Section 8. Clinical Considerations

8. Introduction

This section presents information on the clinical procedures performed in MTN-038. Further clinical considerations related to participant safety monitoring and adverse event reporting are provided in SSP Section 9. Information on performing laboratory procedures is described in SSP Section 10. Instructions for completing data collection forms associated with clinical procedures are provided in SSP Section 12.

The Schedule of Study Visits and Evaluations in Protocol Appendix I indicates when specific clinical and laboratory assessments are to take place. While the protocol dictates the schedule for data capture, the IoR or designee should perform the symptom-directed examination at his/her discretion during any visit if s/he determines it to be clinically necessary, particularly if there are any ongoing medical or mental health conditions that require closer follow-up. The participant's research record should include documentation of these procedures. Throughout this section the term "clinician" will refer to a study doctor or a nurse practitioner in settings where nursing training, scope of practice, and delegation permit nurses to perform clinician activities under doctor supervision.

8.1 Baseline Medical Conditions (Pre-existing Conditions) and Medications

8.1.1 Pre-existing Conditions Collection at the Screening Visit

To establish each participant's medical status at Enrollment (and also assess medical eligibility), pre-existing conditions will be captured starting at the Screening Visit, and documented on the Medical History CRF. Ongoing medical conditions, problems, signs, symptoms, and findings identified prior to enrollment are considered pre-existing conditions. Pre-existing conditions must be graded and are assigned severity grades in the same way that severity is assessed for adverse events (AEs). If a pre-existing condition worsens (increases in severity or frequency) after enrollment, the worsened condition is considered an AE and is reportable on the AE Log CRF. If a pre-existing condition resolves after enrollment, but then recurs at a later date, the recurrence is considered an AE. The purpose of having pre-existing conditions documented is to ensure that abnormalities present at baseline and later observed during follow-up, at the same severity and frequency, are not documented as AEs (see SSP Section 9 AE Reporting and Safety Monitoring for more information).

8.1.2 Participant-Reported Conditions

Participant baseline medical and menstrual history is initially collected and documented at the screening visit and then actively reviewed and updated, as necessary, at the enrollment visit. The purpose of obtaining this information is to:

- Assess and document participant eligibility for the study
- Assess and document the participant's baseline medical and menstrual conditions and symptoms for comparison with signs, symptoms and conditions that may be identified or reported during follow-up (i.e., adverse event identification)

To obtain a complete, accurate, and relevant participant self-reported medical history, it will be necessary to ask the participant about past medical conditions and surgeries, as well as any conditions the participant is currently experiencing at the time of the Screening and Enrollment visits. It is recommended that sites use the MTN-038 Baseline Medical History Guide sheet in conjunction with the Medical History CRF and/or chart notes to guide and document medical history taking. Sites may also use a site-specific form per standard site procedure and should designate which forms will serve as source on the site source document SOP. Site clinicians are encouraged to use their clinical experience and judgment to determine the best phrasing and approach to elicit complete and accurate information from the participant. This is especially important with regard to details about severity and frequency of baseline medical history conditions.

When collecting medical information from the participant, site clinicians should ask probing questions to obtain the most complete and accurate information possible. Details of all relevant conditions identified during the baseline medical history review should be recorded within the Medical History CRF. Relevant conditions include (but are not limited to): hospitalizations; surgeries; allergies; conditions requiring prescription or chronic medication (lasting for more than 2 weeks); and, any condition(s) currently experienced by the participant. The clinician should record as much information as possible about the severity and frequency of any baseline medical condition in the description field within the Medical History CRF to best describe the condition at the time the participant enters the study. In addition to participant-reported conditions, record the following on the Medical History CRF:

- Grade 1 and higher lab values
- Medically-relevant physical exam abnormalities
- Pelvic exam abnormal findings
- Any identified STIs

Generally, it is not expected that conditions less than Grade 1 would be included on the Medical History CRF, unless determined to be relevant by the site clinician. The Medical History CRF can be updated with new or corrected information during follow-up, but only in instances when new information related to the participant's baseline medical history status is obtained after enrollment/randomization. If information is added to the Medical History CRF after the Enrollment Visit, a chart note explaining the update is required.

When collecting medical history, sites should also assess baseline menstrual history including the participant's first and last day of last menstrual period and a description of the participant's menstrual bleeding pattern. If a participant has a menstrual period between screening and enrollment, the dates of the menstrual period should be recorded/updated at enrollment when reviewing menstrual history. This can be documented in chart notes or another site-specific document. Medical history information may also be obtained from reviewing the participant's medical records, in accordance with IRB policies.

Sites should complete an entry on the Medical History CRF for any abnormal genital bleeding patterns (per the DAIDS Female Genital Grading Table for Use in Microbicide Studies (FGGT)) reported by the participant at the Screening and/or Enrollment visit. Site staff should carefully consider any abnormal bleeding patterns since participants must have regular menstrual cycles of at least 21 days in duration to be eligible for study participation. This criterion is not applicable to participants who report using a progestin-only method of contraception at Screening (e.g., Depo-Provera, contraceptive implant, or levonorgestrel-releasing IUD) nor to participants using continuous combination oral contraceptive pills, as the absence of regular menstrual cycles is an expected, normal consequence in this context. Ideally, menses must not coincide with the first seven days of product use. Since expected changes in genital bleeding will not be considered an AE during follow-up, it is important to document a participant's baseline abnormal genital bleeding patterns to the extent possible to monitor for unexpected changes.

8.1.3 Pre-existing Conditions Review and Update at the Enrollment Visit

Information documented on the Medical History CRF at the Screening Visit must be actively reviewed and updated at the Enrollment Visit, especially for those conditions that were ongoing at the Screening Visit. This includes a review and update of the condition's description and severity grade. Make sure the "Is the condition ongoing?" field is completed/updated for each entry prior to final eligibility confirmation.

Chronic conditions should be marked as "yes" for the question "Is the condition ongoing?" at the Enrollment Visit, even if the participant is not currently experiencing an acute event (e.g., intermittent headaches, seasonal or acute allergies). For severity grading, the highest severity experienced for the condition should be used. In the 'Description of medical history condition/event' item, note the typical severity for outbreaks/acute episodes of the condition, and whether the condition is currently being experienced by the participant, or historical.

During screening, if a participant reports having a history of anaphylactic reactions (such as acute anaphylaxis after eating peanuts), even if it has happened only once before in their lifetime, it is still important for the site clinician to document these events as a pre-existing condition on the Medical History CRF. Per the "acute allergic reaction" row of the DAIDS Toxicity Table, an acute anaphylactic event is considered a severity grade 4 as it is by definition a life-threatening reaction. Record the condition/event as "allergic reaction to peanuts" and note types of symptoms (e.g., "throat swelling" or "shortness of breath") indicate the severity grade 4 in the "Description of medical condition/event" field. At the Enrollment Visit, check "yes" to the question, "Is the condition ongoing?" and check "no" for the question "Is condition/event gradable?", as the participant was not experiencing an anaphylaxis event at the time of enrollment/randomization. An AE submission for an anaphylactic reaction is required if this same event occurs after enrollment or during study follow-up. Any acute allergic reaction less than a grade 4 should be documented as a chronic condition.

If a pre-existing condition is resolved as of the date of enrollment/randomization, do not make any changes to the severity grade (similar to what is done when resolving adverse events). In this case, the

response to the question, “Is the condition ongoing?” must be marked “no.” If a pre-existing condition first identified at the Screening Visit is ongoing at the Enrollment Visit, assess the severity at the Enrollment Visit and update the severity grade (up or down) as applicable to reflect the severity at the time of enrollment/randomization.

8.1.4 Baseline Medications

The protocol requires documentation of all medications taken by a study participant, beginning at the Screening Visit and continuing throughout the study follow-up period. The Concomitant Medications Log CRF is used to document all concomitant medications used by a given participant during study participation. Medications include the following:

- Prescription and “over-the counter” medications and preparations
- Vaccinations
- Vitamins and other nutritional supplements
- Herbal, naturopathic, and traditional preparations
- Contraceptive medications
    - Injectable contraceptive (Depo, NET-EN, Cyclofem, etc.): Record each injection that the participant receives during study participation on a new log line. Enter both the start and stop dates as the date of injection. Indicate the frequency as "once". Injections of contraceptive medications used before the Screening Visit are not recorded on the Concomitant Medications Log CRF. This CRF only captures medications used on or after the Screening Visit date
    - Oral contraceptive birth control pills: Record each pill pack confirmed by the participant to have been taken on a new log line. Indicate the start date as the date the first pill of the pack was taken and the stop date as the date the last pill of the pack was taken. If the participant is taking birth control pills at Screening, document this pill pack on the Concomitant Medications Log, as well as any other pill packs she begins during follow-up. If a participant misses a pill, this outage does not need to be recorded on the Concomitant Medications Log CRF
    - Implants/IUD: Record each implant/IUD on a new log line. The start date should be the date of implant or insertion and the stop date should be the date the implant/IUD is removed. Indicate the frequency as “Other” and write “continuous” in the text field. For medical devices with no active medication, such as the copper IUD, indicate the dose as “1”, the dose unit as “Other”, and indicate “device” in the text field. For IUD route, select “Other” and write “intrauterine” in the text field. For Implant route, select “Other’ and write “sub-dermal” in the text field. If the participant has an implant/IUD in place at Screening, document this on the Concomitant Medications Log, as well as any other implants or IUDs she receives during follow-up

Study staff should use the information obtained during the review of the medical history to probe for additional medications that the participant may have forgotten to report.

Participants must not be using or plan to use of anticoagulants or blood thinners (such as heparin, Lovenox®, warfarin, or Plavix® [clopidogrel bisulfate]) during the time of their planned study participation. Use of aspirin should also be avoided 72 hours prior to and after biopsy collection. If site staff have questions about a specific medication and whether or not it is prohibited, they should contact the PSRT for guidance.

In addition, per Protocol Section 5.2, participants must be using an effective form of contraception at the time of enrollment. To be eligible, participants must also state a willingness to refrain from the use of any non-study vaginal products (e.g. spermicides, female condoms, diaphragms, contraceptive VRs, vaginal medications, menstrual cups, cervical caps (or any other vaginally applied barrier method), vaginal douches, lubricants and moisturizers, sex toys etc.) 24 hours prior to enrollment and for the duration of

study participation. Participants may use tampons at any time during the study, except for 24 hours prior to any visit in which CVF is collected (Enrollment visit through Visit 10/Final Contact)

8.2 Clinical Instructions for Checking Ring Placement

Following insertion of the VR at the Enrollment Visit the study clinician or designee should perform a digital exam to check for correct placement of the VR. A digital exam to check placement may be done at other study visits in which a VR is inserted, if needed. The following is the procedure that the IoR or designated clinic staff should use to verify VR placement:

- After VR placement, ask the participant to walk around prior to verification of correct VR placement
- Have the participant lie comfortably on the examination table in supine position (on his/her back)
- Upon genital inspection, ensure that the VR is not visible on the external genitalia. If the VR is visible, the placement is not correct
- Make sure the VR does not press on the urethra
- On digital or bi-manual examination, ensure VR placement at least 2 cm above the introitus, beyond the levator ani muscle
- If, on inspection, the VR is found to be inserted incorrectly, remove and reinsert the VR correctly.

After correct placement is confirmed, the clinician should ask the participant to feel the position of the VR. This will help ensure that the participant understands what correct placement feels like, should they need to check this between study visits. This instruction may be repeated at any visit, as needed. Verification of VR placement should be documented on the Ring Insertion and Removal CRF.

At each follow-up visit, the study clinician must also check for presence and correct placement of the VR worn since the previous visit by visualization with a speculum. This should be done during the Pelvic Exam and documented on the Pelvic Exam CRF. Placement should also be checked as needed at other points during a visit.

8.3 Medical, Menstrual, and Medication History Review at Follow-Up

The Medical History CRF can be updated with new or corrected information during follow-up, but only in instances when new information related to the participant's baseline medical history status is obtained after enrollment/randomization. If information is added to the Medical History CRF after the Enrollment Visit, a chart note explaining the update is required.

8.3.1 Participant-reported Follow-up Medical and Menstrual History

An updated participant self-reported medical and menstrual history is required at each scheduled visit during follow-up. A history should also be performed at interim visits when a participant presents complaining of symptoms or when the purpose of the visit is to re-assess previously-identified AEs. One purpose of the participant-reported follow-up history is to determine whether previously-documented conditions have changed with regard to severity or frequency. Any changes are recorded on the AE Log CRF, as appropriate. A second purpose is to determine whether new symptoms, illnesses, conditions, etc., have occurred since the last medical history was performed. The AE Log CRF itself, chart notes, or a site-specific tool, if desired, may serve as the source document. All newly-identified participant-reported symptoms and conditions will be documented on the AE Log CRF (see SSP section 9 for details regarding AE documentation).

For purposes of this study, "newly-identified" is defined as one of the following conditions:

- not present at baseline (enrollment);

- ongoing at baseline but has increased in severity or frequency during follow-up (includes ongoing baseline conditions or adverse events that increase in severity or frequency during follow-up);
- ongoing at baseline, resolves during follow-up, and then re-occurs (excludes chronic condition which should be reported in accordance section 8.1.2 above)

Any symptoms reported by the participant should be further probed and evaluated. Be sure to ask about ongoing baseline symptoms listed on the Medical History CRF as well as any symptoms listed as "recovering/resolving" on an AE Log CRF.

If, during follow-up, a pre-existing condition resolves or increases in severity or frequency from baseline, this must be documented, but not on the Medical History CRF, which is meant to remain a snapshot of the participant's medical history as enrollment. Document resolution of a pre-existing condition in chart notes or other site-specific tracker. If the pre-existing condition reoccurs or increases in frequency, complete an AE Log CRF to document the change as a new AE and leave the condition's status as 'ongoing" on the Medical History CRF. The AE Log CRF should have the "yes" box marked for the question, "Was this AE a worsening of a baseline medical condition?".

8.3.2 Review of Medications History

At each follow up visit, review the participant's concomitant medications history and document this review by completing the Concomitant Medications Summary and Concomitant Medications Log CRFs. Ask the participant if they have started taking any new medications, and record on the Concomitant Medications Log CRF any new medications started since the last medications assessment. In addition, review all previous entries that do not have a "Date Stopped" entered and ask the participant whether they are still taking the medication (and at the same dose and frequency). If the participant has stopped taking a medication, enter the last date the participant used the medication in the "Date Stopped" field. If the participant is taking the same medication but at a different dose or frequency, enter in the "Date Stopped" field the date the participant last used the medication at the original dose or frequency, and complete a new Concomitant Medications Log entry for the new dose or frequency. Ensure that concomitant medications mentioned in previous parts of the visit are documented correctly and consistently on the Concomitant Medications Log CRF, so that study records are not discrepant.

8.4 Physical Exams

The goal of the physical exam during screening is to collect detailed information on baseline conditions, as well as to evaluate eligibility. A complete physical exam will be conducted at the Screening visit. A targeted physical exam is required at the Enrollment visit and only if indicated at all other in clinic follow-up visits through the final contact visit. Per Protocol Section 7.10 the following assessments are required during the full physical exam:

- General appearance
- Weight (see SSP Section 8.4.1 for further guidance)
- Vital signs:
    - Temperature
    - Pulse
    - Blood pressure (See SSP Section 8.4.2 for further guidance)
    - Respirations*
- Height (See SSP Section 8.4.2 for further guidance)*
- Abdomen*
- Lymph nodes*
- Neck*
- Head, eye, Ear, Nose and Throat (HEENT)*
- Heart*

- Lungs*
- Extremities*
- Skin*
- Neurological*

** Note: Can be omitted during a targeted physical examination, but may be conducted at any time for clinical care.*

At the screening and enrollment physical exams, site staff should assess for any other medical condition that would make participation in the study unsafe or interfere with interpreting the study data or achieving the study objectives. Physical exam assessments should be documented on the Physical Exam and Vital Signs CRFs.

8.4.1 Weight

Participant weight must be measured at the Screening Visit physical exam and additionally when clinically indicated. Weight should be measured in kilograms and can be rounded up to one decimal. Scales should be calibrated at least twice per year, and more frequently if required per local practice standards.

8.4.2 Height

Height should be measured in centimeters and can be rounded up to one decimal.

8.4.3 Blood Pressure

Blood pressure devices are expected to be calibrated regularly per manufacturer's directions.

8.5 Pelvic Exam Overview

The pelvic exam performed during the Screening and Enrollment Visits is necessary to evaluate protocol exclusion criteria and to collect detailed information on baseline genital/genitourinary conditions. A pelvic exam is also required at follow-up visits through PUEV (Visit 9), and if indicated at the Final Contact Visit. Guidance on the conduct of pelvic exams can be found in the remainder of this section. Pelvic exams are documented on the Pelvic Exam CRF, which may be source documented on the Pelvic Exam Diagrams (non-Medidata form) or another site-specific source document, as specified in the site's source documentation SOP.

Note that cervical bleeding associated with speculum insertion and/or specimen collection judged to be within the range of normal according to the clinical judgment of the Investigator of Record (IoR)/designee is not exclusionary, nor considered an AE.

8.5.1 Pelvic Exam Technique

General Technique: Maximize the comfort and privacy of the participant. Position the examination table away from the door or hang a curtain to ensure privacy. Explain what you are doing as you do it. Take as much time as needed to ensure participant comfort and accurate documentation of exam findings. Use clean hand/dirty hand technique, and/or assistants, to avoid contamination. Keep extra gloves available as two hands may be needed at different time points during the exam. Use a speculum of appropriate type and size to permit adequate visualization of the vagina and cervix.

Exams During Bleeding: Routine pelvic exams, i.e., those required at protocol-specified timepoints, should be avoided during menses-like bleeding, as the presence of blood may interfere with visualization of the vagina and cervix, and complicate interpretation of vaginal assays. If a participant is experiencing mild spotting, it is reasonable to proceed with a pelvic exam and collection of samples. If the participant is experiencing greater than mild spotting when presenting for a visit in which a routine pelvic exam is required, perform other protocol-specified procedures at the visit and schedule the participant to return for the pelvic exam as soon as possible after bleeding has stopped, within the visit window (as part of a split visit, if allowable; refer to SSP Section 12). If this is not possible to conduct the pelvic exam, collect all required pelvic specimens (including PK), and note the bleeding in their chart notes and on applicable CRFs (i.e., Pelvic Exam CRF, Cervical Specimen Storage CRF). If a participant is experiencing genital bleeding when presenting for an interim visit and complaining of genital symptoms, every effort should be made to perform a pelvic exam to evaluate symptoms at that time.

8.5.2 Detailed Procedural Instructions

Prior to the Exam: Prepare all required equipment, supplies, and paperwork; label specimen collection supplies as needed. Verify that all equipment is in good working order. Review documentation of prior exams and other relevant documentation from the current visit and prior visits. While the participant is clothed, explain the procedures and answer any questions the participant may have.

If the participant has the VR in prior to the pelvic exam, the VR should remain in for the clinician to check placement during the pelvic exam. The VR may stay in place for the duration of the pelvic exam. If the participant is uncomfortable, the clinician may remove the VR temporarily for the speculum exam and then replace the VR once done.

Examine the External Genitalia:

- Do not insert the speculum before examining the external genitalia.
- Relax the participant's knees as far apart as is comfortable.
- Palpate the inguinal lymph nodes to assess for enlargement and/or tenderness.
- Perform naked eye examination of the external genitalia including the perineum, and perianal area.

Examine the Cervix and Vagina:

- The speculum may be lubricated with warm water if needed. No other lubricant may be used. Gently insert the speculum and open it once past the pelvic floor muscles, using gentle downward pressure, so as to avoid trauma while enabling visualization of the cervical face and upper vagina.
- If the cervix is poorly visualized, to avoid iatrogenic injury, remove the speculum and use a gloved finger (lubricated with warm water if needed) to establish the position of the cervix. Then re-insert the speculum.
- Perform naked eye exam of the cervix, if applicable, and vagina.

Collect Specimens: Collect specimens in the order listed on the sample Pelvic Exam Checklist. The order of specimen collection is critical to ensure that first specimen collections do not affect subsequent specimens. Collect specimens away from apparent abnormalities and/or previously swabbed areas, and avoid touching the VR if in place.

Removal of Visual Obstruction: After collection of vaginal and endocervical specimens, any obstruction (e.g., mucus, cellular debris) may be removed with a large saline-moistened swab (Scopette) in a gentle dabbing fashion to remove the obstruction. Avoid twisting or rolling the swab over the surface of epithelium. Do not use a dry swab to remove any obstruction at any time, as this may cause trauma to the epithelium. If saline is not available, a swab moistened with water will also suffice.

Complete Examination of the Cervix and Vagina: To complete the naked eye examination of the vagina, slowly withdraw the speculum with the blades moderately open, re-focusing as needed. Alternatively, the speculum may be rotated ninety degrees to allow visualization of the anterior and posterior vaginal walls; retract the speculum away from the cervix and close the blades to rotate.

Perform Bimanual Exam: If clinically indicated, after completing all the above-listed examinations and specimen collection and removing the speculum, perform a bimanual exam for adnexal or fundal masses and/or tenderness.

8.5.3 Cervicovaginal Fluid (CVF) Collection

CVF for PK, anti-HSV-2 and biomarkers will be collected from all participants at different points according to the table below. A pre-weighed dacron swab will be inserted into the upper vagina (approximately 5cm/2 inches, near the site of the VR) and held for a slow count to 10 seconds. Collection of the CVF swab for TFV should occur after blood and rectal PK sample collections (when applicable) and prior to the collection of all other vaginal/cervical specimens, and prior to the insertion of the speculum. CVF swabs for biomarkers and anti-HSV-2 should be the last of the CVF and vaginal fluid swabs collected, and with the speculum inserted. Since the VR will remain in during CVF collection, care should be taken to not touch the VR with the swab.

For single PK collection time-points (Days 1, 7, 14, 28, 56, and Final Contact) as well as multiple PK collection timepoints (Enrollment and PUEV/Day 91), CVF for PK should be collected within 30 minutes of blood and rectal PK specimen collection.

Table 8-1

Study Visit/ CVF type	Anti-HSV-2	TFV Levels	Biomarkers
Enrollment	Prior to VR insertion, during pelvic exam	Hours 1 and 4 following VR insertion	Prior to VR insertion, during pelvic exam
Days 1, 7, 14, 28, 42, or 56	**Day 28 and 56 ONLY** During pelvic exam	**Days 1, 7, 14, 28, 56** During pelvic exam	**ALL** During pelvic exam
PUEV/ Day 91	N/A	Prior to VR removal (during pelvic exam) and hour 4 following	Prior to VR removal, during pelvic exam
Final Contact	N/A	Per Visit Checklist	N/A

Refer to SSP Section 10 Lab Considerations for instructions on weighing, processing and storage of the swab for PK.

8.5.4 Cervicovaginal Lavage (CVL) Collection for PK, PD and Biomarkers

CVL for PD and biomarkers will be collected at Enrollment, Day 28 and 56 visits, and additionally for PK, at Day 28 and 56. CVL should be collected after CVF when both specimens are collected at the same timepoint so as to not dilute the CVF sample. Collection timepoints are included in the table below. The study VR should remain in place during sample collection on Day 28 and Day 56. A speculum should be used when CVL is collected during the pelvic exam.

Table 8-2

Study Visit	Timing of Collection
Enrollment	During pelvic exam (prior to VR insertion)
Days 28, 56	During pelvic exam

Suggested Materials

- Drape sheet
- Gloves
- Sterile Normal Saline
- Sterile tubing (4-5 cm in length) (optional)
- Metal specimen rack
- Sterile specimen containers
- Sterile needle-less 30 mL syringe
- Metal speculum
- 2 mL pipette
- 15 mL conical centrifuge tube
- Study source documents
- Clock/timer
- Wet ice or cold packs
- Protective eyewear
- Thermometer

Preparation Notes

- ✓ Prior to examination, have all necessary materials readily available on exam cart or counter near exam table.
- ✓ Check expiration of sterile saline prior to use.

Sample Collection and Transport:

- Draw 10 mL of sterile normal saline into the syringe.
- Carefully insert tip of syringe into the vagina using care not to touch vaginal walls with syringe. With tip of syringe aimed at the cervix or upper end of the vagina, dispense all 10 mL of saline onto the cervix. Gently tilt speculum if necessary to avoid leakage of saline.
- Place tip of a 2 mL pipette onto posterior blade of the speculum and draw fluid into pipette, using care not to touch the vagina or cervix, if applicable.
- Use the 10 mL of saline to lavage the cervix, fornices and vaginal walls. Be sure to lavage each side wall at least twice. Only use the original 10 mL of saline. Do not use any additional saline to perform lavage.
- The saline must be in contact with the vaginal vault for at least 1 minute.
- After at least 1 minute of contact, remove lavage fluid with 30 mL syringe and sterile tubing or 2 mL pipette.
- Save lavage fluid for analysis. Transfer fluid to 15 mL conical centrifuge tube.
- Once lavage procedure is complete, visually inspect cervix and/or vagina.
- Verify labeling of all specimens with study identifiers, visit code, date of collection.
- Place specimen in refrigerator or on wet ice or cold packs immediately after collection. See SSP section 10 Lab Considerations for details on specimen storage and transport.

8.5.5 Cervical biopsies for PK and PD

Participants will be randomized to provide biopsy samples at either visit Days 14 and 56 or visit Day 28 and 91. Collection timepoints and number of biopsies are included in the table below.

Table 8-3

Study Visit	Number of biopsies	Timing of Collection
Days 14, 28	2	During pelvic exam

Day 56	4	During pelvic exam
Day 91/ PUEV	4	Prior to VR removal; during pelvic exam

Using forceps, take samples approximately 3 x 5 mm in size from different locations from the cervix. Biopsy of the cervix does not require an anesthetic; this procedure typically feels like a pinch or a cramp. Bleeding may be controlled through a combination of applied pressure, silver nitrate and/or monsel's solution. The minimum amount of silver nitrate and/or monsel's solution should be used to control bleeding, as excessive use of these agents may impact PK measures. For this reason, cervical biopsy should be the last specimen collected during the pelvic exam. Use of any coagulant used post-biopsy must be recorded on the Concomitant Medications Log CRF.

Participants should be informed that they may experience a small amount of bleeding from the vagina 1-2 days following the procedure. If bleeding is reported as being heavier than the participants' usual menstrual period or if the participant experiences a foul odor or a heavier vaginal discharge (more than usual), they should be instructed to contact the study clinic right away. There is a small risk of the biopsy area becoming infected or having bleeding that is heavier than spotting.

All participants will be instructed to abstain from any receptive vaginal and anal sexual activities for 72 hours prior to each clinic visit and additionally to abstain from such sexual activities for 72 hours after the collection of these samples. Participants will also be counseled to refrain from the use of aspirin (greater than 81mg) that is associated with the increased likelihood of bleeding for 72 hours prior to and following mucosal biopsy collection.

8.5.6 Rectal fluid for TFV levels for PK

At Days 1, 14, 28, 56, and 91/PUEV rectal fluid for PK will be collected from all participants. For single PK collection timepoints (Days 1, 14, 28, 56) as well as multiple PK collection time-points on PUEV/ Day 91, rectal fluid should be collected within 30 minutes of all other PK specimen collection of the same timepoint, and after blood but before CVF collection for PK.

Table 8-4

Study Visit	Timing of Collection
Days 1, 14, 28, 56	During pelvic exam
PUEV/ Day 91	Prior to ring removal and 4 hours following

Rectal fluid will be collected using a swab that is inserted into the rectum through an anoscope. Using study provided lubricant (Good Clean Love lubricant), the clinician should sparingly lubricate the anoscope prior to insertion. The anoscope with obturator should then be inserted into the anal canal until the anoscope 'wings' touch the anal verge. The clinician should maintain pressure on the flange to ensure continued placement of the anoscope and then remove the obturator. Using a lighted instrument (e.g. otoscope or torch) to illuminate the rectum after removing the obturator, the rectal lumen should be visible at the end of the anoscope. The clinician should visually assess the rectum after the anoscope is in place and prior to specimen collection.

The clinician/assistant will open the wrapper containing the swab while ensuring the tip of the swab is not touched. Do not place any fluid or lubricant on swab. After removing the obturator, advance the anoscope slightly then insert the swab into the proximal rectal lumen that is visible at the end of the anoscope. Hold swab against the mucosa for 2 minutes. Fully insert the swab into the transport tube. Carefully snap the swab shaft at the scoreline to fit the tube; use care to avoid splashing of contents. Re-cap tube securely by snapping the cap into place. Following specimen collection, the clinician should assess the anal canal as the anoscope is withdrawn. Refer to SSP Section 10 Lab Considerations for instructions on weighing, processing and storage of the swab for PK.

8.5.7 Documentation of Findings

All exam findings (normal and abnormal) should be documented on the site-designated source document, as specified in the site's source documentation SOP. All abnormal findings must be thoroughly documented (e.g., to include type, size, anatomical location, and severity grade) on the Pelvic Exam CRF, and any other relevant source documents as desired such as the Pelvic Exam Diagrams paper form, to ensure appropriate assessment can be provided during the next pelvic exam.

All abnormal findings observed during the Screening and Enrollment Visits will be documented on the Pelvic Exam CRF and the Medical History CRF. All abnormal findings identified during follow-up will be documented on the Pelvic Exam CRF. All newly-identified abnormal pelvic exam findings will be documented on an AE Log CRF. The results of site local laboratory test results performed using specimens collected during pelvic exams are recorded on the STI Test Results CRF.

All pelvic exam findings consistent with the "Grade 0" column of the FGGT are considered normal. The following also are considered normal:

- anatomic variants
- gland openings
- Nabothian cysts
- mucus retention cysts
- Gartner's duct cysts
- blood vessel changes other than disruption
- skin tags
- scars
- cervical ectopy

Abnormal findings will be classified according to the state of the epithelium and blood vessels associated with the finding, as follows:

Epithelium

Integrity:

- Intact
- Disrupted:
  - Superficial
  - Deep (complete disruption is considered deep and exposes stroma and possibly blood vessels; a bleeding area is often but not always deep)

Color:

- Normal
- Slightly red
- Red
- White
- Other (includes "pale")

Blood Vessels

Integrity:

- Intact
- Disrupted

Pelvic exam findings should be documented using terminology corresponding to the FGGT and the Pelvic Exam CRF. For findings in which the finding term marked on the Pelvic Exam CRF is more specific than the corresponding term on the FGGT, use the more specific CRF term.

8.6 STI/RTI/UTI

Clinical and laboratory evaluations are performed in MTN-038 to diagnose the following STIs and RTIs:

- Chlamydia infection
- Gonorrhea infection
- Trichomonas
- Hepatitis B
- Syphilis infection
- HIV-1
- HSV-1/2

8.6.1 Considerations at Screening/Enrollment

Participants diagnosed during Screening and Enrollment with a symptomatic RTI or UTI may only enroll in the study following completion of treatment and resolution of all symptoms, provided this occurs within 45 days of obtaining informed consent. See Exclusion Criterion #2 in Protocol Section 5.3. Participants diagnosed with an acute STI requiring treatment per CDC guidelines at Screening or Enrollment are ineligible to enroll. See Exclusion Criterion #3, and the note listed underneath, in Protocol Section 5.3.

8.6.2 STI/RTI/UTI Diagnosis

Clinical and laboratory evaluations for gonorrhea, chlamydia, syphilis, hepatitis B, and trichomonas are required at screening, and HSV 1/2 serology is required at enrollment. These evaluations may be repeated, if clinically indicated at all other visits.

If an STI, RTI, or UTI is identified during follow-up, it should be documented as an AE. Infections should be considered "symptomatic" when a participant self-reports or complains of symptoms associated with the infection. Symptoms should not be confused with "signs" of infection that may be observed during clinical examinations performed by study staff.

Genital HSV: Per the FGGT, the term "genital herpes" may only be used for adverse event reporting if laboratory testing is conducted or has been performed in the past; otherwise sites are encouraged to use the most appropriate row in the FGGT which most closely resembles the clinical findings (ulceration, for example).

Urinary tract infections (UTIs): UTIs may be diagnosed in MTN-038 based solely on the presence of symptoms indicative of a possible UTI, or other method of diagnosis (i.e., urine culture or dipstick) as per site standard of care. See SSP Section 9 for guidance on documenting UTI AEs based on symptoms or culture.

The following symptoms are considered indicative of a possible UTI:

- Frequent urge to urinate
- Passage of only a small volume of urine
- Pain and burning during urination
- Lower abdominal pain and/or uncomfortable pressure above the pubic bone
- Milky/cloudy, reddish, or bloody urine

Urine dipstick may be performed per site SOP, however sites are expected to send a urine culture for definitive diagnosis when a UTI is suspected. The results of the urine culture do not need to be returned before presumptive treatment, but the results of the culture will influence how the AE is captured. When the participant initially reports symptoms suggestive of a urinary tract infection, capture each symptom as a separate AE. If urine culture results are positive, update the AE Log CRFs to reflect a single AE for grade 2 Urinary Tract Infection per UTI criteria defined in the FGGT. If urine culture is negative, the AE(s) will remain reported as symptoms only. Record the results of any dipsticks performed on the STI Test

Results CRF; urine culture results must be documented in chart notes and/or other site-specific source documents.

Note that urine dipstick testing is only performed if clinically indicated. At the screening visit, positive dipstick results do not directly impact eligibility, but abnormal protein and glucose parameters should prompt further evaluation or consideration pending IoR review. Abnormal protein and glucose uncovered at the screening visit should be captured on the Medical History CRF. In follow-up, findings of abnormal protein and glucose on the dipstick should be reported on the AE Log CRF as indicated. Grade the severity of the urine glucose value according to the "Proteinuria, random collection" row of the Toxicity Table. Note that findings of LE/nitrites are not gradable per the DAIDs toxicity table, and like other non-gradable labs should not be reported as a baseline conditions or AEs.

When clinically appropriate, investigators should use oral or parenteral medications when at all possible to avoid intravaginal or rectally administered medication use. Observed single dose treatment should be provided whenever possible, per clinician discretion.

8.6.3 STI/RTI/UTI Management

Treatment**:** All participants diagnosed with UTI based on the presence of symptoms should be provided treatment per site standard of care and applicable site SOPs.

All STIs/RTIs should be managed per current CDC guidelines, site standard of care and applicable site SOPs. Current CDC guidelines can be accessed at: http://www.cdc.gov/std/treatment/

Asymptomatic BV does not require treatment per current CDC guidelines. Asymptomatic vaginal candidiasis also should not be treated. During screening, these asymptomatic infections are not exclusionary and during follow-up these asymptomatic infections are not considered AEs, however they will be captured on the STI Test Results CRF.

Syndromic Management: Syndromic management of STIs is acceptable per site SOP and local standard of care; however, a thorough laboratory evaluation is expected in the context of this research study so that a specific diagnosis might be uncovered.

Test of Cure**:** STI/RTI tests of cure are not required in MTN-038, but may be recommended per local guidelines.

8.7 Vaginal Discharge

Both participant complaints and clinical findings of abnormal vaginal discharge are common in microbicide studies. While the evaluation of abnormal vaginal discharge may not differ between the two, whether treatment is offered and how the abnormality is reported may. Abnormal vaginal discharge may be associated with yeast and/or bacterial vaginosis among other conditions. Site clinicians are encouraged to thoroughly evaluate complaints and/or findings of abnormal vaginal discharge as per their discretion. Whether to treat the underlying cause of the abnormal vaginal discharge will depend on:

1. What the underlying diagnosis is; and,
2. Whether the participant is symptomatic.

If the evaluation reveals an underlying sexually transmitted infection such as trichomoniasis, the participant and his/her partner(s) should be offered treatment regardless of symptoms. If the evaluation reveals bacterial vaginosis or yeast, the participant should be offered treatment only if symptomatic. Sites should prescribe non-vaginal treatment when possible.

SSP Section 9 AE Reporting and Safety Monitoring details the reporting of vaginal discharge AEs. Briefly, sites are encouraged to distinguish whether the discharge was initially reported by the participant ("vaginal discharge by participant report") or noted only on pelvic exam by the clinician ("vaginal discharge-clinician observed"). Importantly, in instances when the evaluation of clinician-observed vaginal discharge reveals asymptomatic bacterial vaginosis or asymptomatic yeast, an AE should be reported for "vaginal discharge-clinician observed." Even though asymptomatic yeast and bacterial vaginosis are not considered AEs per protocol, in these instances, the clinician observed vaginal discharge should be captured as an AE.

8.8 Genital Bleeding Assessment

For visits in which cervical PK specimens will be collected, study staff will ask participants whether any genital bleeding was experienced in the preceding seven days and how many days on which bleeding was heavy, defined as soaking a tampon/pad every 2 hours or less. This information is documented on the Cervical Specimen Storage CRF, which is required to be completed at all protocol-specified follow-up visits. In addition, participants will be counseled to report all occurrences of unusual genital bleeding to study staff as soon as possible after identification of the bleeding. Per Protocol Section 8.3.1, expected genital bleeding will not be reported as an AE.

8.9 Management of Laboratory Test Results

CBC with platelets and differential, AST/ALT, and serum creatinine will be performed at Screening and Day 91/PUEV, and if indicated at other follow-up visits. HIV-1 testing will be performed at Screening, Enrollment, and Day 91/PUEV, and if indicated at other follow-up visits. For each study participant, the IoR or designee is responsible for reviewing and monitoring these test results and for ensuring appropriate clinical management of all results. IoR or designee review of laboratory test results should be documented on the lab results report (provided by the lab to the clinic) and/or in chart notes. For tests done at Enrollment through follow-up visits, results should also be documented on CRFs as follows: AST/ALT results on the Chemistry Panel CRF; CBC platelets and differentials on the Hematology CRF; and HIV test results on the HIV Test Results CRF.

Lab results reported on the Chemistry Panel and Hematology CRFs should be entered using the units reflected in the DAIDS toxicity table, corrected version 2.1. If the units present on the source results report do not match the units on the eCRF and in the DAIDS Toxicity Table, values should be converted before entry into the CRF.

The SCHARP Lab Unit Conversions Tool can be found and accessed at the following ATLAS page https://atlas.scharp.org/cpas/project/Collaborators/Lab%20Unit%20Conversion%20Tool/begin.view. This tool will enable sites to convert local lab values and enter them into Medidata Rave using the units reflected in the DAIDS toxicity table. Please note that this tool rounds converted lab values to three (3) digits after the decimal. Site use of this tool is optional, and strongly recommended for sites that must convert local lab values in order to enter them into Medidata Rave for a given study.

In addition to participant-reported conditions, record all abnormal Screening Visit lab values (i.e., severity Grade 1 and higher), regardless of grade, on the Medical History CRF.

At a minimum, all test results of severity Grade 3 and higher and all results requiring product discontinuation should be urgently reported to the site's study clinician.

The IoR or designee should routinely review participant study records to ensure proper monitoring and clinical management of laboratory test results, and documentation thereof. This includes documentation of referrals for abnormal, exclusionary laboratory results that are identified during the screening process.

8.10 Clinical and Product Use Management

Protocol Section 9 provides detailed guidance on clinical and product use management, including general criteria for product hold and discontinuation (Section 9.3), guidance on clinical management in response to observed AEs (Section 9.4), management of STI/RTIs (Section 9.5), management of specific genital events (Section 9.6), HIV infection (Section 9.7), pregnancies (Section 9.8), and guidance on early study termination (Section 9.9). Below is a list of conditions that require temporary or permanent study product discontinuation:

Permanent Discontinuation:

- Acquisition of HIV infection
- Allergic reaction to the vaginal ring
- Pregnancy
- Breastfeeding
- Non-therapeutic injection drug use

Temporary Discontinuation:

- Reported use of PEP for HIV exposure
  - **Note**: Participants who experience a known or potential HIV exposure during study participation will be encouraged to use PEP as soon as possible and within 72 hours of exposure.
- Reported use of PrEP for HIV prevention
- Use of heparin, Lovenox®, warfarin, Plavix® (clopidogrel bisulfate), or other anticoagulant
- Clinical study product hold lasting more than 7 days
- Unable or unwilling to comply with required study procedures, or otherwise might be put at undue risk to their safety and well-being by continuing product use, according to the judgment of the IoR/designee.

All specifications in Protocol Section 9 must be followed. IoRs are encouraged to consult the PSRT with any questions related to proper interpretation of the protocol and proper management of study product use in particular.

All clinical and product use management must be fully documented in participant study records. When the PSRT is consulted in relation to clinical and product use management, completed PSRT query forms (including a response from the PSRT) must be printed and filed in participant study records.

All product holds and discontinuations must be communicated to site pharmacy staff using the Vaginal Ring Request Slip, as described in SSP Section 7 Study Product Considerations. Product discontinuations also must be documented on the Product Discontinuation CRF.