

Section 9. Clinical Considerations

9.	Introduction.....	9-1
9.1	Baseline Medical Conditions (Pre-existing Conditions)	9-1
9.1.1	Baseline Medical Conditions at Screening and Enrollment	9-2
9.1.2	Follow-up Medical History Review	9-3
9.2	Concomitant Medications	9-4
9.3	Prohibited Medications	9-5
9.4	Physical Exam	9-6
9.5	Rectal, Male Genital and Pelvic Exam.....	9-7
9.5.1	Male Genital Exam Instructions.....	9-7
9.5.2	Pelvic Exam Instructions	9-8
9.5.2.1	Pelvic Specimen Collection.....	9-9
9.5.2.2	Documenting Pelvic Exam Findings.....	9-10
9.5.3	Anorectal Exam Instructions.....	9-11
9.5.3.1	Anorectal Specimen Collection	9-11
9.5.3.2	Documenting Rectal Exam Findings	9-14
9.6	STI/RTI/UTI Evaluation, Management and Treatment	9-14
9.7	Clinical and Product Use Management	9-17

9. Introduction

This section presents information on clinical procedures performed in MTN-039. The Schedule of Study Visits and Evaluations in Appendix I of the protocol indicates when specific clinical and laboratory assessments are to take place. The IoR or designee should perform symptom-directed examinations at his/her discretion at any time during any visit if s/he determines it to be clinically necessary, particularly if there are any on-going medical conditions which may require follow-up.

Information on performing laboratory procedures associated with the clinical procedures described in this section is provided in the Laboratory Considerations SSP Section. Instructions for completing data collection forms associated with clinical procedures are provided in the Data Collection SSP section.

9.1 Baseline Medical Conditions (Pre-existing Conditions)

The participants' baseline medical 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 conditions and symptoms for comparison with signs, symptoms and conditions that may be identified or reported during follow-up (i.e. adverse event identification)

In order to obtain a complete, accurate, and relevant participant self-reported medical history, it will be necessary to ask the participant about significant past medical conditions as well as any current conditions. Medical history information may also be obtained from reviewing the participant's medical records, in accordance with IRB policies.

It is recommended that sites use the MTN-039 Baseline Medical History Guide (available on the MTN-039 web page under Study Implementation Materials) in conjunction with the Baseline Medical History Log CRF and/or chart notes to guide and document medical history taking.

When collecting medical information from the participant, site clinicians should ask probing questions to obtain the most complete and accurate information possible. This is especially important regarding details about severity and frequency of baseline medical conditions. Details of all relevant conditions identified during the baseline medical history review should be recorded on the Baseline Medical History Log CRF.

Baseline medical conditions are a subset of a participant's medical history and consist of all ongoing and/or relevant medical conditions, problems, signs, symptoms and abnormal findings that are observed and/or reported at enrollment or before a potential participant is enrolled (randomized). 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 on the Baseline Medical History Log CRF to best describe the condition at the time the participant enters the study. In addition to participant-reported conditions, the following should be recorded on the Baseline Medical History Log:

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

Note: Generally, it is not expected that conditions less than Grade 1 would be included on the Baseline Medical History Log, unless determined to be relevant by the site clinician.

The baseline medical history should explore in detail any medical conditions or medications that are deemed exclusionary for this study. At the enrollment visit, a participant's history should be reviewed and updated as needed. Refer to protocol section 5.2 and 5.3 for a complete listing of study inclusion and exclusion criteria.

9.1.1 Baseline Medical Conditions at Screening and Enrollment

The Baseline Medical History CRF is completed based on all screening source documents including, but not limited to, the Physical Exam CRF, Anorectal Exam CRF, Pelvic Exam CRF, Pelvic Exam Diagrams, and site-specific laboratory reports.

Information documented on the Baseline Medical History Log 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.

If a baseline medical 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 selected "*no.*" If a baseline medical condition first identified at screening is ongoing at enrollment, 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.

Recurrent Chronic Conditions: Recurrent 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. When assessing chronic conditions, it is important to note what, if any, medications a participant may take for a reported chronic condition and whether taking this medication during study participation may result in product discontinuation. For example, if a participant suffers from chronic asthma and uses an anti-inflammatory medication or an immunomodulatory to control his/her condition, site staff are asked to use their discretion with evaluating the eligibility of this participant.

Menstruation and Bleeding Events (for female participants): When collecting baseline medical history, sites should also ascertain the participant's first and last day of bleeding and a description of the participant's typical 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.

Site staff should carefully consider any bleeding patterns; menses should not coincide with enrollment, dosing and post-dosing visits and for this reason, site staff should ensure the participant's menstrual cycle coincides with the study product washout period between doses. Expected changes in genital bleeding (changes in genital bleeding deemed to be related to the participant's contraceptive use or menstruation postpartum) 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.

Note that any bleeding abnormalities ongoing at baseline (e.g. menorrhagia, metrorrhagia, or menometrorrhagia) should be selected as "not gradable" on the Baseline Medical History Log CRF. This is because the Female Genital Grading Table (FGGT) grades these bleeding events relative to each participant's baseline bleeding pattern. In the "Description of medical condition/event" field, include text similar to what is in the FGGT row to describe the severity and frequency of the condition, and whether it is attributed to a participant's current contraceptive method. Infrequent bleeding at baseline should also be captured, using the terms "missed menses", "oligomenorrhea" or "amenorrhea" as appropriate. If infrequent bleeding is explained by contraceptive method, note this in the description field and select 'not gradable'. If infrequent bleeding is unexplained, assign a severity grade from 1-2 per the FGGT.

Anaphylactic Reactions: 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 Baseline Medical History Log 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.

9.1.2 Follow-up Medical History Review

An updated participant self-reported medical history is required at each scheduled visit during follow-up. A history should also be performed at interim visits when a participant complains 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 in severity or frequency. A second purpose is to determine whether new symptoms, illnesses, conditions, etc., have occurred since the medical history was last assessed. The applicable CRF, 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 considered AEs and documented in the participant chart.

For purposes of this study, a “newly-identified” condition is defined as one of the following:

- not present at baseline (enrollment);
- ongoing at baseline but has increased in severity or frequency during follow-up (includes ongoing baseline conditions or AEs 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 SSP Section 9.1.1 above)

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

If, during follow-up, a baseline medical condition resolves or increases in severity or frequency from baseline, this is not updated on the Medical History Log CRF. If the condition increases in severity or frequency from baseline, and meets requirements for AE reporting, complete an AE Log CRF to document the new AE (i.e., the baseline condition at an increased severity and/or frequency). The AE Log CRF should be selected “yes” for the question, “Was this AE a worsening of a baseline medical condition?”.

The Baseline Medical History Log 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 Baseline Medical History Log CRF after the Enrollment Visit, a chart note explaining the update is required.

Participants will be counseled to report all occurrences of unusual genital bleeding that is different from baseline reports and not attributable to contraceptive method to study staff as soon as possible after identification of the bleeding.

9.2 Concomitant Medications

The Concomitant Medications Log CRF is used to document all concomitant medications used by a given participant during his/her study participation.

Protocol section 6.6 requires site staff to document all medications taken by study participants beginning at screening and continuing throughout the duration of the study. 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, if applicable
 - 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

Use of sexual lubricants should be recorded on the on the Concomitant Medications Log CRF as well.

Note: Alcohol consumption and recreational drug use should not be reported as concomitant medications on the Concomitant Medications Log. Instead, excessive alcohol consumption (defined as binge drinking, heavy drinking, and any drinking by pregnant women or people younger than age 21 (as per the CDC: <https://www.cdc.gov/alcohol/fact-sheets/alcohol-use.htm>) and recreational drug use may be considered baseline medical conditions, per site clinician judgment, in which case they should be recorded on the Medical History Log CRF.

It is helpful to ascertain the baseline medication information in the context of the baseline medical history. Site staff should ask open-ended questions to elicit participant report of current medications, and use the information obtained in the medical history to probe for additional medications that the participant may otherwise forget to report.

To help ensure accurate reporting of concomitant medications information, participants should be encouraged to bring a list of all medications to study visits.

At each follow-up visit, review the participant’s concomitant medications history and document this review by completing the item “Is the participant taking any concomitant medications that have not been previously reported?” on the Follow-up Visit Summary CRF and/or Interim Visit Summary CRF. Ask the participant if s/he has started taking any new medications, and record on the Concomitant Medications Log CRF any new medications s/he reports having started since her last medications assessment.

In addition, review all previous entries that do not have a “Date Stopped” entered and ask the participant whether s/he is 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 the date the participant last used the medication at the original dose or frequency in the “Date Stopped” field, 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.

9.3 Prohibited Medications, Products and Practices

Certain medications, products and practices are contraindicated during the study participation because they may be harmful to the participant, impact product safety and drug

concentration/PD/biomarker safety parameters or confound adverse event determination. Participants will be counseled on avoiding using protocol specified medications and engaging in the certain practices during study participation.

These include the following: PrEP for HIV prevention, PEP for potential HIV exposure, rectally administered products or any product containing N-9, strong or moderate CYP3A inhibitors and inducers, and anticoagulants.

Use of aspirin (greater than 81 mg) and other non-steroidal anti-inflammatory drugs (NSAIDs) within 72 hours prior to and following a PK sample collection visit should be restricted. Should a participant report taking these medications within 72 hours prior to biopsy collection, the visit should be rescheduled within the visit window, if possible. If it is determined that rescheduling the visit within the window is not possible, the visit may proceed at IoR discretion after proper participant counseling has occurred. Rapid PSRT consultation may be requested at IoR discretion to assist in determining whether biopsy collection should be delayed or proceed as scheduled.

Sexual activity (RAI, oral, etc.) and use of other products (i.e. personal lube or regular enemas for routine cleansing practices used rectally, etc.) are only restricted 72 hours prior to and following study visits.

Tampon use is prohibited during study participation 24 hours prior to each visit.

Site staff are requested to consult the MTN-039 Management Team on protocol deviation considerations should a participant report using a prohibited medication. Site IoRs should use their discretion when determining whether or not to enroll participants using medications not explicitly prohibited by the protocol but that may impact study results or participant safety.

9.4 Physical Exam

Protocol Section 7.8 outlines all required physical exam assessments which are done as part of a comprehensive physical examination. A comprehensive physical exam is required at Screening and Enrollment. At baseline, during a physical exam, 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 exams may identify additional baseline medical information that participants inadvertently do not report in their baseline medical history. In such situations, the clinician should add the information to the Medical History Log CRF as well, since the condition was present at the time of enrollment.

A targeted physical examination (to include assessment of general appearance and vital signs at a minimum) will be done, if clinically indicated, at all other follow-up visits and interim visits. Site clinicians may use their discretion to determine whether or not to conduct a more comprehensive physical exam in response to reported symptoms or illnesses present at the time of the exam.

Physical exam assessments should be documented on the Physical Exam and Vital Signs CRFs.

Weight

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

Height

Participant height should be measured in centimeters at the Screening and Enrollment Visit and additionally when clinically indicated.

Blood Pressure

Blood pressure is measured as a component of the Vital Sign assessment; devices are expected to be calibrated regularly per manufacturer's directions.

Pharyngeal Swab

One swab (pharyngeal) will be collected to test for chlamydia and gonorrhea at Screening and when clinically indicated at all other visits. To collect the swab, the participant should be instructed to open his or her mouth as widely as possible, allowing the clinician to make adequate contact with key areas of the throat (posterior wall, tonsils and uvula). If needed, a tongue depressor may be used. Insert the swab and rub the tonsillar pillars and posterior pharynx (behind the uvula). When removing the swab following collection, carefully ensure that the swab does not touch any area of the mouth (including the tongue, cheeks or teeth) before placing the swab in the appropriate specimen collection tube. See Laboratory Considerations SSP section for collection details.

9.5 Rectal, Male Genital and Pelvic Exam

The rectal (anorectal) exam during the Screening and Enrollment visits is necessary to evaluate protocol exclusion criteria and to collect detailed information on baseline rectal conditions. This exam scheduled during follow-up visits is necessary to assess for safety and collect required laboratory specimens. Pelvic exams and male genital exams are only performed if indicated at any study visit.

Protocol Section 7.8 outlines the assessments that may be included for the rectal exam. Detailed guidance on performing the rectal, pelvic and male genital exams, documentation of findings, and conduct of the anorectal and pelvic specimen collection can be found in the remainder of this section.

The rectal exam as well as the male genital or pelvic exam (if done) procedures must be performed in the order shown on the Genital Exam Checklist and at designated area(s) of the genitalia as noted on the checklist. At the Dosing Visits, any genital exams done should occur prior to insert administration and associated PK/PD/Biomarker specimen collection should occur at the assigned time points after insert administration. Note that specimen collection may be required at a visit even if the associated exam is not performed i.e. pelvic specimen collection required without an indicated pelvic exam. The order of specimen collection is critical to ensure that the first specimens collected do not affect subsequent specimens. Collect specimens away from apparent abnormalities and exclude swabbed areas from subsequent examination.

Prior to the exam, prepare all required equipment, supplies, and paperwork; label specimen collection supplies as needed. Review documentation of prior exams and other relevant documentation from the current visit and prior visits. Explain the procedure to the participant and answer any questions s/he may have.

9.5.1 Male Genital Exam Instructions

Male genital exams are only conducted if clinically indicated at all study visits and may include assessments of the following (via naked eye and hand-held magnifying glass):

- Entire penile surface
 - Internal and external foreskin (if present)
 - Shaft
 - Glans
 - Urethral meatus
- Scrotum
- Inguinal lymph nodes

There is no specific CRF for the male genital exam. All findings (normal and abnormal) can be documented on the Anorectal Exam CRF under “Other, Specify,” chart notes, or site-specific form.

9.5.2 Pelvic Exam Instructions

Pelvic exams are only conducted if clinically indicated at all study visits for participants assigned female sex at birth and if anatomy allows. Pelvic exams and pelvic samples ideally should not be collected if the participant is experiencing menstrual-like bleeding as this may interfere with visualization of the vagina and cervix and complicate interpretation of lab assays. The washout period between doses (7-49 days) should be timed to coincide with the participants’ menses to minimize the chance of participants being on their menses during any of the dosing, 24-, 48- or 72-hour post-dosing visits.

See below for special circumstances in the event a participant is experiencing menses or any vaginal bleeding at the time of an exam.

- During enrollment, if the participant is experiencing or reports vaginal bleeding beyond mild spotting, reschedule the exam and associated baseline sample collection to be completed within the 45-day screening window.
- During a scheduled follow-up visit, the pelvic exam and associated sample collection, and vaginal swabs, should still be completed as long as bleeding is no greater than mild spotting and the participant is comfortable. If the participant is experiencing greater than mild bleeding, perform other protocol-specified procedures at the visit and schedule the participant to return for the sample collection as soon as possible after menses, within the visit window (as part of a split visit, if allowable).
- If a participant presents for an interim visit complaining of genital symptoms, perform a pelvic exam to evaluate symptoms at that time. If the participant is not comfortable with completing an exam, they should be scheduled to return for a pelvic exam as soon as possible after vaginal bleeding stops.

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. If not standard of care, consider having an additional person (medical assistant or nurse) present during the examination to ensure participant comfort.
- 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. For most participants, a Graves speculum is preferred to enable visualization of all anatomic areas and tissues. Record the type and size of the speculum used on the Pelvic Exam Diagrams form. Prior to insertion, ensure that the speculum functions properly and has no rough edges.

Position the Participant:

- Drape the participant and establish a comfortable examination position that allows for appropriate examination of the genitalia such as dorsal lithotomy with or without use of stirrups; position should allow for the perineum and vulva to be inspected. Make all necessary adjustments to equipment and room to ensure participants comfort: i.e. adjust stirrups and back elevation as needed.

Examine the External Genitalia:

- For pelvic exams, a visual exam (i.e. a naked eye examination) should be performed of the external genitalia including the perineum, and perianal area. Palpate the inguinal lymph nodes to

assess for enlargement and/or tenderness. Do not insert the speculum before examining the external genitalia.

Examine the Internal Genitalia (Cervix and Vagina):

- The speculum may be lubricated with warm water only, 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 and vagina, noting any abnormal findings.
- 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.
- Removal of Visual Obstruction: After collection of vaginal and endocervical specimens, any obstruction (e.g., mucus, cellular debris) may be removed using 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.

Perform Bimanual Exam:

- After completing all of the above-listed tissue examinations and specimen collection and removing the speculum, perform a bimanual exam for adnexal or fundal masses and/or tenderness.

9.5.2.1 Pelvic Specimen Collection

Clinicians should collect pelvic specimens following a pelvic exam, if performed, and without a speculum in place. Sample collection will occur for participants assigned female sex at birth and if anatomy allows.

Cervicovaginal Fluid Collection for PD and PK: Cervicovaginal fluid will be collected from the posterior fornix to capture EVG/TAF concentration.

Site staff should weigh each swab and document the pre-collection weight on the LDMS Tracking Sheet. Following collection, site staff should place the swab back in the designated pre-weighed cryovial, obtain the post weight for each swab using an analytical balance, and document the post weight on the LDMS Tracking Sheet. Detailed instructions for weighing may be found in Section 11 Lab Considerations of the SSP.

Chlamydia trachomatis (CT)/ Neisseria gonorrhoea (GC)/Trichomonas (TV): Collection of two vaginal swabs for NAAT for GC/CT/TV will be done at Screening and at all other visits if clinically indicated. The clinician/assistant will use the collection swab provided in the appropriate testing kit outlined in the Laboratory Considerations SSP section. The clinician/assistant will open the peel pouch containing the swab. Insert the swab 1 ½ inches into vagina and rotate 360° against lateral vaginal wall. After specimen collection, put the swab in the transport medium and break the shaft at the painted breakpoint. Re-cap tube securely by snapping the cap into place.

Cervicovaginal Fluid for Microflora/Gram Stain: Vaginal fluid will be collected from the lateral vaginal wall for microflora/gram stain analysis. If the PK and PD swabs were collected first, then collect in a different location in the vagina preferably closer to the introitus. Insert the swabs and rotate three times. After specimen collection, use one swab to prepare the gram stain slides and put remaining swab for microflora in the transport medium, break the shaft at the painted breakpoint and re-cap tube securely by snapping the cap into place.

9.5.2.2 Documenting Pelvic Exam Findings

All findings (normal and abnormal) should be documented using the Pelvic Exam Diagrams form. If an exam is conducted at baseline, abnormal findings will be documented on the Pelvic Exam CRF and the Baseline Medical History Log CRF. When an exam is conducted during follow up, all abnormal findings identified will be documented on the Pelvic Exam CRF and AE Log CRF, as appropriate. Supplemental information may also be recorded in chart notes or on other designated source documents as needed.

Note: 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

IUCD strings may be visible upon exam and are also considered a normal finding. If present, they should be documented on the Pelvic Exam Diagrams form. Sites may determine whether they choose to consistently document the presence of IUCD strings (best practice) or not. It is recommended that if a participant has an IUCD, but the strings are not visible upon exam, this should be documented and followed up on.

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 term. All AEs should be documented per the term marked on the Pelvic Exam form. Always include the specific anatomical location of pelvic exam findings (e.g., vaginal, vulvar) in the AE term.

9.5.3 Anorectal Exam Instructions

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. If not standard of care, consider having an additional person (medical assistant or nurse) present during the examination to ensure participant comfort.
- 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.

Position the Participant

- Position the participant in the left lateral decubitus position (fetal position) with both legs flexed allowing a full view of the anus, perianus and buttocks.

External anorectal examination

- A visual perianal exam should also be performed during routine rectal exams. With gloved hands, the clinician should separate the participant's buttocks as far apart as is comfortable for him/her. Perform a naked eye examination of the perianal area and evaluate any abnormalities including but not limited to hemorrhoids, lesions, lumps, or rashes.

Internal anorectal examination (Digital Rectal Exam)

This examination is performed prior to the insertion of the anoscope or flexible sigmoidoscopy. The purpose of this exam is two-fold. First, this examination is intended to relax the anal sphincter around the opening of the anus in preparation for the subsequent anoscopy/ flexible sigmoidoscopy and specimen collection. In addition, the examination enables the clinician to assess potential findings such as lumps/areas of discomfort. The clinician will lubricate a gloved finger with Good Clean Love lubricant. The clinician will then gently and slowly insert a gloved index finger down) into the anus. The clinician should sweep the finger circumferentially around the entire anal/distal rectal surface. Any abnormal findings or unexpected discomfort should be noted. It is not required for this exam to assess the prostate gland.

Potential participants identified at screening with abnormalities of the rectal mucosa, or anorectal symptoms that represent a contraindication to study participation are not eligible for the study. For participants who enroll in the study, abnormal anorectal exam findings (that are not exclusionary) identified at the Screening and Enrollment Visits should be recorded as a baseline medical condition.

Note: An anoscopy is only required when an anoscope is inserted for rectal sample collection and may be excluded from the rectal exam when sampling with an anoscope is not done. For the Dosing Visits, the anoscopy should occur at the time of the assigned post-dose rectal swab collection when the anoscope is inserted, unless there is indication to do an anoscopy prior to product administration, such as to reassess an AE or a potential AE per participant report or digital or visual rectal exam finding.

9.5.3.1 Anorectal Specimen Collection

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. Following specimen collection, the

clinician should assess the anal canal as the anoscope is withdrawn.

Chlamydia trachomatis (CT)/ Neisseria gonorrhoea (GC)

Collection of the rectal swab for NAAT for GC/CT is required at Screening and at all other visits if clinically indicated. While other FDA approved platforms, validated for rectal samples, are acceptable, it is recommended that the GenProbe Aptima or Cepheid GeneXpert NAAT method be used. When using the Gen-Probe Aptima NAAT method, clinicians should use the Gen-Probe Aptima Unisex Swab (blue swab). The clinician/assistant will open the peel pouch and/or 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. Rotate it 360 degrees and remove. After specimen collection, put the swab in the transport medium and break the shaft at the painted breakpoint. Re-cap tube securely by snapping the cap into place.

Rectal Fluid Collection for PK, PD, Microbiome

Site staff should plan to allow sufficient time to prepare for the rectal swab procedure.

To collect specimens, (these should immediately follow the GC/CT swab)

- PK and PD samples should be collected by, introducing the swab into the proximal rectal lumen (in touch with the rectal walls). Hold (or leave) swab in place for 2 minutes. Remove the swab and place it into the appropriate collection tube.
- Microbiome samples should be collected by introducing the swab into the rectal lumen and rotating several times over the lateral wall.

If the collection swab is pre-cut, sites may utilize an extender pipette assembly method to aid in fluid collection (also see image below):

- Cut the distal end of the transfer pipette at the second gradation for swabs. These will serve as extension/holder device for the swab. Attach the swab, via the stick, to the transfer pipette and ensure that they are secure.



Rectal Enema (In-Clinic)

At Enrollment/baseline (for all participants), during the 48-hour post-dose for Group 1 **or** the 72-hours post-dose for Group 2 (when assigned to have rectal tissues samples collected), participants will have a rectal enema performed in the clinic **after** swab collection but **prior** to each rectal biopsy procedure and sigmoidoscopy. An enema is a procedure, which involves instilling a sterile saline solution to wash the rectum to cleanse the lower bowel and remove any obstruction (stool).

In the event the enema does not provide instructions for use, the following procedures should be performed:

- Fill enema bottle with 125 mL (about 4 ounces) of sterile normal (0.9%) saline, if not pre-packaged

- Have participant position themselves on the exam table in a position that best suits them for enema administration (e.g. rotate onto his or her left-hand side with right knee bent.)
- If enema bottle is not pre-lubricated, apply a small amount of Good Clean Love water-based lubricant. (DO NOT USE Surgilube or other chlorhexidine containing lubricants)
- Gently insert the tip of the enema bottle into the anus
- Slowly instill the solution into the rectum
- After holding the fluid in the rectum for about 3-5 minutes (or until the participant has the urge to expel), the participant should be asked to expel the enema fluid into the toilet

Note: In accordance with LoA#02, all participants will be given an enema in-clinic on the day of dosing (Visits 3 and 7) at least 45 minutes prior to dose administration. The intent of this enema is to minimize as much as possible the presence of stool/obstructions during tissue biopsy collection for participants in Group 1, for whom collection occurs the day of dosing, and for participants in Group 2, for whom tissue biopsy collection occurs at Visits 4 and 8. If a participant already administered their at-home enema the day prior to Visits 3 and 7, the in-clinic enema will serve as a second (additional) enema.

Rectal Enema (At-Home)

To prepare for biopsy collection on the day of study product dosing, study participants will be provided an enema that should be used at home. Clinic staff will provide the saline enema kit on the day of Enrollment and at Visit 6 and will instruct study participants to use the solution to cleanse their bowels prior to their scheduled Visit 3 and 7 visits. Detailed instructions will be provided and reviewed with the participant upon provision of the saline enema kit. See SSP section 8.3.3 for additional details.

If a participant has not used the at-home enema prior to their clinic visit, this should be documented as a protocol deviation.

Preparation of the Sigmoidoscope

Check to ensure the sigmoidoscope light is switched on, suction is on, and air flow is working. With the participant in the left lateral decubitus position, a digital rectal exam is performed as above, the sigmoidoscope tip is lubricated with Good Clean Love lubricant and gently inserted to ~15 cm from the anal verge.

Rectal Tissue Collection for PK, PD and biomarkers (via flexible sigmoidoscopy)

All participants will be instructed to abstain from inserting anything into the rectum, including having receptive anal intercourse for 72 hours (3 days) after the collection of these samples. Participants will also be counseled to refrain from the use of NSAIDs, aspirin and/or other drugs that are associated with the increased likelihood of bleeding for 72 hours (3 days) prior to and following biopsy collection. Should a participant report the use of such drugs or engagement of such practices within 72 hours prior to a scheduled sample collection, the collection of biopsies will be performed at the discretion of the IoR. Staff may also consult the PSRT for guidance on whether/how to proceed with scheduled study visit procedures.

Note: It is suggested that participants be instructed to drink lots of water the night before study product dosing visits and to not eat anything for at least 8 hours prior to tissue biopsy collection.

The following procedures should be performed:

- Introduce endoscopic 'jumbo' forceps into the sigmoidoscope channel and commence mucosal specimen collection at ~15 cm from the anal verge. The forceps need to be washed (dipped) in water between every biopsy. Forceps measuring approximately 3.7 mm with a 3.2 mm jaw will be required in order to obtain a 15 mg biopsy.
- Each individual biopsy should be obtained before the next one is collected. See the Laboratory Considerations SSP section for details on how many rectal biopsies should be collected and how samples should be handled.

- Prior to and following tissue collection and if local standard of care, staff may obtain participants' vital signs and document in chart notes or on another site-specific tool any abnormal findings, which should be further evaluated.

Participants should also be informed that they may experience a small amount of bleeding from the rectum (noticeable when wiping after a bowel movement) for 2 to 3 days following the procedure. Excessive bleeding is not expected. In the unlikely event that excessive bleeding occurs, it is likely to be noticed when having a bowel movement or when wiping following a bowel movement.

If the participant presents with any of the following after the flexible sigmoidoscopy procedure, the participant should be referred for assessment at the emergency department of the nearest hospital:

- Bleeding that continues after the flexible sigmoidoscopy procedure that is uncontrolled (occurring between bowel movements) and results in the passage of large blood clots
- Local or systemic features compatible with infection (fever, localized anorectal pain, anal discharge)
- Abdominal pain, swelling or fever that is consistent with perforation of a hollow viscus or any local or systemic clinical features suggestive of this condition.

In the case of any life-threatening event, participants should be instructed to seek immediate emergency care. Where feasible and medically appropriate, participants will be encouraged to seek evaluation where the study clinician is based, and to request that the clinician be contacted upon their arrival. Sites should make every effort to obtain and use records from non-study medical providers to complete any safety related documentation, pending written permission from the participant.

Note: Rectal tissue biopsies (for PK) are required to be weighed. Site staff should weigh each cryovial and document the pre-collection weight on the LDMS Tracking Sheet. Following collection, site staff should obtain the post weight for each cryovial containing the biopsy and document the post weight on the LDMS Tracking Sheet. Complete details can be found in the Laboratory Considerations SSP section.

9.5.3.2 Documenting Rectal Exam Findings

All rectal exam findings, including findings noted during the anoscopy and flexible sigmoidoscopy, should be documented using the Anorectal Exam CRF.

All abnormal findings must be thoroughly documented and include location and severity of the finding to ensure appropriate assessment can be provided during subsequent examinations. Supplemental information may also be recorded in chart notes or on other designated source documents as needed.

As previously mentioned, all abnormal non-exclusionary findings identified at Screening and Enrollment will be documented as baseline medical conditions on the Medical History Log CRF as well.

Any abnormal findings identified during follow-up will be documented on the Anorectal Exam CRF, as appropriate and as an adverse event, if applicable, on the AE Log CRF. Any unexpected discomfort should also be noted in chart notes.

The results of laboratory tests performed using specimens collected during follow-up rectal exams are recorded on the STI Tests CRF.

9.6 STI/RTI/UTI Evaluation, Management and Treatment

Clinical and laboratory evaluations are performed in MTN-039 to diagnose the following STIs and RTIs:

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

All participants diagnosed with an active sexually transmitted or reproductive tract infection (STI/RTI) or UTI based on the presence of symptoms should be provided treatment and or referral for treatment per site standard of care and applicable site standard operating procedures (SOPs). STIs/RTIs will be treated in accordance with current CDC guidelines which can be accessed at (<http://www.cdc.gov/std/treatment>).

Potential participants presenting with an active (symptomatic or per laboratory or clinical diagnosis) infection requiring treatment at Screening or Enrollment will be excluded from study participation. Per current CDC guidelines, the following symptomatic infections require treatment and are exclusionary: gonorrhea, chlamydia, syphilis, active herpes simplex virus (HSV) lesions, anogenital sores or ulcers, or symptomatic genital warts, chancroid, pelvic inflammatory disease (PID), other vaginitis, and trichomoniasis.

Participants who are otherwise eligible but are diagnosed with BV and/or candida may be treated, re-tested during the screening process and enrolled if all symptoms have resolved.

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.

Participants’ partners should be offered treatment, or referrals, as per sites’ SOPs.

Urinary tract infections (UTIs)

Participants who are otherwise eligible but have symptoms consistent with a UTI at screening may be treated and enrolled if all symptoms have resolved. The following symptoms are considered indicative of a possible UTI:

- Frequent urge to urinate
- Pain and burning during urination
- Lower abdominal pain and/or uncomfortable pressure above the pubic bone

A urine culture is not required at screening.

In follow-up, suspected UTIs may be clinically managed based solely on the presence of symptoms indicative of a possible UTI however the AE term “urinary tract infection” should be reserved for participants with a positive urine culture.

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 during follow-up. 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 or the Male Genital Grading Table, as applicable. If the urine culture is negative, the AE(s) will remain reported as symptoms only. Results of any urine cultures and dipsticks performed 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 Baseline Medical History Log 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.

HIV Testing

At Screening and/or Enrollment (prior to randomization), all participants will undergo HIV serology testing. Note at Enrollment, sites will perform HIV rapid test to screen for HIV status prior to randomization. Participants who have a rapid HIV test is positive or indeterminate should have confirmatory testing completed. If confirmatory testing confirms HIV infection, the participant is not eligible for enrollment. If confirmatory testing is negative or indeterminate, the MTN LC should be consulted.

Participants who have a reactive HIV test result during follow-up visits will be temporarily held and confirmatory testing conducted. In addition, if a participant has signs or symptoms consistent with acute HIV infection, or expresses a concern about recent HIV acquisition, testing will be performed immediately. Any participant who is found to have confirmed HIV infection after enrollment, product use and study participation will be permanently discontinued. All participants with confirmed HIV infection will be counseled and referred to available resources for medical and psychosocial care and support. If the participant opts to remain in follow up, site staff must follow-up on all referrals at each subsequent follow-up visit to determine if the participant actually sought the care to which s/he was referred, the outcome of the referral, and whether additional referrals are needed. All referrals, outcomes, and follow-up plans and actions must be fully documented in participant study records.

Protocol-specified examinations and laboratory tests will provide information upon which appropriate clinical care decisions can be made. Study staff must refer participants to non-study HIV care providers. Study staff will provide and explain all study examination findings and test results to participants. They also will provide copies of laboratory test result reports to participants and their non-study providers (if the participant grants approval). Study investigators will be available to consult with non-study providers on optimal clinical care and treatment decisions for participants.

Syphilis testing

Prior to enrollment in the study, appropriate clinical management action is required with any positive syphilis test and confirmation found at screening. Action required prior to enrollment depends on the current health status of the participant and the availability of medical records documenting prior infection, as follows:

- If the participant has clinical signs or symptoms of syphilis, s/he is not eligible for enrollment.
- If the participant has no clinical signs or symptoms of syphilis, and credible medical records are available to document adequate treatment of a prior syphilis infection (per CDC guidelines), and the participant's current RPR titer is 1:4 or lower, the participant may be enrolled in the study without providing treatment at the discretion of the IoR or designee, without consulting the PSRT.

If syphilis is diagnosed during screening, 'syphilis seropositivity' should be recorded within the Medical History Log CRF, and the screening RPR titer included ("RPR titer: 1 to X"). A baseline medical history condition of syphilis seropositivity should be documented on the "ongoing at time of assessment" at baseline. A test or cure (i.e., four-fold decrease in titer) is not required prior to enrollment; however, repeat serology is expected 6 months after treatment for clinical management purposes.

9.7 Clinical and Product Use Management

Protocol Section 9 provides detailed guidance on clinical and product use management, including general criteria for product discontinuation (Section 9.3), guidance on product discontinuation in response to observed AEs (Section 9.4), HIV (Section 7.5.1), pregnancy (Section 9.5) and early study termination (Section 9.6).

All specifications of protocol sections 6 and 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. Conditions requiring permanent discontinuation are summarized below.

The protocol specifies that permanent product discontinuation should be initiated if a participant reports prohibited medication use as listed in Section 9.3 of the protocol. When possible, treatment options that are not prohibited by the protocol should be pursued, however clinical management of the participant should be prioritized if alternative treatment is not available. If prohibited medications, other than those listed in Section 9.3 of the protocol, are used, sites should consult the PSRT.

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. Unless otherwise specified in protocol section 9, the IoR/designee should immediately consult the PSRT for any product discontinuations. Product discontinuations must be communicated to site pharmacy staff using the Study Product Request Slip as described in Section 8 of this manual. Product discontinuations also must be documented on the Product Discontinuation CRF.

Criteria	Permanent Discontinuation	Temporary Hold
Acquisition of HIV infection (confirmed HIV infection)*	X	[X] <i>*Note: Product should be temporarily held upon the first reactive rapid test</i>
Anogenital STIs	X	
Reported use or need of PEP*	X	
Reported use or need of PrEP	X	
Pregnancy	X	
Breastfeeding	X	
Reported use of prohibited medications and medications, including N-9 products)		X
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.	X	
Use of anticoagulants (e.g., heparin, Lovenox, warfarin and Plavix)	X	
Use of certain CYP3A inhibitors and inducers (as specified in protocol)	X	
Related Grade 2 and Grade 3 AEs		X
Grade 4 AE not addressed in Section 9.5 (regardless of relationship)	X	

** Participants who experience a known or potential HIV exposure during study participation or have a recognized risk of exposure and thus need PEP will have study product permanently discontinued and will be referred for PEP or PrEP initiation. Those who need PEP will be encouraged to start it as quickly as possible and within 72 hours of potential exposure.*