Section 8. Clinical Considerations

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This section provides information on the clinical procedures performed in MTN-017. 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 Investigator of Record 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 or mental health conditions which may require follow-up.

Information pertaining to participant safety monitoring and adverse event reporting procedures are provided in Section 9 of this manual. Information on performing laboratory procedures is described in Section 10 of this manual. Further instructions on completing data collection forms associated with clinical assessments are provided in Section 11 of this manual.

8.1 Participant-Reported Medical History (Baseline [Pre-Existing Conditions] and during Follow up)

In order to obtain a complete, accurate, and relevant medical history at screening and enrollment and to assess medical eligibility, it will be necessary to ask the participant about his/her past medical conditions as well as any conditions s/he is currently experiencing at the time of the Screening and Enrollment visits (i.e., pre-existing conditions).

Information pertaining to the participants' medical history (particularly symptoms, conditions, and diagnoses that occurred in the time since he/she has become sexually active or that affect eligibility) should be obtained. This includes, but is not limited to, a history of hospitalizations, surgeries, allergies, any condition that required prescription or chronic medication (that is, more than 2 weeks in duration), and acute conditions occurring prior to Enrollment. "Sexually active" refers to the time point that the participant first had anal sex with another partner. The purpose of obtaining this information during screening and enrollment is to:

- assess whether the participant is medically eligible to participate in the study
- evaluate the participant's baseline signs, symptoms and conditions in comparison with those that may be identified or reported during study participation

When collecting past medical history from the participant, the clinician should ask probing questions in order to collect the most complete and accurate information possible, especially with regard to severity and frequency. It is recommended that sites use the MTN-017 Baseline Medical History Questions sheet (Word version available on the MTN-017 web page under Study Implementation Materials) to best capture this information; however, alternative site-specific forms may be used.

8.1.1 Pre-existing Conditions Review at Screening

Pre-existing 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). .

All ongoing and/or relevant participant-reported symptoms, clinical signs (including gradable lab results), diagnoses, and medically-relevant chronic conditions will be recorded on the Pre-existing Conditions CRF. This form is completed based on all screening source documents including, but not limited to, the Baseline Medical History Questions Sheet, Physical Exam form, Anorectal Exam form, Safety Laboratory Results form, and STI Test Results form. The clinician should record as much information as possible about the severity and frequency of any pre-existing condition in source documents as well as in the comments field of the Pre-existing Conditions CRF to best describe the condition at the time the participant enters the study. This allows for greater objectiveness in noting any severity increase of the pre-existing condition.

8.1.2 Pre-existing Conditions Review at Enrollment (time of randomization)

Medical history information first obtained at Screening must be actively reviewed and updated at Enrollment (prior to randomization). This includes a review and status update of the conditions described, severity grade, and comments noted for the entry. If new signs/conditions are identified at Enrollment (prior to randomization), these newly identified conditions should be documented on the Pre-existing Conditions CRF as needed.

By definition, pre-existing conditions are any symptoms, conditions, or diagnoses present at baseline/enrollment (prior to randomization). Participants have not yet been exposed to study product at this time, thus, these conditions are not considered AEs. However, new conditions identified during follow-up that were not present at enrollment and pre-existing conditions that increase in severity (grade) or frequency during follow-up, are considered AEs.

8.1.3 Medical History Review during Follow Up

It is necessary to review and document updates to the participant's self-reported medical history at each follow-up clinic visit (and any interim visit) for the following reasons:

- to determine whether previously reported and/or documented conditions are ongoing or have changed with regard to severity or frequency
- to determine whether newly-identified symptoms, illnesses, or condition have occurred since the last medical history was performed

Note: For purposes of this study, "newly-identified" is defined as a condition that:

- was not present at baseline
- was present at baseline (ongoing at enrollment) and has now increased in severity grade or frequency or has resolved after enrollment and prior to the current report;
- has already been reported as an adverse event on an AE Log CRF and has increased in severity grade/frequency

It is recommended that sites use the MTN-017 Follow-up Medical History Log (Word version available on the MTN-017 web page under Study Implementation Materials) to best capture medical history during follow-up visits; however, alternative site-specific forms may be used. Study participants medical 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 adverse events (AEs). Once a participant is enrolled and before his/her first follow-up visit, site staff should transcribe all entries on the Pre-Existing Conditions CRF that are marked as "ongoing" at Enrollment onto a new Follow-up Medical History Log designated for use for that participant.

At each follow-up visit, site clinicians will begin the follow-up medical history by reviewing with the participant and eliciting updates (resolution, outcome date, severity grade, etc.) on those symptoms/conditions that were documented as ongoing since the participant's last visit (i.e., missing an "Outcome Date" on the Follow-up Medical History Log and/or marked as outcome- "continuing" on an Adverse Experience Log CRF. Site clinicians should then probe and evaluate for any new onset conditions/symptoms since the participant's last visit. Clinicians should use their clinical experience and judgment to elicit complete and accurate medical history information from participants.

- New onset conditions/symptoms that began since the last visit should be recorded as new entries on the Follow-up Medical History Log, and may require completion of an AE Log CRF. This includes any reoccurrences of conditions/symptoms that were previously reported and had resolved at a prior visit, Documentation should include the current severity grade.
- Ongoing conditions that have increased in severity grade or frequency should be recorded as new entries on the Follow-up Medical History Log.
- Ongoing conditions that have not changed in severity or frequency, or have improved but not yet resolved, do not warrant any changes to the entries on the Follow-up Medical History Log.

- Ongoing conditions that have resolved since the last visit should have their entries updated with an “Outcome Date” on the Follow-up Medical History Log. If the ongoing condition was pre-existing, the resolution may be documented in the “Comments” field of the Pre-existing Conditions CRF.

If during follow-up a condition is identified as being present at baseline and the participant inadvertently did not report it in his/her baseline medical history, the clinician should add the information to the Pre-existing Conditions CRF. A chart note should also be documented to explain why the information is recorded on the Pre-existing Conditions CRF retrospectively.

8.2 Participant-reported Medication History (Baseline and during Follow Up)

The MTN-017 protocol requires medication information to be obtained from the participant at Screening and actively reviewed and updated at Enrollment and throughout study participation.

8.2.1 Baseline Medication History

Documentation of all medication taken within 12 weeks (3 months) prior to Screening and during study participation is required for each participant. All medications (prescription and non-prescription), alternative medications (e.g., herbs, vitamins) will be collected. The following is not an exhaustive list of all concomitant medications used which should be documented.

- Prescription and “over-the counter” medications and preparations
- Pre-exposure Prophylaxis(PrEP) or post-exposure prophylaxis (PEP) for prevention of HIV
- Vaccinations
- Lubricants (except study provided lubricant*)
- Douches and/or enemas*
- Vitamins and other nutritional supplements
- Herbal, naturopathic, and traditional preparations
- Alcohol and recreational or street drugs

Note: participants who report the use of post-exposure prophylaxis (PEP) for HIV exposure within the 12 weeks prior to screening or anticipated use during study participation are excluded from study participation.

** Per protocol, participants will be provided with lubricant when indicated. However, it is not required that participants use study-provided lubricant. Use of lubricants douches and/or enemas is permitted in this study as long as these products do not contain N-9 or corticosteroids. Participant-reported use of lubricants (including study provided lubricant), enemas and douches should be documented on the Rectal Practices CRF. This CRF will capture use of these products at Enrollment and at each regularly scheduled follow up visit.*

In order to avoid adverse events caused by drug interactions, whenever a concomitant medication is taken, site staff should review the concomitant medication's and study product‘s most recent package insert and investigators brochure to obtain the most current information on drug interactions and contraindications.

It is helpful to assess medication information in the context of the participants’ reported medical history. Site staff should use the information obtained during the review of the participants’ medical history to probe for additional medications that the participant may have forgotten to report. For example, if the participant reports headaches as part of his/her medical history, but does not report any medication taken for headaches, ask if s/he takes any medications for headaches. Similarly, if a participant reports taking a medication for a condition that s/he

inadvertently did not report when providing medical history information, the condition should be considered a pre-existing condition.

Note: Hormones used by participants should be recorded as indicated on the Concomitant Medications Log.

Medication history information documented on the Concomitant Medications Log at the Screening Visit must be actively reviewed and updated at the Enrollment Visit (prior to randomization). Review the information on this CRF with the participant at the Enrollment Visit and update as applicable.

8.2.2 Medication History Review during Follow-Up

At each follow up visit, site staff should retrieve and review the completed Concomitant Medications Log CRF, record any new medications the participant reports starting since his/her last medications assessment. All previous entries should be actively reviewed with the participant. The participant should be asked whether s/he is still taking all previously recorded medications, at the same dose and frequency.

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

8.2.3 Prohibited Medications and Products

Many medications interact and should not be used together, and additionally some medications may alter the parameters that are measured in MTN 017. For this reason, certain medications are contraindicated and should not be used during study participation because it may be harmful to the participant. The following medications are prohibited and/or restricted during study participation:

- Any investigational products
- Systemic immunomodulatory medications (e.g. corticosteroids)
- Warfarin or heparin
- Rectally-administered medications or products, containing N-9 or corticosteroids (including over-the-counter preparations)
- Any drug associated with the increase likelihood of bleeding after mucosal biopsy (rectal fluid/tissue subset only)

If a participant reports using any of the prohibited medications listed above during the study, this must be recorded on the Concomitant Medications Log CRF. In the event a participant reports use of any prohibited medication listed above, the site should consult the PSRT for guidance on study product management.

Potential participants who agree to take part in the rectal tissue/fluid subset must be willing to restrict their use of any non-steroidal anti-inflammatory drug (NSAID) during study participation. If a participant reports using aspirin or aspirin-containing products and other non-steroidal anti-inflammatory drugs such as ibuprofen (Advil, Motrin) and naproxen (Aleve, Naprosyn) within 72 hours (3 days) prior to a study visit in which endoscopic examinations and/or biopsies are obtained, the study visit should be rescheduled. In the event that rescheduling is not possible, the site should consult the PSRT immediately for guidance on the best course of action. Study product use may be discontinued at the discretion of the IoR/clinician in consultation with the PRST.

8.3 Physical Exams

Protocol Section 7.11 outlines the required physical exam assessments. A targeted physical examination is required at Screening, Enrollment/Period 1 Product Use Initiate visit, and at the Period 3 Product Use End Visit/Final Clinic visit.

At all other visits during follow up, a physical examination is conducted if clinically indicated. Site clinicians may use their discretion in determining whether to conduct a physical exam in response to clinically indicated and/or reported symptoms presenting at other study visits.

The Abbreviated Physical Exam CRF will be provided to sites to document the conduct of the physical examination.

Physical exams may identify medical conditions that participants inadvertently do not report in their baseline medical history review. For example, the clinician may identify a skin condition during the physical exam and upon further inquiry learn that the participant has had the condition since age 16. In such situations, the clinician should add the newly identified information to the Abbreviated Physical Exam CRF, and the Pre-existing Conditions form as well, since the condition was present at the time of enrollment (prior to randomization).

8.4 Rectal Exam

A rectal exam during the Screening and Enrollment Visits is necessary to evaluate protocol exclusion criteria and to collect detailed information on baseline anorectal conditions. Rectal exams are also performed to ensure the ongoing safety of study participants and when clinically indicated to evaluate anorectal symptoms.

A rectal exam is required at every study visit. A rectal examination consists of visual inspection of the anus and perineum, a digital examination and an anoscopic rectal exam.

At each visit when a rectal exam is performed, rectal fluid and tissue (if applicable) will be collected for clinical/laboratory evaluations specified in protocol Section 7.12.
Potential participants identified at screening with abnormalities of the rectal mucosa, or anorectal symptoms that represent a contraindication to study participation (including participation in the rectal tissue/biopsy subset if applicable) 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 pre-existing conditions.

Any abnormal findings found during rectal exams performed during follow-up should be documented and/or reported as an adverse event as described in Section 9 of this manual.

Scheduled rectal exams should be performed according to the guidance provided in the remainder of this section. Exam procedures must be performed in the order shown on the exam checklist provided. Rectal exams performed at interim visits or in response to symptoms should be targeted to symptoms. In this instance, staff are not required to complete all components of the complete rectal exam.

8.4.1 Documentation of Rectal Exam Findings

All rectal exam and anoscopic findings should be documented using the Anorectal Exam CRF. For participants in the rectal fluid/biopsy subset, any findings noted via flexible sigmoidoscopy should be documented on the Rectal Biopsy/Fluid Subset Specimens 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 pre-existing conditions on the Pre-existing Conditions CRF as well. Any abnormal findings identified during follow-up will be documented on the Anorectal Exam CRF or Rectal Biopsy/Fluid Subset Specimens CRF, as appropriate. All newly-identified abnormal rectal/anal exam findings will be documented as an Adverse Event on the Adverse Experience Log (AE-1) CRF.

Note: Any unexpected discomfort should also be noted on the Rectal Exam CRF as well in chart notes.

Note: Rectal bleeding observed on follow-up exam after anoscope insertion or swab/sponge collection that is consistent with rectal bleeding observed at the baseline exam after anoscope insertion or swab/sponge collection is not considered an AE. In addition, per Clarification Memo #2 to protocol version 1.0, new rectal bleeding observed on follow-up exam (i.e., not seen at baseline) that is judged to be within the range of normal, according to the clinical judgment of the IoR/designee, is not an AE and therefore should not be reported as such. If the bleeding exceeds the amount considered normal and/or is observed or reported in subsequent day(s) following anoscope insertion or swab/sponge collection, it is considered an AE and should be reported accordingly.

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

8.4.2 General Technique (Preparation and Position)

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 procedure to him/her and answer any questions he/she may have.
- The clinician should wash his/her hands and put on disposable latex free gloves for each procedure.

Establish Participant Comfort

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

Position the Participant

- Participants should undress from the waist down and be provided with a sheet/drape to cover up.
- Position the participant in the left lateral decubitus position (fetal position) with both legs flexed.
- Adjust the sheet/drape to allow a full view of the anus, perianus and buttocks.

8.4.3 Visual Examination of the Perianal Area

A visual exam should be performed during routine scheduled 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.

8.4.4 Swab Collection for HSV Detection and HPV Typing

The following specimens should be collected after visual examination of the perianal area and prior to the digital examination.

8.4.4.1 HSV Detection

The swab for detection of HSV 1/2 is done only if clinically indicated (i.e. the presence of shallow perianal ulceration or vesicle crops). This testing will only be performed if it is the local standard of care. Perform as indicated per local SOP.

8.4.4.2 Anal HPV Typing

The swab for anal HPV typing is required for all participants at the Enrollment/Initiate Period 1 Visit. The examiner should use one hand to spread the buttocks and expose the anus. Insert a water-moistened Dacron swab from the Digene Female HPV Swab Specimen Collection Kit a minimum of 6 cm into the anus. If there is difficulty inserting the swab, the participant should also retract the buttocks. With pressure on the distal end of the swab, rotate it gently and slowly in a circular fashion as it is withdrawn over 10-15 seconds. Do not retract the buttocks when the swab is close to the verge to ensure that it is sampled as well. After the sample has been obtained, place the swab in the bottom of the specimen transport tube. Snap off shaft so that the tube may be capped. Re-cap tube securely by snapping the cap into place. For storage and/or shipping instructions, please see section 10 of this manual.

8.4.5 Digital Rectal Examination (DRE)

A digital rectal exam should be performed during routine rectal exams after each visual inspection. The clinician will perform a digital rectal exam prior to the insertion of the anoscope or flexible sigmiodoscopy (in the subset) for the collection of specimens. 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 provided by the clinic staff. The clinician will then gently and slowly insert a gloved index finger (palmar surface 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 on the Anorectal Exam CRF.

Note: It is not required for this exam to assess the prostate gland.

8.5 Specimen Collection (applicable to all study participants)

Rectal fluid will be collected for the detection of Neisseria gonorrhea/ Chlamydia trachomatis (GC/CT). Rectal fluid will also be collected for the assessment of adherence to study product use [adherence pharmacokinetics (PK)] and resistance to HIV in a laboratory [pharmacodynamics (PD)]. To collect the above-mentioned samples the clinician and/or designee will need to insert an anoscope into the rectum prior to collection. Instructions for preparation and insertion of anoscope are described in section 8.8.1 below.

Specimens will be collected in the appropriate order as outlined on the rectal exam checklist which is available on the MTN-017 Study Implementation under 'Checklist' (http://www.mtnstopshiv.org/node/4524). The order of specimen collection is critical to ensure that the initial specimens collected do not affect and/or contaminate subsequent specimens. For further information on specimen collection, processing and testing, see section 10 of this manual (Laboratory Considerations).

8.5.1 Preparation of the Anoscope

Rectal fluid will be collected using either a rectal sponge (PK/PD) or swab (GC/CT) 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 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.

8.5.2 Chlamydia trachomatis (CT)/ Neisseria gonorrhea (GC)

The rectal swab for NAAT for GC/CT is required for all participants at Screening, Enrollment/Initiate Period 1 Visit, and at the Period 1, 2, and 3 End Visits. Collection of the rectal swab for NAAT for GC/CT is done if indicated at all other scheduled visits.

It is recommended that the GenProbe Transport kit or Cepheid GeneXpert NAAT method. When using the Gen-Probe Aptima NAAT method, clinicians should use the Gen-Probe Aptima Unisex Swab (blue swab).

Instructions for collection and transport of rectal swabs for GC/CT testing with Gen-Probe:

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 GC/CT swab into the proximal rectal lumen that is visible at the end of the anoscope. Rotate it 360 degrees and remove. 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. For storage and/or shipping instructions, please see section 10 of this manual.

Instructions for collection and transport of rectal swabs for GC/CT testing with GeneXpert:

The clinician/assistant will use the Xpert collection swab. The clinician/assistant will open the peelpouch containing the 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.

Immediately following insertion into the transport tube, elute material from the swab while avoiding foaming by gently shaking or inverting the tube 3-4 times. For storage and/or shipping instructions, please see section 10 of this manual.

8.5.3 Rectal Sponge Collection for Adherence PK and PD

The rectal sponge for PD is required for **all participants** beginning at the Enrollment/Period 1 Initiate Visit and at every scheduled visit during follow up. The rectal sponge for adherence PK is required for **all participants** beginning at the Mid Period 1 visit and at every scheduled visit during follow up. This swab will be collected simultaneously with the sponge for PD at timepoints when both are required.

At Enrollment/Initiate Period 1 Visit, collect the **rectal sponge for PD** only for **all participants**. One sponge will be inserted through the anoscope. The sponge should be placed into the proximal rectal lumen (in touch with the rectal walls) and will remain in place for exactly two (2) minutes before removing. Refer to section 10 of this manual for further instructions on assembly of the sponge prior to and following collection of rectal fluid.

Beginning at the Period 1 Mid Period visit and at every scheduled visit during follow up at visits when the r**ectal sponges for adherence PK and PD** are required, two pre-weighed sponges (one marked for PD and one marked for adherence PK) will be inserted through the anoscope simultaneously. The sponges should be placed into the proximal rectal lumen (in touch with the rectal walls) and will remain in place for exactly two (2) minutes before removing.

Site staff should plan to allot sufficient time to prepare for the rectal sponge procedure. Prior to collection, site staff should weigh the dry sponge and labeled cryovial. The pre-weight should be documented on the LDMS Tracking Sheet.

To collect specimens, the clinician should use the Good Clean Love (water-based) lubricant to lubricate the anoscope. With the participant in the left lateral recumbent position (laying on the left side with the knee and thigh drawn upward), slowly insert the anoscope with obturator in place through the anus and advance the instrument until the flange is flush with the participant's skin. Maintain pressure on flange to ensure continued placement of the anoscope. Once in place, remove obturator and introduce the sponge (attached to the pipette sponge holder extension: see picture below) through the anoscope into the rectum. Hold (or leave) sponge in place for 2 minutes.

Sponge assembly (see below) The rectal sponge is a triangular dry sponge that is mounted on the end of a blue plastic stick. Cut the distal end of the transfer pipette at the second gradation. These will serve as extension/holder device for the sponge. Attach the sponge, via the stick, to the transfer pipette and ensure that they are secure.

Following specimen collection, slowly remove the anoscope and disengage the sponge from holder (plastic pipette), discarding the plastic pipette. Place the sponge back into the original

weighed cryovial (by matching the number of the sponge to the tube) and ensure that the cap is fully tightened. The site clinician should document the collection time on the LDMS Tracking Sheet. Once placed back into the cryovial, the sponge and labeled cryovial should be weighed. Site staff should document the weight (post-weight) on the LDMS Tracking Sheet.
Refer to section 10 of this manual for further instructions on the weighing, storage and processing of the sponges for adherence PK and PD prior to and following collection of rectal fluid.

8.6 Specimen Collection (applicable to Rectal Fluid/Tissue Subset only)

Among a subset of 36 participants, rectal biopsies and fluid specimens will be collected for secondary and exploratory analysis (e.g. PK and mucosal immunity). Sites participating in the collection of these specimens must follow sections 8.6.1-8.6.4.

Rectal tissue specimens (biopsies) will be collected at each of the following time points: Enrollment, Period 1 End, Period 2 End and Period 3 End Visits. Specimen collection, as well as any findings identified via sigmoidoscopy, will be documented on the Rectal Biopsy/Fluid Subset Specimens CRF.

Approximately 15 biopsies will be collected at Enrollment and approximately 20 biopsies will be collected at all other time points in order to obtain sufficient material for processing and testing at each time point.

All participants who agree to taking part in the Rectal Fluid/Tissue Subset will be instructed to abstain from inserting anything into the rectum, including usage of the study gel and 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 mucosal biopsy collection.

No special preparation, including dietary, is needed before having these specimens collected. Participants may follow their regular daily routine and eat/drink as they normally would prior to arriving to the visit (with the exceptions as stated above). Participants should be instructed not to douche or take any laxatives to cleanse the rectum prior to biopsy collection as any required cleansing procedures will be conducted in clinic. Such practices may change the cells in the rectum, which must be left undisturbed in order to get an accurate sampling.

8.6.1 Coagulation Testing (INR or PT)

In preparation for participation in this subset, potential participants who agree to take part in the rectal tissue/fluid subset will have their blooded tested at Screening to determine how quickly their blood clots and if bleeding problems are present to ensure the biopsies are taken safely. Participants with abnormal coagulation test results will be ineligible to participate in the rectal tissue/fluid subset. Test results will be recorded on the Safety Laboratory Results CRF.

The values listed below are representative of normal values per DAIDS Toxicity Table.

- International Normalized Ratio (INR): 1.1-1.5x site laboratory ULN (Grade 1)
- Prothrombin Time (PT): 1.1-1.25x site laboratory ULN (Grade 1)

8.6.2 Rectal Sponge Collection for Mucosal Immunology

The rectal sponge for mucosal immunology is required only for participants taking part in the rectal fluid/tissue subset at the Enrollment/Period 1 Initiate visit, and at Period 1, 2, and 3 End Visits only.

For those participants who agree to take part in the Rectal Fluid/Tissue Subset, one pre-weighed sponge will be inserted through the anoscope. The sponge should be placed into the proximal rectal lumen (in touch with the rectal walls) and will remain in place for exactly two (2) minutes before removing.

Note: This sponge may be taken at the same time (simultaneously) as the adherence PK and PD sponges.

Refer to section 10 of this manual for further instructions on assembly, weighing and storage of the sponge for mucosal immunity.

8.6.3 Enema

Prior to each rectal biopsy procedure and sigmoidoscopy, each participant will have a rectal enema performed.

A rectal 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). This enema should take place at the study site in order for staff to document that the enema was performed.

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 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, ask the participant to expel the enema fluid into a restroom toilet.

8.6.4 Rectal Tissue (biopsies) Collection

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, the sigmoidoscope tip is lubricated with *Good Clean Love* lubricant and gently inserted to ~15 cm from the anal verge

Rectal Tissue (biopsies) Collection:

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 3.3 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.

At **Enrollment/Period 1 Initiate visit**, the following biopsies are required:

- Collect two (2) biopsies for Mucosal Gene Expression Array Determination
- Collect one (1) biopsy for Histology
- Collect four (4) biopsies for PD
- Collect seven (7) biopsies for Mucosal T Cell Phenotyping
- Collect one (1) biopsy for Proteomics

During **follow up**, the following biopsies are required:

- Collect five (5) biopsies for PK (refer to section 10 of this manual for further instructions on the weighing and storage of the rectal biopsy for PK.)
- Collect two (2) biopsies for Mucosal Gene Expression Array Determination
- Collect one (1) biopsy for Histology
- Collect four (4) biopsies for PD
- Collect seven (7) biopsies for Mucosal T Cell Phenotyping
- Collect one (1) biopsy for Proteomics

Following tissue collection, participant vital signs should be obtained and documented and any abnormal findings 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 unlikelihood 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.

Participants will be contacted by telephone 48-72 hours (2-3 days) after sample collection after the Period 1 End and Period 2 End Visits to assess for any AEs. Participants will be encouraged to contact the site to report any new or worsening AEs within 7 days following the Period 3 End Visit.

8.7 STI/RTI/UTI Management

STI/RTI Diagnosis: Clinical and laboratory evaluations are performed throughout the course of MTN-017 to diagnose the following STIs and RTIs:

- Chlamydia trachomatis (CT)
- Herpes simplex virus (HSV1/2 detection)
- Human papillomavirus (anal HPV)
- Neisseria gonorrhea (GC)
- Syphilis
- Hepatitis B
- Hepatitis C

All participants diagnosed with active anorectal, 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).

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.

STI/RTIs will be treated in accordance with current World Health Organization (WHO) guidelines which can be accessed at:
http://www.who.int/reproductivehealth/topics/rtis/evidence/en/index.html.

Potential participants presenting with an active (symptomatic) infection requiring treatment at Screening will be excluded from study participation. Per current WHO guidelines, the following symptomatic infections require treatment and are exclusionary: symptomatic Chlamydia trachomatis (CT) infection, Neisseria gonorrhea (GC), syphilis, active herpes simplex virus (HSV) lesions, anogenital sores or ulcers, or symptomatic genital warts.

Note: HSV-1 or HSV-2 seropositive diagnosis with no active lesions is allowed, since treatment is not required. In cases of non-anorectal GC/Chlamydia identified at screening, one re-screening no earlier than two months after the screening visit will be allowed.

8.8 Syphilis

If a reactive Rapid Plasma Reagin (RPR) or Venereal Disease Research Laboratory (VDRL)is identified during screening, a confirmatory FDA approved test (MHA-TP or TPHA, or other treponemal test) result must be received and appropriate clinical management action taken, prior to enrollment in the study. Action required prior to enrollment depends on the current health status of the participant and the availability of medical records documenting his/her 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 WHO 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.

8.9 HIV Testing at Screening

At Screening and/or Enrollment (prior to randomization), all participants will undergo HIV-1 serology testing. Sites will perform HIV rapid test to screen for HIV status. Participants will be ineligible for enrollment regardless of subsequent/confirmatory test results if:

- One or both of the rapid HIV tests are positive
- Rapid HIV tests are discordant

8.9.1 Assessment of Acute HIV Infection

If at Screening and/or Enrollment, a potential participant has signs or symptoms consistent with acute HIV infection, the participant is not eligible for enrollment. Acute HIV infection is defined as the period of rapid viral replication that immediately follows the initial establishment of infection with HIV. Symptoms of acute HIV infection may be indistinguishable from a typical viral syndrome. These symptoms may include:

- fever
- fatigue
- headache
- myalgia
- weight loss
- pharyngitis or sore throat
- lymphadenopathy
- rash
- diarrhea

Clinicians should assess the possible causes of these symptoms, length of time the participant has been experiencing these symptoms, and severity grade. Symptoms should be managed clinically per standard of care and participant will not be eligible for study participation.

Participants for whom were reported as a screen failure due to concern for acute HIV infection should have repeat HIV testing no earlier than two months following the prior negative HIV test. If the HIV antibody test is negative at that point, and the participant no longer has symptoms suggestive of acute viral infection, the participant may undergo a second screening attempt for the study, assuming no other interim contraindications are noted. If an alternative diagnosis for the symptoms is identified (for example, malaria or influenza) then a second screening attempt may be scheduled two-months following the initial attempt, once all symptoms have been resolved. If symptoms are not adequately resolved by the second screening attempt, and HIV testing is negative, assess for additional possible causes of symptoms and refer for further evaluation if necessary. Participants may only be rescreened a maximum of one time. If during the second screening attempt other contraindications for eligibility are present, the participant is not eligible for study participation.

8.9.2 HIV Testing during Follow Up

During follow-up, HIV testing will be performed as described in Section 10 of this manual. Participants who have a reactive HIV test result during follow-up visits will be instructed to temporarily hold study product immediately and will be further tested using Western blot (WB).

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 will permanently discontinue product use. If the participant is willing, s/he can be followed for all remaining scheduled study visits and complete modified study procedures per their original study schedule until their originally scheduled study exit date.

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 (see section 7.6.1 of the protocol) 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.

If a participant requests post-exposure prophylaxis (PEP) for HIV exposure, they will be referred to their primary care provider for evaluation and possible treatment per local standard of care. If the participant starts PEP, study product use must be permanently discontinued per protocol Section 9.3.

Truvada for PrEP should only be used as part of a comprehensive prevention strategy that includes other prevention measures such as safer sex practices. A comprehensive prevention strategy includes consistent and correct use of condoms, the individual knowing both his/her own status and his/her partner's HIV status, getting regular testing for HIV and other sexually transmitted infections, and informing individuals about and supporting their efforts to reduce sexual risk behavior.

Plasma storage is required at the End Period Visits. It is required for further Laboratory Center HIV testing (CD4, HIV RNA, and HIV drug resistance) of enrolled participants in the event of a positive HIV rapid or positive HIV EIA test result, and when additional samples (e.g., Sample 2) are collected as part of algorithm testing at the site local lab to confirm a participant’s HIV infection status.

8.10 Hepatitis B Testing

All participants undergo screening for HBV with assessment of hepatitis B surface antigen (HBsAg) and hepatitis B surface antibody (HBsAb) at Screening.

Hepatitis B Surface Antigen (HBsAg): If this test is positive, then the hepatitis B virus is present in the blood. This means that the participant has either an acute or chronic hepatitis B infection.

Hepatitis B Surface Antibody (HBsAb or anti-HBs): this test is used to detect previous exposure to HBV. Antibodies produced in response to HBV surface antigen can also be acquired from successful vaccination. This test is done to determine the need for vaccination or to determine if a person has recovered from an infections and is immune. If this test is positive, then the participant is immune to (or protected against) hepatitis B.

Those with active HBV infection as evidenced by detection of HBsAg receive standardized counseling relevant to natural history and transmission risks of HBV, and are excluded from enrollment. Those who test positive for HBsAb and thus have pre-existing immunity to HBV

(either due to resolved natural infection or prior immunization) are eligible for enrollment. Those who test negative for both HBsAg and HBsAb are offered immunization against HBV and considered eligible for enrollment.

Participants who decline immunization for HBV, and thus will remain vulnerable to HBV infection over the course of the trial, are monitored for HBV seroconversion with serologic testing. Those with newly detected HBsAg will have study product discontinued, and are followed monthly for an additional three months with transaminases to ensure that post-cessation of study product hepatitis flares are diagnosed and managed appropriately.

All study sites should maintain adequate supplies of Hepatitis B vaccine for study participants and should store and administer vaccine according to package insert instructions. All applicable local policies and guidelines for Hepatitis B vaccination also should be followed.

Participants with a negative HBsAg test at Screening are susceptible to Hepatitis B infection. If they do not have evidence of immunity (or protection from the infection), they may be offered an HBV vaccination at any time during follow up.

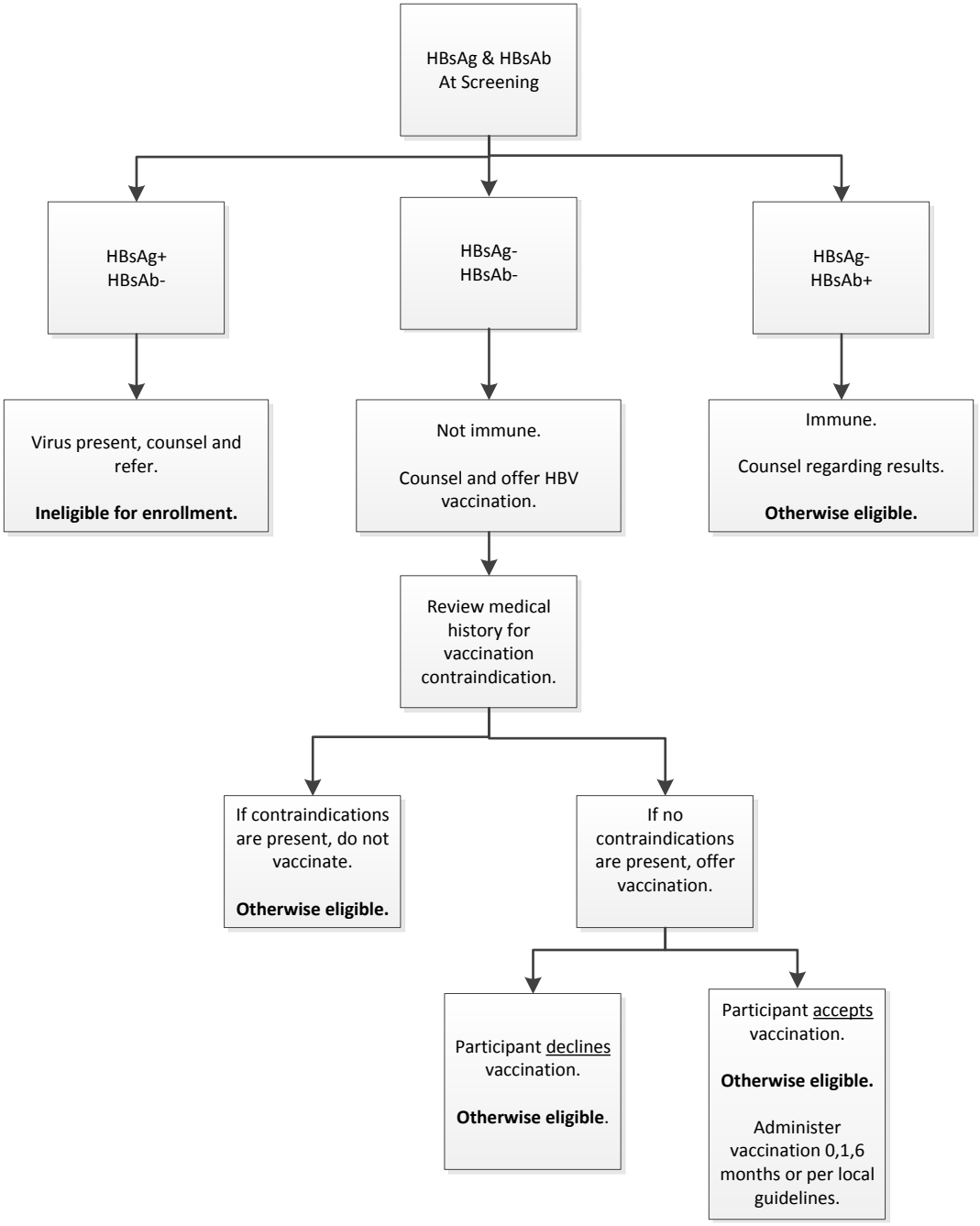
Hepatitis B vaccination is not required in order to be eligible for enrollment in the study. However, enrolled participants who are susceptible should receive the first vaccination of the three-dose vaccine series on the day of enrollment. The second and third vaccinations should then be provided per local policies and guidelines. In the event there is an interruption between vaccinations, per recommendations of the US Centers for Disease Control and Prevention, the vaccine series does not need to be restarted. If vaccination is interrupted after the first dose, the second dose should be administered as soon as possible. However, the second and third doses should be separated by at least four weeks.

All vaccinations should be recorded on the Concomitant Medications Log CRF. Each injection should be recorded as a separate entry, to ensure the “Date Started” and “Date Stopped” are the same date. Instructions for CRF completion are below:

- Mark the “once” box for “Frequency”
- Mark the appropriate box for “Route” (e.g., “IM”, or “Other” for subcutaneous injections)

Participants who decline vaccination at enrollment should continue to be offered vaccination throughout follow-up and, if they later accept vaccination, may initiate the vaccine series at any time.

Figure 8.1
Algorithm For Management of Hepatitis B Serologic Assays



8.11 Hepatitis C antibody testing at Screening

Hepatitis C (HCV) antibody testing will likewise be performed at Screening. Participants with a positive HCV antibody test are not eligible for study participation and will be referred to their primary provider for management.

8.12 Management of Laboratory Test Results

Hematology and liver and renal function (AST/ALT/Creatinine) testing will be performed at Screening and the Final Clinic visit, and when clinically indicated. Creatinine testing will be performed at Screening and at End Period Visits, and when clinically indicated. Results will be recorded on the Safety Laboratory Results CRF.

For each study participant, the IoR or designee is responsible for monitoring these test results over time and for ensuring appropriate clinical management of all results. All reviews of laboratory test results should be documented on the lab results report (provided by the lab to the clinic) and/or in chart notes.

The IoR or designee should routinely review MTN-017 participant study records to ensure proper monitoring and clinical management of laboratory test results, and documentation thereof. All reviews performed by the IoR or designee should be documented in participant study records.

8.12.1 Calculating Creatinine Clearance Rates (Screening Only)

At Screening, when a participant's serum creatinine level is tested, his/her Creatinine clearance rate must be calculated, using the Cockcroft-Gault formula provided in protocol Section 5.3 (see exclusion criterion 3f). To facilitate proper calculation, all sites are encouraged to use the creatinine clearance calculation worksheet provided in the Study Implementation Materials section of the MTN-017 web page (http://www.mtnstopshiv.org/node/4524) .

At all sites, participant weight (in kg) and participant age should be entered into the worksheet in whole numbers (no decimal places). When entering age, the number of completed years achieved at the time when the calculation is performed should be entered.

8.13 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 product hold and discontinuation in response to observed AEs (Section 9.4), early study termination (Section 9.5), HIV infection (Sections 9.6), Hepatitis B infection (Section 9.7) and management of STI/RTI (Sections 9.8).

All specifications of protocol Sections 7 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.

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. Product holds and discontinuations also must be documented on Product Hold/Discontinuation Log case report forms.

8.14 Pre-Exposure Prophylaxis (PrEP)

Truvada for PrEP should only be used as part of a comprehensive prevention strategy that includes other prevention measures such as safer sex practices. A comprehensive prevention strategy includes consistent and correct use of condoms, the individual knowing both his/her own

status and his/her partner's HIV status, getting regular testing for HIV and other sexually transmitted infections, and informing individuals about and supporting their efforts to reduce sexual risk behavior.

Per protocol Section 6.6, use of emtricitabine, tenofovir disoproxil fumarate or tenofovir disoproxil fumarate/ emtricitabine or any other medication for pre- exposure prophylaxis for possible HIV exposure may be permitted at the discretion of the IoR only if it becomes standard of care for HIV prevention.

In the event a participant reports using Truvada as a pre- exposure prophylaxis (PrEP), during the daily oral FTC/TDF product use period, participants will be instructed to use only the study provided FTC/TDF for the duration of the product use period and refrain from using their routine oral PrEP medication. At the conclusion of the daily oral FTC/TDF product use period, participants may resume taking their routine oral PrEP medication. Given that oral FTC/TDF as PrEP is approved by the US FDA, if a participant took part in a PrEP research study within 12 weeks prior to Screening, oral FTC/TDF as PrEP would not be considered an investigational product (and thus, not prohibited under protocol section 6.7).