAC: Port-a-Cul transport tube
CXS: cervix

12.5 Urine Testing for Pregnancy and Urinary Tract Infection

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 pregnancy testing first, then the urine dipstick.

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 20-30 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.

12.5.2 Pregnancy Testing

Note: If the urine is too dark from blood to read the pregnancy test, the urine can be centrifuged or another urine sample can be collected.

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

The Quidel QuickVue One-Step hCG urine or Quidel QuickVue Combo hCG urine/serum pregnancy test must be used at all sites. This test was selected for use in MTN 005 because of its ease of use and prior validation. 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 005. 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

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.

Any of the Siemens Multistix urine test strips can be used at all sites. Perform this test according to site SOPs and the package insert. Assess and record results for 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.

12.6 Blood Testing for HIV and Syphilis

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 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 HIV testing.

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 (US Labs) or to DAIDS GCLP standards (Non-US Labs). 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 005 HIV testing algorithm (see appendix II in the current version of the MTN 005 protocol and in the appendix 12-1 of this section of the SSP). If the EIA is non-reactive, the participant will be considered HIV-uninfected. If the EIA is reactive or indeterminate, 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 (sample 2) will be drawn for confirmatory testing, except for screening visits, see below. 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.

If a participant has a positive sample 1 Western Blot at a screening, they are not eligible for MTN 005. Do not proceed to sample 2. The participant should be referred for treatment and counseling per local guidelines.

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 005 Protocol Safety Review Team.

12.6.8 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 at $\leq -70^{\circ}\text{C}$ until requested for shipping and/or testing by the MTN NL.

For routine plasma archive, standard processing per site SOPs should be performed. Prepare as many ~1.5 mL aliquots as possible.

- If the volume is less than 4.0 mL notify the MTN NL.
- If volume is less than 2.0 mL redraw as soon as possible.
- Use LDMS to label and track all aliquots.
- Store all aliquots frozen on site $\leq -70^{\circ}\text{C}$.
- The MTN NL will send instructions when shipping and/or testing is required.

- If samples are hemolysed, store the aliquots as per normal and enter comments in LDMS.

12.7 Testing of Cervical Specimens

12.7.1 *Chlamydia and Gonorrhea Testing*

Note: Sites can choose to use the BD Probetec, Gen-Probe Aptima, or Roche Amplicor. If the site does not have access to any of these tests they can send the samples to the NL for testing. Contact the NL prior to sending specimens for GC/CT testing.

Collect cervical swabs and transport to the local laboratory according to the specific manufacturer's recommendations.

Testing will be done at the local laboratories according to the site SOP.

12.7.2 Endocervical Swabs for Biomarker Analysis (US sites only)

At the enrollment and 16 week/study termination visits, 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).

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), and cap the vial. (NL will provide cryovials with PBS for the US sites)

Deliver the tube 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 Table 12-2 for LDMS for additive and derivative codes) and label the vial with a LDMS label.

Freeze at $\leq -70^{\circ}\text{C}$ within 8 hours of collection.

12.7.3 Papanicolaou (Pap) Test

Pap smears are only required if clinically indicated or if a participant has not had a documented normal test within 12 months. If a Pap smear is required, ecto- and endocervical cells will be collected after all tissues have been visually inspected and all other required specimens have been collected. The testing will be done at the site's local laboratory. 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.

12.8 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.

12.8.1 Rapid Test for Trichomoniasis

This testing will be done using the Genzyme 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; 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.

12.8.2 Vaginal pH

Vaginal pH will be assessed as part of on-site evaluations for bacterial vaginosis. pH Indicator Strips (pH range 3.6 to 6.1) with brand names S/P Cardinal Health, or Baker-pHIX, or Machery-Nagel must be used at all sites, as follows:

- During the pelvic examination, a Dacron swab will be used to collect vaginal fluids from the lateral wall and then swabbed onto the pH strip (Do not inserting the pH strip into the vagina).
- 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.

12.8.3 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 and candidiasis, as summarized in Table 12-4 below.

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.

CLIA regulations require semi-annual wet mount proficiency testing; therefore the MTN NL will administer a web-based proficiency test 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 laboratory staff is hired, so that appropriate training can take place prior to such staff performing wet mounts for study purposes.

**Table 12-4
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)
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 (one for KOH and one for clue cells). 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, 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.

12.8.4 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 either shipped to the MTN NL (US sites) or read locally (India site) and the other will be archived on site until written notification is received from the SDMC that the slide may be discarded. The second slide can be used if the first slide has insufficient material, broken, or lost when transported to the NL. Instructions for slide preparation and shipping are provided below.

India site only:

An excel spreadsheet will be provided for recording the results. The results will be sent to the NL monthly.

12.8.4.1 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.
- The US sites will 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 India site will process and read the slides at the NARI laboratory. 10% of the slides read at the NARI lab will be sent to the MTN Network laboratory for quality assurance testing. The MTN NL will provide a list of slides to be sent for QA.
- 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).

Note: The MTN 005 protocol requires that dried smears be prepared for all potential study participants at Screening, however all slides will not have Gram stains done. 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.

12.8.5 Vaginal Swab for Quantitative Culture (US site only)

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 slides 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.

If sending Monday through Thursday Send to:

Lorna Rabe
Magee-Womens Research Institute
204 Craft Ave, Room A530
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 (lrabe@mwri.magee.edu and kstoner@mwri.magee.edu) when the shipment has been picked up from the site by the courier/shipping company and the tracking number.

Attach the LDMS shipping manifest to the email notification.

12.8.6 Vaginal swab for PCR (Pune site only)

A single vaginal swab will be collected and stored at the Pune site for future testing of microflora.

- Collect the specimen for culture by rotating a single flocked swab several times over the lateral wall of the vagina. Insert the swab into a cryovial (labeled with a SCHARP label) and breaking off the shafts of the swab, and capping.
- Transport the tube on ice.
- Deliver the tube and an LDMS Specimen Tracking Sheet to the local LDMS laboratory within 4 hours.
- Using the LDMS Tracking Sheet, log the cryovial into LDMS (specimen type = VAG. See Table 12-3 for LDMS for additive and derivative codes) and label the vial with a LDMS label.
- Freeze at $\leq -70^{\circ}\text{C}$ within 4 hours of collection.

12.8.7 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.

12.9 Testing of Intravaginal Ring (IVR) for Biofilms (US sites only)

The IVR will be tested by culture to quantify and identify the bacteria adhering to the ring. Sections of the ring will also be evaluated using scanning electron microscopy (SEM) to view and photograph any biofilms present on the rings and to measure the size of the biofilms. Another section of the ring will be stained using fluorescence in situ hybridization (FISH) with a universal oligonucleotides probe that targets most bacteria to detect the bacteria present in the film. **Only rings that have been removed by the clinician will be tested.** This is to ensure the viability of the organism on the ring and eliminate the possibility of external organisms contaminating the ring.

The clinician will remove the ring at the exit visit, place the ring in a sterile container (urine cup or petri dish), and deliver to the lab immediately for processing. The ring will be cut in half and one half will be swabbed for culture; this swab will be placed in a Port-A-Cul transport tube. The other half will be placed in a tube with 2.5% paraformaldehyde for 1 hour and then transferred into a tube with phosphate buffered saline (PBS). The Port-a-cul and the tube with PBS and section of the ring will be sent to the MTN NL in Pittsburgh

12.9.1 Supplies needed to process:

- Sterile petri dish

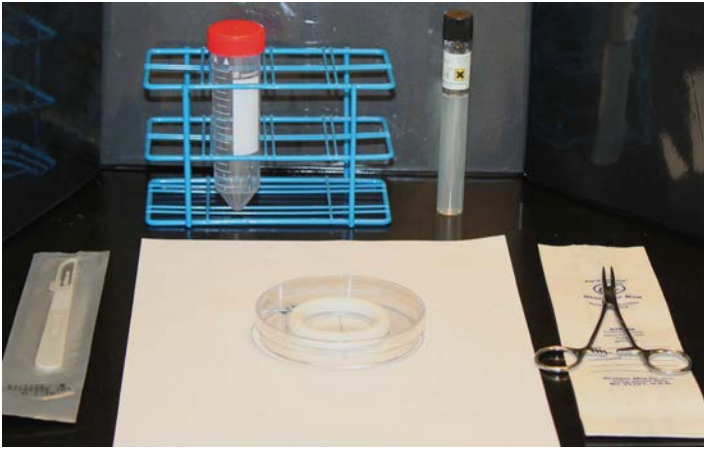
- Sterile forceps
- Sterile scalpel
- Port-a-cul tube
- Sterile Dacron swab
- 1-50 cc tube containing PBS
- 1-50 cc tube containing 2.5% paraformaldehyde
- 1 sponge stopper

(Network Lab will provide all the above except forceps and scalpel)

12.9.2 Processing procedure

- Remove the IVR from the vagina and place in a sterile container (urine cup or petri dish) labeled with a SCHARP label.
- Immediately transport the ring to the local laboratory. The ring must be processed within 1 hour of removing from the vagina.
- With the ring in a petri dish cut the ring in half with sterile scalpel.
- **Gently**, insert 1/2 of ring into tube with 2.5% paraformaldehyde, cap tightly. (2.5% paraformaldehyde will be provided by the NL)
- After 1 hour **gently** transfer the ring section from the tube with paraformaldehyde to a tube with enough PBS to cover the ring completely (approximately 15-20 mL). Insert a sponge cork into the tube and push down to the top of the ring to prevent the ring from moving. Cap tightly. (the cork does not need to be sterile)
- Using the sterile forcep to hold the remaining ½ of the ring, swab the inner and outer surfaces with a sterile Dacron swab. See diagram below.
- Insert the swab into a Port-A-Cul transport tube (labeled with a SCHARP label), submerging the swab into the gel and breaking off the shaft of the swab, and capping.
- The Port-a-cul and the tube containing the section of IVR in PBS may be kept at controlled room temperature for up to 4 hours. They must be refrigerated after 4 hours and shipped with ice packs.
- Deliver the Port-A-Cul, IVR section, and the LDMS specimen tracking sheet to the local LDMS laboratory.
- Using the LDMS Tracking Sheet, log the IVR into LDMS (See table 12-2 for LDMS codes) and label the Port-A-Cul tube and IVR container with LDMS labels.
- Use LDMS to generate a shipping manifest for the specimens to be shipped.
- Ship the swab of the IVR and the tube containing the section of IVR along with the vaginal culture and the vaginal smear for gram stain the same day of collection by overnight courier.
- See shipping instructions listed in section 12.8.5

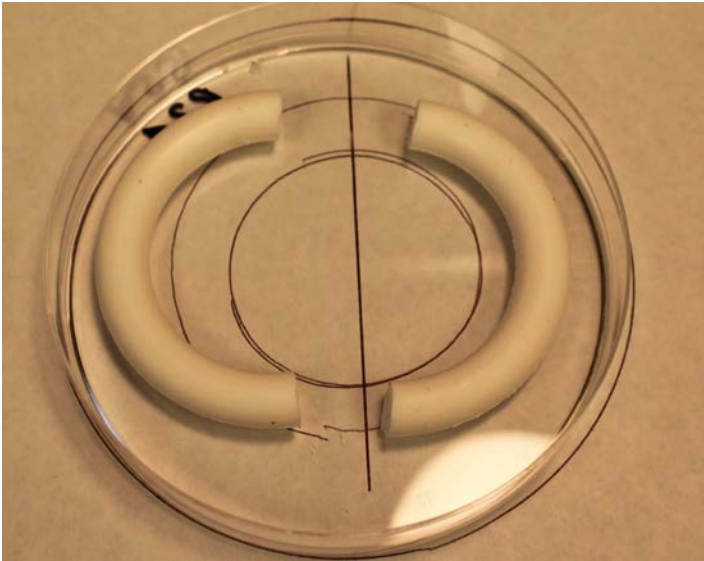
Diagram for processing the IVR for culture and stains



Use sterile scalpel, forceps, and petri dish for processing the ring.



Gently, hold the ring with the forceps and cut the ring in half. See next photo



½ for culture and ½ for biofilms staining



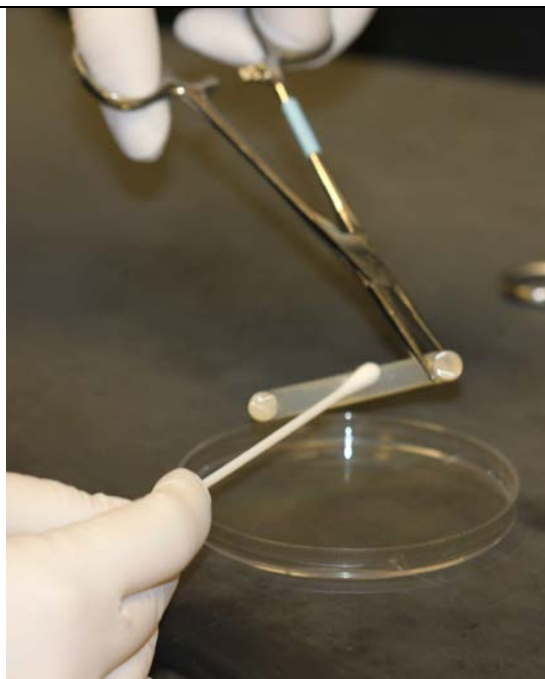
Gently insert ½ section into tube with paraformaldehyde and leave for 1 hour to fix the cells



After 1 hour transfer the section into a tube with enough PBS to cover the ring for transport. Discard the paraformaldehyde in chemical discard



Insert a sponge cork into the tube and push it down to the ring to prevent the ring from moving. Cap tightly.



Second half of ring:
Swab the entire ½ section of ring with sterile swab.



Insert the swab into a Port-a-Cul transport tube. Push the swab about half way into the tube, break off the shaft, and cap tightly.



- Place the 2 Port-a-cul tubes in a biohazard bag with a paper towels wrapped around the tubes to protect from breakage and to absorb fluid in case of leakage. Add the slide holder to the bag with the port-a-cul tubes.
- Place the tube containing the ring section in a separate biohazard bag with a paper towel.
- Add ice packs to the package.
- Ship to the NL the day of collection.
- See shipping instructions listed in section 12.8.5 above

APPENDIX 12-1: HIV ANTIBODY TESTING ALGORITHM



Note:

US Sites: 1 rapid EIA

India Site: 1 non-rapid EIA

Note:

Indeterminate Sample 2 WB or IFA- refer to Network Lab for guidance

