A Randomized, Double-Blind, Placebo Controlled, Phase 2 Study to Assess Treatment of Gardnerella Vaginalis Vaginal Colonization with Amoxicillin

DMID Protocol Number: 16-0073

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STATEMENT OF COMPLIANCE

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46; 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312)
- ICH E6; 62 Federal Register 25691 (1997)
- NIH Clinical Terms of Award

All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Principal Investigator and Site Investigator:

Date:

Jane Schwebke, MD
University of Alabama at Birmingham

Site Investigator:

Date:

Candice McNeil, MD, MPH
Wake Forest University Health Sciences

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LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
BV	Bacterial Vaginosis
CFR	Code of Federal Regulations
CoC	Certificate of Confidentiality
CRF	Case Report Form
CROMS	Clinical Research Operations and Management Support
CSR	Clinical Study Report
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
EIC	Enrollment Informed Consent
FDA	Food and Drug Administration
FWA	Federalwide Assurance
GCP	Good Clinical Practice
GV	Gardnerella Vaginalis
HIPAA	Health Insurance Portability and Accountability Act
IATA	International Air Transport Association
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDES	Emmes Corporation's Internet Data Entry System
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ITT	Intent-to-Treat
JAMA	Journal of the American Medical Association
MedDRA®	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NAAT	Nucleic Acid Amplification Test
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
OCRA	Office of Clinical Research Affairs, DMID, NIAID, NIH, DHHS
OHRP	Office for Human Research Protections
OHSR	Office for Human Subjects Research

ORA	Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics
PP	Per-Protocol
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event/Serious Adverse Experience
SDCC	Statistical and Data Coordinating Center
SI	Site Investigator
SIC	Screening Informed Consent
SOP	Standard Operating Procedure
STD	Sexually Transmitted Disease
STI	Sexually Transmitted Infection
T. vaginalis	*Trichomonas vaginalis*
ToC	Test of Cure
US	United States

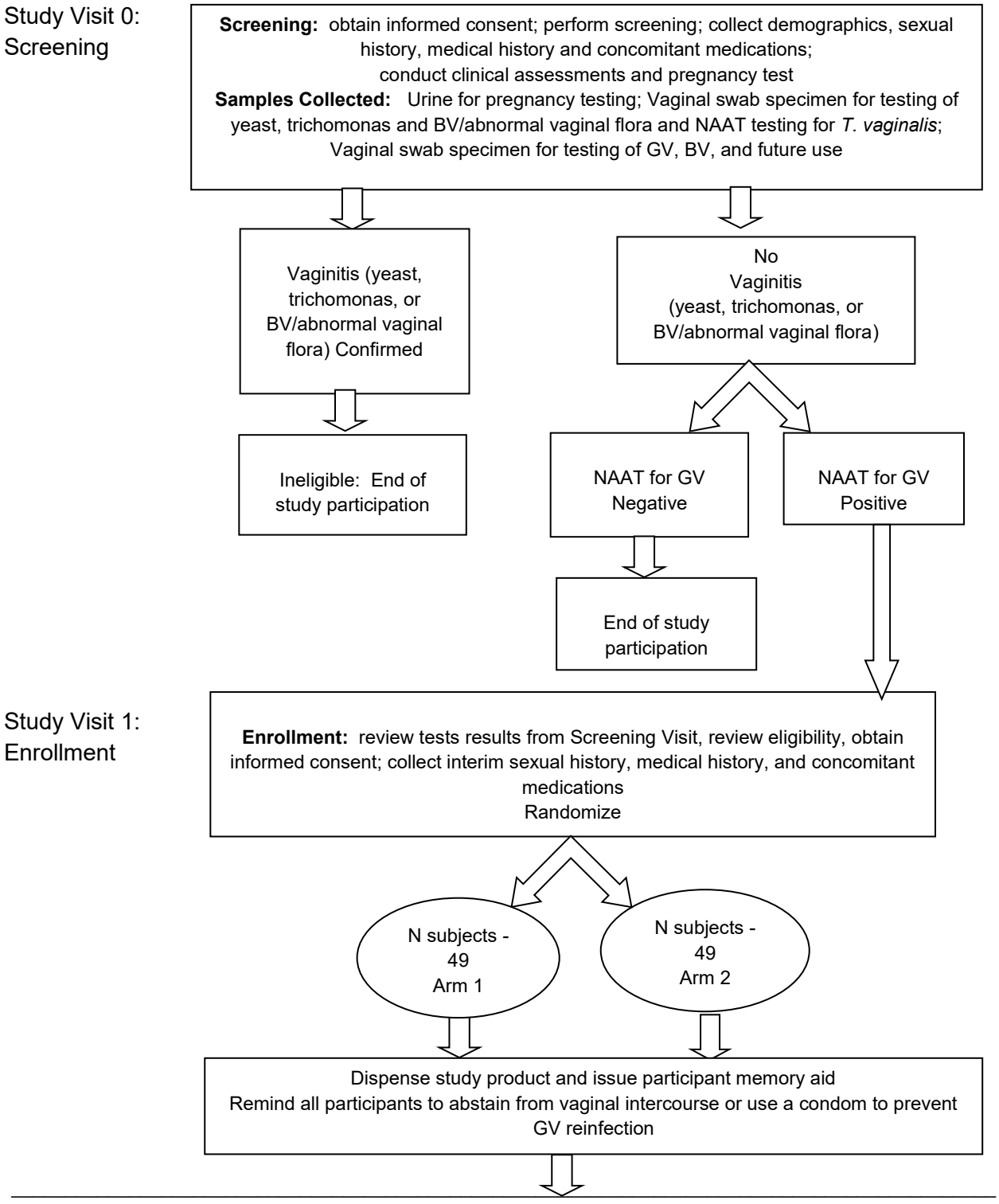
PROTOCOL SUMMARY

Title:	A Randomized, Double-Blind, Placebo Controlled, Phase 2 Study to Assess Treatment of Gardnerella vaginalis Vaginal Colonization with Amoxicillin
Phase:	II
Population:	Approximately 245 healthy women will be screened to enroll 98 participants (to achieve 82 evaluable participants) ages 18-45 with no evidence of vaginitis (yeast, trichomonas, bacterial vaginosis (BV)); presence of Gardnerella vaginalis (GV) detected by NAAT; willing to use condoms during the study; not currently menstruating.
Number of Sites:	Two US clinics: University of Alabama at Birmingham and Wake Forest University Health Sciences
Study Duration:	Approximately 24 months is anticipated for enrollment and completion of participation.
Participation Duration:	It is anticipated that each participant will be part of the protocol for approximately 22 days (screening visit, enrollment visit, and 1 follow-up visit).
Description of Agent or Intervention:	Amoxicillin 2 x 250 mg capsules PO bid 7 days or placebo
Objectives:	Primary: To determine if treatment with amoxicillin eradicates GV in women who are colonized/infected with GV but have no clinical evidence of BV. Secondary: • To evaluate the safety and tolerability of amoxicillin compared to placebo. Exploratory: • To determine the demographic and behavioral factors associated with vaginal colonization of GV;

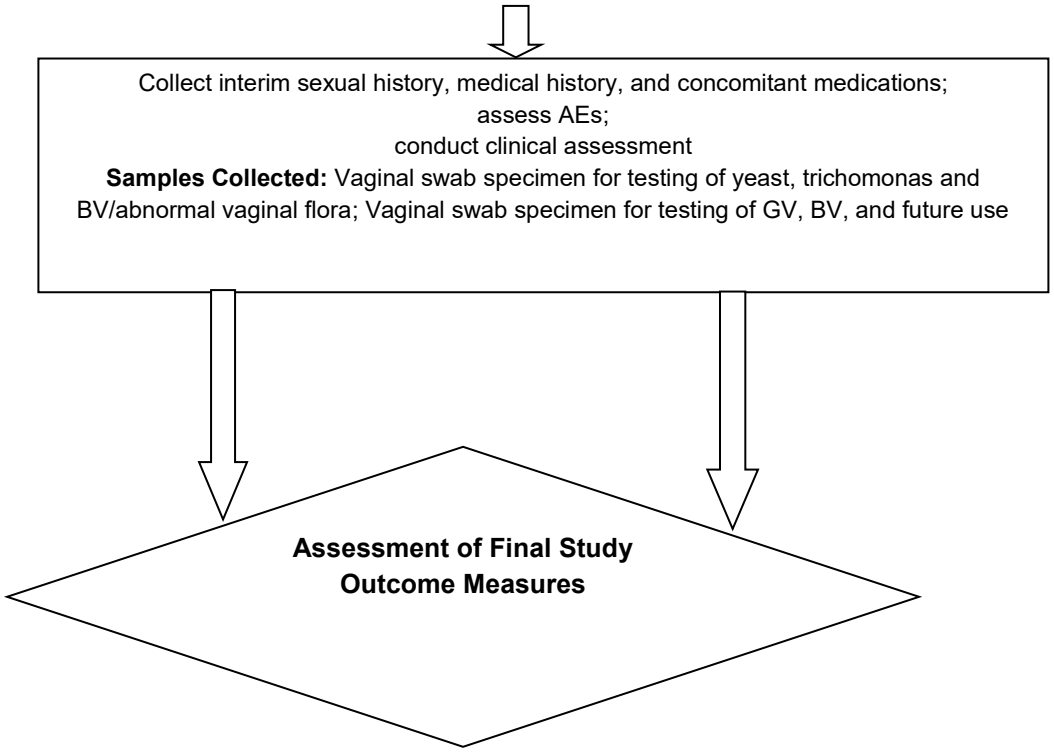
Description of Study Design:	This is a randomized, double-blind, placebo-controlled Phase 2 study. It is anticipated that 245 women will be screened in order to enroll 98 participants to achieve 82 evaluable participants. Participants will be randomized to amoxicillin 2 x 250 mg PO bid or placebo, for 7 days. Participation will include three visits in total – a Screening Visit, an Enrollment Visit, and 1 Follow- up visit. Total participation for each woman will be approximately 22 days.
Estimated Time to Complete Enrollment:	Approximately 24 months

Schematic of Study Design:

Total N: ~245 screened to enroll 98 (to achieve 82 evaluable)



Study Visit 2:
Follow-up (ToC)



1 KEY ROLES

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Bacterial Vaginosis (BV) is the most common cause of vaginitis worldwide and is strongly associated with important public health issues including preterm birth and acquisition/transmission of HIV and other STDs(1, 2). Although the exact cause of BV is unclear, the current epidemiologic data strongly support the hypothesis that BV is sexually transmitted(3). Further, there is mounting data to suggest that *Gardnerella vaginalis* (GV) is the founder organism that is sexually transmitted(3). Once GV is established, there is a resultant change in the vaginal microbiome characterized by loss of lactobacilli and an increase in anaerobic organisms leading to the syndrome of BV(3, 4). GV is nearly universally found in women with BV. However, it may also be found in women who do not meet the current definition of BV, leading some to question its pathogenicity. Others have hypothesized that there are strain differences among GV in terms of virulence factors(5).

2.2 Rationale

If GV is necessary for the development of BV, then eradication of GV in women who do not meet the clinical criteria for BV should abort the future development of BV. Amoxicillin was chosen as the therapeutic intervention because it is highly active against GV but has limited efficacy against anaerobes associated with BV. This Phase 2 study will determine if GV in the vaginal flora will be eradicated by amoxicillin. If amoxicillin successfully eradicates GV, we will develop the next step to examine prevention of BV through screening and treatment of GV.

The amoxicillin dose will be 2 x 250 mg capsules PO twice a day for 7 days. Placebo will be similarly packaged.

Generally healthy women between ages 18 and 45 presenting to Health/STD clinics and responding to advertisements will be invited to participate in this study. Women who are pregnant will not be permitted to enroll.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

The primary associated risk with this study is a possible Adverse Event, AE, secondary to amoxicillin. These could include allergic reaction, rash, nausea, vomiting, diarrhea or other unforeseen events. Amoxicillin may lead to penicillin allergy in the infant of nursing mothers. Amoxicillin can reduce the effectiveness of hormonal contraceptives.

Potential risks may include irritation from vaginal swabs or minor bleeding if the swabs are not used according to instructions. Information concerning participants' sexual history is necessarily detailed given the study's objectives and may provoke some minor psychological or emotional stress when requested. Participation in research may involve a loss of privacy. Participant records will be kept as confidential as is possible under the law. Individual identity will not be used in any reports or publications resulting from this study.

There is no current standard of care for a positive GV test result and women who test positive but do not have symptoms are usually not given any treatment. Thus, if a participant's GV test result remains positive after completion of the study, no further participant contact by study staff is required.

2.3.2 Known Potential Benefits

Participants may benefit from this study by finding out more about the specific cause of any vaginal symptoms they may be experiencing. Participants may benefit from pre- and post-test counseling, treatment, and referrals, as necessary. It has previously been observed that participants are empowered by knowing, for example, more about what BV is and how it can be prevented.

3 OBJECTIVES

3.1 Study Objectives

3.1.1 Primary objective:

- To determine if treatment with amoxicillin eradicates GV in women who are colonized/infected with GV but have no clinical evidence of BV.

3.1.2 Secondary objective:

- To evaluate the safety and tolerability of amoxicillin compared to placebo.

3.1.3 Exploratory objective:

- To determine the demographic and behavioral factors associated with vaginal colonization of GV.

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measures

- The proportion of participants with eradication of GV in each study arm at Visit 2 (Day 15-21) as assessed by NAAT.

3.2.2 Secondary Outcome Measures

- The proportion of participants reporting related adverse events (AEs and SAEs) in each study arm following the first dose of study product through Visit 2 (Day 15-21).

3.2.3 Exploratory Outcome Measures

- The demographic and behavioral factors among women in the NAAT screening population without vaginitis who are baseline GV positive and women who are baseline GV negative

4 STUDY DESIGN

This is a randomized, double-blind, placebo-controlled Phase 2 study screening approximately 245 adult females 18-45 years of age to enroll approximately 98 participants to achieve 82 evaluable participants at the test of cure (ToC) visit. The study is designed to determine if amoxicillin will eradicate vaginal colonization/infection with GV when administered to women who are colonized/infected with GV but have no clinical evidence of BV. Women who consent to screening participation will undergo pelvic examination on entry and vaginal specimens collected for pH, whiff test and wet prep microscopy (Amsel criteria with the exception of evaluation of vaginal discharge), Nugent score, NAAT for trichomonas, and quantitative NAAT for GV. At Visit 1 (Enrollment - Day 1) women who tested positive for GV by NAAT, negative for BV by Nugent and negative for trichomonas by NAAT will be enrolled and randomized to one of two groups (amoxicillin or placebo). A ToC will be completed at Visit 2 (Day 15-21).

Baseline information will be collected from all women at the screening visit (Visit 0 (Day -14 to -7). This information will consist of demographics, medical and sexual history, concomitant medications, and clinical and laboratory findings. Vaginal swabs will be collected as noted above. Women who test positive for vaginitis at the screening visit will be screen failures. At the Enrollment Visit (Visit 1, Day 1) women who tested positive for GV, negative for abnormal flora/BV, and negative for trichomonas will be enrolled and randomized. Those who were negative for GV will be screen failures and will not continue in the study, but, their, demographic, medical, and sexual history as well as baseline swabs will be used for comparisons to the GV positive women for an exploratory objective.

Women who are enrolled will be asked to return for one further visit, Visit 2 (Day 15-21), where a ToC will be completed (a pelvic exam completed with the same specimens taken as those at baseline with the exception of the trichomonas NAAT).

Participants will be queried regarding any adverse outcomes and all AEs will be collected through Visit 2. Safety oversight will be provided by an Independent Safety Monitor (ISM) at each clinical site and by a Data Monitoring Safety Board (DSMB). The DSMB members will meet at specified times during the course of the study as defined in the DSMB Charter.

Laboratory testing will include nucleic acid amplification test (NAAT) for trichomonas at each site’s local lab; Nugent score for BV at Dr. Schwebke’s Lab; quantitative NAAT for GV at LabCorp Research and Development Laboratory; and assessment of vaginal pH, saline microscopy (yeast, trichomonas, clue cells) at each site’s local lab.

The duration of the study for participants who are enrolled and randomized will be approximately 22 days. For those not enrolled, participation will end at their post screening follow up phone call. It is expected that enrollment will be completed in approximately 12 months.

Women will be asked to abstain from vaginal intercourse or use condoms at all times for vaginal intercourse during their participation independent of birth control use or if participant is of non-childbearing potential. This restriction is to prevent participants from GV reinfection.

For details on study procedures and evaluations and study schedule by study visits, see Sections 7, 8, and Appendix A: Schedule of Events.

5 STUDY ENROLLMENT AND WITHDRAWAL

Approximately 245 adult females ages 18-45, who are in good health, will be screened to enroll approximately 98 participants to achieve 82 evaluable participants. No exemptions are granted on inclusion/exclusion criteria in DMID sponsored trials.

Women will be recruited from Health/STD clinics.

Participants screened who test positive for GV by NAAT, negative for BV by Nugent (Nugent score 0-3) and negative for trichomonas by NAAT, will be enrolled and randomized.

Participants screened who test positive for vaginitis (i.e., yeast, trichomonas, or BV/abnormal vaginal flora) will be notified during the screening visit that they are not eligible to participate in the study.

Participants screened who test negative for GV by NAAT will be notified by a post screening visit telephone call that they are not eligible to participate in the study. They will be counseled that asymptomatic GV does not require medical treatment and given instructions on relevant reasons to pursue medical care.

5.1 Participant Inclusion Criteria

Section 8 has details of all testing.

Women eligible to enroll in this this study must meet all of the inclusion criteria:

1. Women ages 18-45, inclusive;
2. No evidence of vaginitis (yeast, trichomonas, and BV/abnormal vaginal flora) or other vaginal conditions which in the opinion of the investigator could be confounders*;
   *These causes will initially be detected by wet mount microscopy with trichomonas during the screening procedures and later confirmed by NAAT and BV/abnormal vaginal flora confirmed by Nugent scoring (Nugent score of 4-10) (see Section 8);
3. Presence of GV detected by NAAT*;
   *results of NAAT testing will be available prior to return for Enrollment visit;
4. Willing to use condoms during vaginal intercourse while participating in the study;
5. Not currently menstruating at screening visit;
6. Willing and able to provide written informed consent;
7. Negative urine pregnancy test on all participants of childbearing potential at study screening;

8. Participant must be of non-childbearing potential* or must be using highly effective birth control** to avoid becoming pregnant.

   *Non-childbearing potential is defined as being post-menopausal for at least 1 year, status after bilateral tubal ligation, or status after bilateral oophorectomy or status after hysterectomy.

   **In addition to the required use of condoms by the male partner during study participation, participants must agree to avoid becoming pregnant by using one of the following acceptable method of birth control for 30 days prior to screening and for the duration of the study;

   - Intrauterine contraceptive device; OR
   - Oral contraceptives; OR
   - Hormonal injections; OR
   - Hormonal implants; OR
   - Contraceptive patches; OR
   - Monogamous relationship with vasectomized partner; OR
   - Exclusively same-sex relationships; OR
   - Abstinence;

9. Participant is not planning on taking antibiotics or using any intravaginal microbicides from the Screening visit through the Visit 2 Follow-up (ToC);
10. Participant is willing and able to cooperate to the extent and degree required by this protocol at the discretion of the investigator.

5.2 Participant Exclusion Criteria

Participants eligible to participate in this study must not meet any of the following exclusion criteria:

1. Pregnant or nursing;
2. Allergic to penicillin, amoxicillin, cephalosporins, or other β-lactam antibiotic;
3. Use of antibiotics in the past 14 days prior to screening visit;
4. HIV infected;

5. Women taking immunosuppressive agents;
6. History of renal impairment;
7. Use of any investigational drug within the past 30 days prior to screening;
8. Any other condition that, in the opinion of the investigator, would interfere with participation in the study.

5.3 Treatment Assignment Procedures

Enrollment of participants will be done online using the enrollment module of AdvantageEDC^{SM}. Participants will be randomized using a 1:1 ratio to receive product or placebo after providing consent and confirmation of eligibility based on the study inclusion and exclusion criteria.

The study will use a stratified, permuted block randomization scheme. Permuted block randomization is used to avoid the potential for serious imbalance in the number of participants assigned to each group, an imbalance that can occur in the simple randomization procedures. Stratification will be by study site.

5.3.1 Randomization Procedures

The list of randomized treatment assignments will be prepared by statisticians at the Emmes Corporation and provided to the research pharmacist at UAB who will prepare the study treatment. The research pharmacist will label each vial of study treatment with a blinded treatment number according to the randomization scheme. The list of randomized treatment assignments will be included in the enrollment module of The Emmes Corporation's Internet Data Entry System (IDES). IDES will assign each enrolled participant a blinded treatment code from the list after demographic and eligibility data have been entered into the system.

Instructions for use of the enrollment module are included in the IDES User's Guide.

Each site will have a supply of blinded vials pre-labeled with treatment numbers, each containing sufficient doses to treat a participant for seven days. Once a participant is assigned a treatment number, the corresponding vial will be distributed to the participant.

5.3.2 Masking Procedures

The active and placebo arms of this 2-arm study will be masked by providing identical study vials to participants in the two arms. All participants and clinical investigators participating in this trial will be blinded with respect to treatment assignment. The research pharmacy at UAB will over-encapsulate the amoxicillin capsules for the active treatment arm and will create identical appearing placebo capsules containing lactose.

The participants, the study personnel who perform study assessments after administration and those dispensing study medication, data entry personnel at the sites, and laboratory personnel will be blinded to treatment assignment. The DSMB may receive data in aggregate and presented by treatment group, but without the treatment group identified. The DSMB may be unblinded to individual study treatment assignments, as needed, to adequately assess safety issues. Refer to the MOP for unblinding procedures.

5.3.3 Reasons for Withdrawal

Participants may withdraw voluntarily from participation in the study at any time. Participants may also withdraw voluntarily from receiving the study intervention for any reason. An investigator may also withdraw a participant from receiving further study intervention. Any participant who has received at least one dose of study drug will be continued in efficacy and safety follow-up, if the participant agrees.

Any enrolled participant may withdraw or be withdrawn from the study for the following reasons:

- The participant withdraws consent
- The study is terminated
- For any reason that, in the opinion of the investigator, precludes the participant's participation in the study
- Participant is lost to follow-up

If a participant withdraws or is withdrawn prior to completion of the study, the reason for this decision must be recorded in the case report forms (CRFs). A participant who voluntarily withdraws from the study or has been discontinued from receiving further treatment will be encouraged to permit continued follow-up of AEs and to follow scheduled visits. Participants should be asked to continue scheduled evaluations, complete an end-of-study evaluation, and be given appropriate care under medical supervision until the symptoms of any AE resolve or the participant’s condition becomes stable.

Refer to Section 7.4 for procedures to be followed if a participant withdraws from the study.

Withdrawal of participants from analysis populations is discussed in Section 11.4.1.

5.3.4 Handling of Withdrawals

Participants who withdraw, or are withdrawn from the efficacy endpoint analysis, or are lost to follow-up after signing the Enrollment Informed Consent (EIC) form, randomization, and receipt of study product will not be replaced. Participants who withdraw consent after signing the EIC form and randomization but before receipt of study product may be replaced.

If withdrawal occurs after study treatment, the participant will be asked to continue scheduled study procedures including safety and efficacy evaluations, if possible, and be given appropriate care under medical supervision if symptoms of any AE related to participation in the study are continuing. The participant will be followed until the AE is resolved or until the participant's condition becomes stable.

Participants who request to withdraw their consent after study treatment from further participation in the study will be reminded of the importance of continuing in the study for safety evaluations and will be encouraged to complete the Early Termination Visit if they choose not to complete the remaining study visits. The Early Termination Visit procedures are listed in Section 7.4. Participants who choose to decline continuation in study participation will no longer be contacted for follow-up.

In the case of participants who fail to appear for a follow-up safety assessment, extensive effort (i.e., three documented contact attempts via phone calls and/or e-mails, made on separate occasions) will be made to locate or recall them or at least to determine their health status. Participants who cannot be located after extensive effort will no longer be contacted for follow-up. These efforts will be documented in the participant's records.

If a participant is withdrawn from the study due to an AE that prohibits continued participation in the study, she will be given appropriate care and treatment under medical supervision until the condition has resolved or becomes stable.

Safety and efficacy data will be collected on any participant who is withdrawn from the efficacy endpoint analysis. Refer to Section 11 for details on how participants who are withdrawn will be handled during analysis.

5.3.5 Termination of Study

DMID reserves the right to terminate the study at any time for clinical or administrative reasons.

6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1 Study Product Description

Amoxicillin, USP is a semisynthetic antibiotic, an analog of ampicillin, with a broad spectrum of bactericidal activity against many gram-positive and gram-negative microorganisms. Amoxicillin is similar to penicillin in its bactericidal action against susceptible bacteria during the stage of active multiplication. It acts through the inhibition of cell wall biosynthesis that leads to the death of the bacteria.

6.1.1 Acquisition

The antibiotic to be used in this study is FDA-approved for use in the US for gynecological and other infections. Amoxicillin 250 mg generic capsules and placebo will be purchased in bulk from the UAB Research Pharmacy.

The research pharmacy will over-encapsulate the amoxicillin capsules for the active treatment arm and will create identical appearing placebo capsules containing lactose. The UAB pharmacy will package the capsules (amoxicillin or placebo) into individual doses. This includes encapsulation, packaging, blinding and labeling according to the randomization schedule for each site and applicable regulatory requirements. The UAB pharmacy will ship the medication to Fisher BioServices at the following address:

DMID Clinical Materials Services (CMS) Contract
Fisher BioServices
20439 Seneca Meadows Parkway
Germantown, MD 20876
Phone : (240) 477-1350
Fax : (240) 477-1360
Email : DMID.CMS@thermofisher.com

Study products will be shipped from the DMID Clinical Materials Services (CMS) to the investigational site upon request and approval by NIAID DMID.

6.1.2 Formulation, Packaging, and Labeling

Amoxicillin
Amoxicillin will be supplied as 250 mg amoxicillin trihydrate. Each active capsule of amoxicillin will be placed in a gelatin capsule. The gelatin capsules are Swedish orange capsules from Capsugel. Each vial contains 28 capsules.

Placebo for Amoxicillin

Each placebo capsule will be filled with lactose only and will be identical in appearance to the capsule with the active ingredient. The lactose filler is Lactose U.S.P. and the small amount should not be a problem even for persons who are lactose-intolerant. Participants may experience some mild bloating. Each vial contains 28 capsules.

The active and placebo capsules will be placed into amber prescription vials and labeled with a treatment number and allocated according to the randomization scheme generated by Emmes.

Vials will be placed into plastic bags with a sheet of instructions for each arm.

6.1.3 Product Storage and Stability

All study medications are stable at room temperature and will be stored at 20-25°C/68-77°F.

6.2 Dosage, Preparation and Administration of Study Intervention/Investigational Product

6.2.1 Dosage

Participants will be randomized to one of two study products as follows:

- Amoxicillin 2 x 250mg capsules PO twice a day for 7 days
- Placebo 2 capsules PO twice a day for 7 days

6.2.2 Preparation

A Site Research Pharmacist may be delegated the responsibility of study product dispensation. The Research Pharmacist must be a licensed, registered pharmacist and is the preferred healthcare practitioner to be delegated to perform this activity. If a Research Pharmacist is not available, a physician, nurse practitioner, physician assistant, registered nurse, or other authorized healthcare practitioner who is a member of the clinical study staff may be delegated to dispense the study product. These personnel must be licensed, trained, and qualified to prepare investigational study product and must be authorized to dispense the study product under state and local rules and regulations.

6.2.3 Administration

All active and placebo study products will be orally administered.

Each participant will receive a single vial of medication. Section 6.1.2 explains the packaging. Section 4 explains the design and study arms. After randomization of the participant, the study clinician will obtain the next available drug number and vial of medication for that drug number. The participant will be given instructions on how and when to take the medication and to bring the vial back to the clinic at the next follow-up visit.

6.3 Accountability Procedures for the Study Intervention/Investigational Product(s)

Fisher will ship packaged study medication to the investigational sites. At each site, study product will be kept in a locked storage cabinet at the clinic and administered by the study personnel. A drug accountability log should be filled out for all study medications at the sites.

After receipt of the study product, the site PI is responsible for its distribution and disposition and has ultimate responsibility for study product accountability. The site PI may delegate to a Site Research Pharmacist or an appropriately qualified staff member the responsibility for study product accountability. The designee will be responsible for maintaining complete records and documentation of product receipt, accountability, dispensation, temperature and storage conditions, and final disposition of study product. All study product, whether administered or not, must be documented on the appropriate study product accountability record or dispensing log. The sponsor's monitoring staff will verify the participating clinical sites' study product accountability records and dispensing logs per the study monitoring plan.

Unused dispensed study product returned to the site will be accounted for at the site and participant compliance will be recorded. Any unused returned study product will be disposed of according to the site's SOPs. Upon completion or termination of the study and after the final monitoring visit, final disposition of the unused never dispensed study product will be determined by DMID and communicated to the participating sites by the DMID Clinical Project Manager.

6.4 Assessment of Participant Compliance with Study Intervention/Investigational Product

Participants will be asked to bring the vial of medication and memory aid to the study personnel at their follow-up visit. Study personnel will review adherence to the study medication schedule with the participants, including the participant's memory aid, and record this on the appropriate data collection form at Visit 2 (ToC) and any Unscheduled Visits. Study personnel will record the number of pills returned. The participant's memory aid may be discarded by the study personnel post review with the participant.

6.5 Concomitant Medications/Treatments

Administration of any medications, therapies, or vaccines will be recorded on the appropriate data collection form. Concomitant medications will include all medications taken 14 days prior to the screening visit through Visit 2 or early termination, whichever occurs first. Prescription and over-the-counter drugs will be included, as well as intravaginal products, herbs, vitamins, and supplements. Previously recorded medications will be updated as appropriate.

Participants who have received study drug and are subsequently diagnosed with a concomitant infection that requires systemic antibiotics will receive treatment according to the local clinic's standard protocols.

At the discretion of the site PI, use of new medication should prompt evaluation for the presence of a new diagnosis of chronic medical disease or condition.

Medications that might interfere with the evaluation of the study product should not be used unless absolutely necessary.

Refer to Section 5 for medications that are prohibited for study eligibility and throughout study participation.

7 STUDY SCHEDULE

Study visit information is listed in this section, Section 8 (Study Procedures/Evaluations), and the Appendix A: Schedule of Events. Further instructions are described in the protocol-specific Manual of Procedures (MOP).

7.1 Visit 0 (Day -14 to -7) Screening

- Potential participants will be provided with a description of the study (purpose and study procedures) and asked to read and sign the Screening Informed Consent form, (SIC). The SIC form will be signed prior to performing any study eligibility procedures.
- Eligibility criteria will be reviewed with participants.
- Demographic information will be collected from the participant.
- Sexual history for the past 90 days will be collected.
- Medical history will be collected only on active conditions within one year prior to screening. All concomitant medications taken in the last 14 days prior to the screening visit will be recorded on the appropriate data collection form.
- A urine pregnancy test will be performed on all participants of childbearing potential and must be negative prior to administration of study product.
- A pelvic examination will be conducted for the collection of vaginal swab specimens for:

  pH, whiff test, and wet prep microscopy (Amsel criteria with the exception of evaluation of vaginal discharge), Nugent score, NAAT for trichomonas, quantitative NAAT for GV, and future use. If the on-site microscopy reveals a vaginal infection (i.e., yeast, trichomonas, and BV/abnormal vaginal flora), the remaining specimens that were collected will be discarded as the participant is not eligible for the study. For this study, vulvovaginal candidiasis will be defined by the presence of budding yeast and/or pseudohyphae; trichomoniasis will be defined by the presence of motile trichomonads on wet prep or a positive NAAT for T. vaginalis; abnormal flora/BV by the presence of at least 2/3 of the following criteria – pH >4.5, positive whiff test, ≥ 20% clue cells. These stricter criteria will eliminate those women with intermediated flora as well as BV. We will not evaluate the presence of vaginal discharge per se due to the subjective nature of this observation. If these vaginal infections are found on the screening wet prep the participant will not be eligible for the study.

AFTER VISIT 0 ENDS AND PRIOR TO VISIT 1

- Women whose tests from the screening visit return positive for GV by NAAT, negative for abnormal flora/BV by Nugent score of 0-3 and negative for trichomonas by NAAT

will return for the Enrollment visit, which is scheduled at the end of the screening visit.

- Women whose tests from the screening visit return negative for GV by NAAT will be called and informed that they are no longer eligible.

- Women whose tests from the Screening visit return positive for GV by NAAT and also test positive for either BV or trichomonas will be called and informed that they are no longer eligible.

7.2 Visit 1 (Day 1) Enrollment

- Test results from the screening visit will be reviewed and the following actions completed:

  - Participants whose tests from the screening visit return positive for GV by NAAT, negative for abnormal flora/BV by Nugent (Nugent score 0-3) and negative for trichomonas by NAAT, are considered eligible for study participation.

- Study personnel will review study eligibility to ensure unchanged since the screening visit. Pregnancy test result from screening visit will be reviewed and confirmed to be negative.

- Screened participants who are deemed eligible will be asked to read and sign the Enrollment Informed Consent form, (EIC). The EIC form will be signed prior to performing any study procedures.

- Interim sexual history since the screening visit will be collected.

- Medical history will be reviewed and updated as appropriate.

- All concomitant medications taken since the screening visit will be recorded on the appropriate data collection form. Previously recorded medications will be updated as appropriate.

- Participants will then be enrolled and randomized to treatment vs. placebo (via Advantage EDC) and instructed to take the medication twice per day for 7 days.

- Study personnel will schedule randomized participants to return for follow-up at Visit 2 (Day 15-21).

- Study drug, memory aid and instructions will be provided to participants prior to departure from clinic. Remind participant to abstain from vaginal intercourse or use condoms at all times for vaginal intercourse until next visit independent of birth control use or if participant is of non-childbearing potential. This restriction is to prevent participants from GV reinfection.

7.3 Visit 2 (Day 15 [Window: Days 15-21]) Follow-up (ToC)

- Interim sexual history since the last clinic visit will be collected.
- Medical history will be reviewed and updated as appropriate.
- All concomitant medications taken since Visit 1 will be recorded on the appropriate data collection form. Previously recorded medications will be updated as appropriate.
- Compliance with the study protocol criteria will be assessed via review of participant memory aid, drug return, and review with participant.
- Study personnel will discuss with participants and assess and record all AE/SAEs.
- A pelvic examination will be conducted for the collection of vaginal swab specimens as done at screening, with the exception of trichomonas NAAT.
- If the Visit 2 GV specimen is still positive by NAAT no further participant contact is needed.

7.4 Early Termination Visit

The following assessments will be performed at the early termination visit for participants who withdraw, or are withdrawn or terminated from this study:

- Interim sexual history since the last clinic visit will be collected.
- Medical history will be reviewed and updated as appropriate.
- All concomitant medications taken since the last study visit will be recorded on the appropriate data collection form. Previously recorded medications will be updated as appropriate.
- Compliance with the study protocol criteria will be assessed via review of participant memory aid, drug return, and review with participant.
- Study personnel will discuss with participants and assess and record all AEs/SAEs. Previously recorded AEs/SAEs will be updated as appropriate.
- If indicated for symptoms, a pelvic examination will be conducted for the collection of vaginal swab specimens for:
    - pH, whiff test, and wet prep to determine the etiology of current symptoms.

7.5 Unscheduled Visit

Participants may be seen for unscheduled visits as necessary. For example, for problems with the medication, development of intercurrent vaginal symptoms, or need for treatment of trichomonas.

Any of the following activities may be conducted at the discretion of the site PI:

- Interim sexual history since the last clinic visit will be collected.
- Medical history will be reviewed and updated as appropriate.
- All concomitant medications taken since the last study visit will be recorded on the appropriate data collection form. Previously recorded medications will be updated as appropriate.
- Compliance with the study protocol criteria will be assessed via review of participant memory aid, drug return, and review with participant.
- Study personnel will discuss with participants and assess and record all AEs/SAEs. Previously recorded AEs/SAEs will be updated as appropriate.
- If indicated for symptoms, a pelvic examination will be conducted for the collection of vaginal swab specimens for:
    - pH, whiff test, and wet prep to determine the etiology of current symptoms.

8 STUDY PROCEDURES/EVALUATIONS

8.1 Clinical Evaluations

Medical history only on active conditions within one year prior to screening will be obtained by interview of the participants at Visit 0 and will be updated at each clinic visit. At the follow-up visits, an interim medical history will be obtained by interview of the participants noting any changes since the previous visit.

Sexual history will be obtained by interview of the participants at Visit 0. Participants will be queried at Visit 1 and Visit 2 (and as needed at unscheduled and early termination visits) regarding sexual activity and condom use since the previous clinic visit.

Medications history (concomitant medications) will include a review of all current medications and medications taken 14 days prior to the screening visit and through study completion. This will occur at Visits 0, 1, and 2, and as needed at unscheduled and early termination visits. Prescription and over-the-counter drugs will be included (lubricants, intravaginal products, vitamins, herbs and supplements). Assessment of eligibility will also include a review of all permitted and prohibited medications per the Participant Inclusion and Exclusion Criteria (see Section 5.1).

Study drug, memory aid and instructions will be provided to participants at Visit 1. Compliance with the study drug will be assessed at Visit 2 (and as needed at unscheduled and early termination visits) via review of participant memory aid, drug return, and review with participant.

AE/SAE assessment will be reviewed with participants at Visit 2 (and as needed at unscheduled and early termination visits) and recorded.

A pelvic examination will be performed on Visit 0 and 2, and as indicated at unscheduled or early termination visits. All pelvic examinations will be performed by a qualified study clinician.

Pelvic Examination:

During pelvic examination, the following will be used for the clinical diagnosis of BV by Amsel criteria. Instructions for obtaining and determining the presence of Amsel clinical criteria can be found in the protocol-specific MOP and consist of the following:

1) A fishy odor of the vaginal discharge with the addition of a drop of 10% KOH (positive whiff test);

2) Clue cells ≥ 20% of the total vaginal squamous epithelial cells on saline microscopy;

3) Vaginal fluid pH > 4.5.

8.2 Laboratory Evaluations

8.2.1 Clinical Laboratory Evaluations

A urine pregnancy test will be performed at the site during the screening visit and must be negative prior to participant being eligible for enrollment. NAAT for trichomonas will be performed by the local lab. Nugent score for BV will be performed in Dr. Schwebke's lab. Quantitative NAAT for GV will be performed at LabCorp Research and Development Laboratory.

Bedside Evaluation of Vaginitis

A vaginal swab specimen should be used to determine the vaginal pH using ColorpHast pH paper. Vaginal secretions should be placed into 0.3 cc of saline and examined within 10 minutes of collection by microscopy to detect the presence of yeast, trichomonas, and clue cells. A drop of the suspension would be mixed with 10% KOH for the "whiff" test and KOH fungal prep.

Nugent Criteria for Determination of BV:

A vaginal swab for microbiologic assessment of BV by Nugent criteria should be performed. The Nugent score utilizes a 0-10 point scale for evaluation of vaginal flora and is based on the weighted sum score of the following three bacterial morphotypes calculated from the slide exam under oil immersion (1000x):

- *Lactobacillus*: Large Gram-negative rods
- *Gardnerella*/*Bacteroides* spp: thin, curved, Gram-variable rods
- *Mobiluncus* spp: thin, curved, Gram-variable rods

The Nugent score is interpreted as follows: 0-3 normal; 4-6 intermediate; and 7-10 BV. For the purposes of this study, a score > 3 is considered abnormal, and a score of 0-3 is considered normal.

NAAT testing for *T. vaginalis*

This is to be performed by the site lab using FDA cleared methods.

8.2.2 Special Assays or Procedures

The specific details regarding the preparation of the samples collected for GV NAAT testing can be found in the protocol-specific MOP.

8.2.3 Specimen Preparation, Handling, and Shipping

8.2.3.1 Instructions for Specimen Preparation, Handling, and Storage

Specimen preparation, handling, and storage will be done according to local clinic SOPs. Additional details can be found in the protocol-specific MOP.

8.2.3.2 Specimen Shipment

Gram stain specimen shipment for Nugent scoring to the UAB Central laboratory will be performed according to all applicable International Air Transport Association (ATA) requirements. The UAB Central laboratory address is as follows:

UAB Infectious Diseases Laboratory
1900 University Blvd
THT 220
Birmingham, AL 35294

Shipment of GV NAAT isolates to LabCorp will be performed according to all applicable International Air Transport (IATA) requirements. LabCorp address is as follows:

ATTN: Molecular R&D 6500
LabCorp
1447 York Court
Burlington, NC 27215

9 ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

Safety will be monitored throughout the study by a pelvic examination. Safety will be assessed by the frequency and severity of:

1. Serious adverse events occurring from the time of the study dose through Visit 2.
2. Non-serious adverse events occurring from the time of the study dose through Visit 2.

9.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

9.2.1 Adverse Events

Adverse Event:

ICH E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation participant administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for “serious adverse events” should be captured on the appropriate CRF. Information to be collected includes event description, time of onset, clinician’s assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis, which would include MD, PA, Nurse Practitioner, DO, or DDS), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the patient is screened should be considered as baseline and not reported as an AE. However, if it deteriorates at any time during the study, it should be recorded as an AE.

All AEs must be graded for severity and relationship to study product.

FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Severity of Event: The following guidelines will be used to quantify intensity:

Gastrointestinal side effects which include nausea, vomiting, diarrhea, and abdominal pain will be assessed on a scale of 1 to 3 according to the toxicology table in Appendix B: Toxicology Table of this protocol.

Systemic reactions which include allergic reaction, headache, fatigue, and myalgia will be assessed on a scale of 1 to 3 according to the toxicology table in Appendix B: Toxicology Table of this protocol.

All other AEs will be graded using the following general criteria:

Mild: events require minimal or no treatment and do not interfere with the patient's daily activities.

Moderate: events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

Severe: events interrupt a patient's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

Relationship to Study Products: The clinician's assessment of an AE's relationship to study medication is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to study product assessed using the terms "related" or "not related". In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

9.2.2 Serious Adverse Events

Serious Adverse Event (SAE):

An adverse event or suspected adverse reaction is considered “serious” if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death,
- a life-threatening adverse event*,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

* Life-threatening adverse event. An adverse event is considered “life-threatening” if, in the view of either the investigator or sponsor, its occurrence places the participant at immediate risk of death. It does not include an adverse event, had it occurred in a more severe form, might have caused death.

All SAEs will be:

- recorded on the appropriate SAE form and eCRF
- followed through resolution by a study clinician as defined in section 9.4
- reviewed and evaluated by a study clinician

9.2.3 Procedures to be Followed in the Event of Abnormal Clinical Findings

The site PI is responsible for reporting all AEs/SAEs that are observed or reported during the study, regardless of their relationship to study product. AEs/SAEs or abnormal clinical findings will be documented, reported and followed appropriately.

9.3 Reporting Procedures

The Protocol Chair will monitor all adverse events and report serious adverse events to DMID and each site's local IRB within 24 hours.

9.3.1 Serious Adverse Events

SAEs will be followed until resolution even if this extends beyond the study-reporting period. Resolution of an SAE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

Any AE that meets a protocol-defined serious criterion must be submitted immediately (within 24 hours of site awareness) on an SAE form to the DMID Pharmacovigilance Group, at the following address:

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20817, USA
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)
SAE FAX Phone Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible.

The DMID medical monitor and clinical protocol manager will be notified of the SAE by the DMID Pharmacovigilance Group. The DMID medical monitor will review and assess the SAE for regulatory reporting and potential impact on study participant safety and protocol conduct.

At any time after completion of the study, if the investigator becomes aware of an SAE that is suspected to be related to study product, the investigator will report the event to the DMID Pharmacovigilance Group.

9.3.2 Regulatory Reporting for Studies Not Conducted Under DMID-Sponsored IND

As this study will not be conducted under an IND, MEDWATCH will be used to report related AEs. DMID should be copied simultaneously when an alternate method of reporting is utilized.

9.3.3 Reporting of Pregnancy

Participants of child-bearing potential will be counseled to continue using acceptable forms of birth control as well as condoms during the study period. If a participant becomes pregnant during the study, dosing will be discontinued immediately. A pregnancy reporting form will be completed for any study participant who becomes pregnant during the study period. The site will maintain contact with pregnant study participants to obtain pregnancy outcome information. The pregnant participant will be followed until delivery or until the end of pregnancy (in the case of miscarriage or pregnancy termination). Infants born to these study participants will also be monitored for SAEs (congenital anomalies or other birth defects) and other complications for up to two months after birth. Pregnancy reporting forms will be limited to collecting data on the following information:

- prior maternal history including congenital abnormalities or pregnancy complications;
- estimated date of conception;
- estimated and actual date of delivery or end of pregnancy;
- pregnancy outcome (live birth, stillbirth, miscarriage or elective termination);
- mode of delivery;
- maternal complications; and
- neonatal complications (i.e., lethal or nonlethal congenital abnormality).

Pregnancies occurring in study participants will be reported via AdvantageEDC^{SM} on the Pregnancy Report form.

9.4 Type and Duration of Follow-up of Participants after Adverse Events

AEs and SAEs will be followed from the time of study treatment through resolution even if this extends beyond the study reporting period, Visit 2. Resolution of an AE/SAE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

Follow-up procedures, evaluations, and outcomes will be recorded on the appropriate data collection form.

9.5 Halting Rules

Further enrollment will be halted for DSMB review/recommendation if any two participants experience an adverse event that is judged to be Severe (Grade 3) and related to study product.

DMID retains the authority to suspend additional enrollment and administration of study product during the entire study, as applicable.

9.6 Safety Oversight (ISM plus DSMB)

An Independent Safety Monitor (ISM) at each clinical research site will oversee the safety of research participants at that site and will provide independent written evaluation of SAEs and related Grade 3 AEs to the DMID Clinical Project Manager and DMID Medical Monitor. The ISM will serve as an independent consultant for the site PI on participant-related issues. The ISM will communicate with the site PI and study PIs to resolve any issues.

Safety oversight will be conducted by a DSMB that is an independent group of experts that monitors participant safety and advises DMID. The DSMB members will be separate and independent of study personnel participating in this trial and should not have scientific, financial or other conflict of interest related to the study. The DSMB will consist of members with appropriate expertise to contribute to the interpretation of the data from this trial.

The DSMB will review study progress and participant, clinical, and safety data at the following time points:

- At specified times during the course of study as defined in the DSMB Charter,
- Ad hoc when a halting rule is met, for immediate concerns regarding observations during the study, or as needed.
- A final closeout meeting will be held at the end of the study after the database is locked and when the final study report is available.

The DSMB will operate under the rules of a DMID-approved charter that will be written at the organizational meeting of the DSMB. As an outcome of each review/meeting, the DSMB will make a recommendation as to the advisability of proceeding with study administrations (as applicable), and to continue, modify, or terminate the study. The DSMB members will meet at specified times during the course of the study as defined in the DSMB Charter.

DMID or the DSMB chair may convene the DSMB on an ad hoc basis according to protocol criteria or if there are immediate concerns regarding observations during the course of the study. The DMID Medical Monitor is empowered to stop enrollment and study treatment if adverse events that meet the halting criteria are reported. The DMID Medical Monitor will be

responsible for reviewing SAEs in real time. The DSMB will review SAEs on a regular basis and ad hoc during the study.

10 CLINICAL MONITORING

10.1 Site Monitoring Plan

DMID, the sponsoring agency, or its designee will conduct site-monitoring visits as detailed in the clinical monitoring plan and in accordance with DMID policies. Site monitoring is conducted to ensure that the human subject protections, study and laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, ICH/GCP guidelines and applicable regulations; and that the study is conducted in accordance with the protocol, protocol-specific manual of procedures and applicable sponsor standard operating procedures. Monitoring visits will include, but are not limited to, inspection of study facilities, review of regulatory files, accountability records, CRFs, IC forms, printouts of medical and laboratory reports from the electronic medical records (EMR) system, and protocol and GCP compliance. Site monitors will have access to the study site, study personnel, and all study documentation according to the DMID-approved site monitoring plan. Study monitors will meet with PIs to discuss any problems and actions to be taken and document visit findings and discussions.

Site visits will be made at standard intervals as defined by DMID and may be made more frequently as directed by DMID.

11 STATISTICAL CONSIDERATIONS

11.1 Study Hypotheses

There is one planned formal test of hypothesis which compares amoxicillin to placebo with respect to the primary efficacy outcome measure. The hypothesis test compares the proportion of participants with eradication of GV in each study arm through Visit 2 (Day 15-21) as assessed by NAAT.

The null hypothesis for the comparison is that there is no difference in proportions between study arms, with a two-sided alternative. The test will be conducted using a Pearson Chi-Square test with Yates continuity correction at the 5% two-sided significance level. The hypothesis will be tested in the Intent-to-Treat (ITT) efficacy analysis population for the primary analysis and repeated as a secondary analysis in the Per-Protocol (PP) analysis population.

11.2 Sample Size Considerations

Sample size calculations are based on detecting a decrease in GV colonization rate from 80% among participants receiving placebo to as large as 44% among participants receiving amoxicillin. Using the two-sided Pearson Chi-Square test with Yates continuity correction, significance level of 0.05, and assuming an amoxicillin to placebo allocation ratio of 1:1, 82 women (41 in each arm) would provide 90% power to detect a difference in colonization rates of 36% (i.e. 44% colonization rate in the amoxicillin arm).

Assuming a 15% drop-out rate between enrollment and the ToC visit approximately 98 women will need to be randomized to obtain 82 women evaluable for the primary analysis. Assuming 40% of women without BV will have a positive NAAT test for GV at screening, approximately 245 women without BV will need to be screened to randomize 98 women in the study.

11.3 Planned Interim Analyses

11.3.1 Safety Review

The DSMB may review study progress and participant safety data at specified times during the course of study, as defined in the DSMB Charter. The study will be monitored to determine if any of the safety halting rules described above in Section 9.5 are met. The halting rules do not utilize any statistical criteria and no formal hypothesis testing is planned to occur for the safety and enrollment data reviews

11.3.2 Efficacy Review

There are no planned interim efficacy analyses for this study.

11.4 Final Analysis Plan

A separate statistical analysis plan document will be generated that will contain the details of the analyses. This section outlines the major components of the analyses.

11.4.1 Analysis Populations

Intent-to-Treat (ITT) Population: This analysis population includes all randomized participants.

The ITT population will be used as the primary efficacy analysis population.

Safety Population: This analysis population includes all randomized participants who received at least one dose of study treatment.

Per-Protocol (PP) Population: This analysis population includes all randomized participants who met all inclusion/exclusion criteria, are evaluable, adhered to the assigned study treatment regimen (as defined below), and returned to the study site for the ToC visit within the specified window (Visit 2 [Window: Day 15 -21]) and did not experience any of the following after enrollment:

- Unprotected vaginal sexual intercourse between Visit 1 and Visit 2
- New pregnancy reported by the subject
- Receipt of any antibiotic or intravaginal microbicide other than study drug

Participants are deemed evaluable if their BV and GV status can be determined at screening and their GV status can be determined at Visit 2. Participants who are missing Gram stain, GV NAAT, or Amsel results at screening are not evaluable. Participants who are missing GV NAAT results at the Visit 2 are not evaluable.

A Participant is considered adherent to the study treatment regimen if she uses at least 80% of the scheduled doses (i.e. at least 12 of the 14 doses) of study treatment, as assessed by the number of pills returned. If pill count cannot be assessed, participant report will be used to assess adherence.

In the unlikely event of an error in randomization or study product administration, participants will be grouped by the formulation they actually received in the PP and Safety analyses but will be grouped by their intended randomized assignment in the ITT analysis.

NAAT Screening Population: This analysis population includes all screened women who are evaluated for GV by NAAT.

The NAAT screening population will be used as the exploratory efficacy analysis population.

11.4.2 Baseline Characteristics

Baseline and demographic characteristics will be summarized overall and by treatment. For both continuous and categorical variables, appropriate summary statistics will be applied. For continuous variables, descriptive statistics will include the number of non-missing values, mean, standard deviation, median, minimum, and maximum. For categorical variables, descriptive statistics will include counts and percentages per category.

11.4.3 Safety Analysis Plan

Safety evaluations will be based on the incidence, severity, and type of adverse events and serious adverse events as detailed in Section 9. Safety variables will be tabulated and presented for all participants in the Safety population, grouped by treatment.

Unsolicited AEs will be coded by MedDRA® for preferred term and system organ class. The proportion of participants and exact 95% confidence intervals of AEs will be computed in aggregate, as well as by MedDRA® categories. The number of SAEs will be reported by a detailed listing showing the type, MedDRA® coding, relevant dates (treatment dosing dates and AE onset and resolution dates), severity, relatedness, and outcome for each event.

In addition to the descriptive analyses described above, the proportion of participants who experience related adverse events in each study arm following the first dose of study product through Visit 2 (Day 15-21) will be summarized overall and by treatment group. In addition, the proportion of participants who discontinue study product early due to adverse events will be summarized overall and by treatment group.

11.4.4 Efficacy Analysis Plan

11.4.4.1 Primary Efficacy Analysis

For the primary analysis of the efficacy of amoxicillin in women who are infected with GV but have no clinical evidence of BV, the number and proportion of women with eradication of GV at Visit 2 (Day 15-21) will be summarized overall and by treatment group. The point estimates for the arm-specific proportions and difference in proportions as well as corresponding 95% confidence intervals will be reported. A test of hypothesis will be conducted using Pearson Chi-Square test with Yates continuity correction at the 5% two-sided level of significance level to

formally compare the treatment groups. The primary analysis will be performed in the ITT analysis population and repeated as a secondary analysis in the PP analysis population.

Eradication of GV is defined by a negative NAAT result for GV at Visit 2 (Day 15-21). Participants who have a missing GV NAAT result at the ToC visit are excluded from the PP analyses, and will be classified as treatment failures in the ITT analyses.

11.4.4.2 Exploratory Efficacy Analysis

Details of the exploratory efficacy analysis, as well as any additional exploratory analyses that may be performed to supplement the primary analysis, will be described in the separate Statistical Analysis Plan. As an example of the latter, demographic and baseline characteristics of participants who received amoxicillin and were positive for BV by Nugent Score and Amsel Criteria at Visit 2 (Day 15-21) will be summarized.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of participants. Each site will permit authorized representatives of the DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. These representatives will be permitted access to all source data, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and participant files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial. Data collection forms will be derived from the eCRFs and be provided by the Statistical and Data Coordinating Center (SDCC).

13 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, the participating sites are responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. Each site PI will provide direct access to all trial-related sites, source data/data collection forms, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. Each site PI will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

The SDCC will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the site(s) for clarification and resolution.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

The site PI will ensure that this trial is conducted in full conformity with principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR 46, 21 CFR 50 and 56, and ICH E6; 62 Federal Regulations 25691 (1997); and future revisions, if applicable. The site PI's institution will hold a current Federal Wide Assurance (FWA) issued by the Office of Human Research Protection (OHRP) for federally funded research.

14.2 Institutional Review Board

Prior to enrollment of participants into this trial, the approved protocol and informed consent forms will be reviewed and approved by the appropriate IRB listed on its FWA. A copy of the IRB letter of approval will be provided to DMID.

The IRB Federal Wide Assurance number will be provided to DMID.

Should amendments to the protocol be required, the amendments will be written by the sponsor and provided to the site PI for submission to the IRB.

14.3 Informed Consent Process

The site PI, or designated study staff, will choose participants in accordance with the eligibility criteria detailed in Section 5. Before any study procedures are performed, participants must sign the Screening Informed Consent Form and, if applicable the Enrollment Informed Consent form that both comply with the requirements of 21 CFR Part 50 and 45 CFR 46 and the local IRB.

Informed consent is a process that is initiated prior to an individual agreeing to participate in a trial and continuing throughout the individual's trial participation. There will be two consent forms: Screening Informed Consent (SIC) for the screening procedures (determination of eligibility confirmed upon receipt of the GV results within 7-14 days post specimen collection) and an Enrollment Informed Consent (EIC) for the full study. Upon receipt of the GV test results and review of the original screening criteria, the participant is confirmed to be eligible, they will then need to be consented using the EIC prior to continuation.

Before any screening or study procedures are performed, participants will receive a comprehensive explanation of the proposed screening procedures, study procedures and study interventions/products, including the nature and risks of the trial, alternate therapies, any known AEs, the investigational status of the components, and the other elements that are part of

obtaining proper informed consent. Participants will also receive a detailed explanation of the proposed use and disclosure of their protected health information (PHI), including specifically their specimens. Participants will be allowed sufficient time to consider participation in the screening portion and enrollment in the trial, after having the nature and risks of both the screening and trial explained to them. Participants will have the opportunity to discuss the trial with their family, friends, or legally authorized representative or think about it prior to agreeing to participate.

Both the SIC and EIC will describe in detail the study interventions/products, study procedures, risks, and possible benefits given to participants. Neither informed consent form can include any exculpatory statements. Both informed consent forms will be IRB-approved, and participants will be asked to read and review the appropriate document. Upon reviewing the appropriate document, the site PI (or designee) will discuss the research study to participants and answer any questions that may arise. Participants must sign the informed consent form(s), and written documentation of the informed consent process is required prior to starting any study procedures/interventions being done specifically for the trial, including administering study product.

Study personnel may employ IRB-approved recruitment efforts prior to obtaining the participant's consent; however, before any study procedures are performed to determine protocol eligibility, the SIC must be signed. Participants will be given a copy of all informed consent forms that they sign.

By signing the SIC and EIC, participants agree to complete all evaluations required by the trial, unless the participant withdraws voluntarily or is terminated from the trial for any reason.

The rights and welfare of participants will be protected by emphasizing to participants that the quality of their medical care will not be adversely affected if they decline to participate in or withdraw from this trial.

14.4 Exclusion of Women, Minorities, and Children (Special Populations)

This trial will be inclusive of female adults who meet the Participant Inclusion/Exclusion Criteria, regardless of religion or ethnic background. Should the outcome of this trial be deemed acceptable, additional trials may be initiated in other populations.

14.5 Subject Confidentiality

Subjects will be identified by a participant number and not by name. Subject confidentiality is strictly held in trust by the participating site PIs, their study personnel, the sponsor, and their

agents. This confidentiality is extended to cover testing of biological samples, in addition to the clinical information relating to subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the trial or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All information provided by the sponsor and all data and information generated by the participating sites as part of the trial (other than a participant's medical records) will be kept confidential by the site PI and other study personnel to the extent permitted by law. This information and data will not be used by the site PI or other study personnel for any purpose other than conducting the trial. These restrictions do not apply to: (1) information that becomes publicly available through no fault of the site PI or other study personnel; (2) information that is necessary to disclose in confidence to an IRB solely for the evaluation of the trial; (3) information that is necessary to disclose in order to provide appropriate medical care to a study subject; or (4) study results that may be published as described in Section 16. If a written contract for the conduct of the trial that includes confidentiality provisions inconsistent with this statement is executed, that contract's confidentiality provisions shall apply rather than this statement.

The study monitor, or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the site PI. This includes, but is not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this trial. The participating sites will permit access to such records.

To protect privacy, we have received a Certificate of Confidentiality, CoC. With this CoC, the participating sites cannot be forced to release information that may identify the participant, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The participating sites will use the CoC to resist any demands for information that would identify the participant, except as explained below.

The CoC cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of federally funded projects, like this or local laws, such as for reporting of communicable diseases.

A CoC does not prevent the participant from voluntarily releasing information about themselves or their involvement in this research. If any person or agency obtains a written consent to receive study information, then the participating sites may not use the CoC to withhold that information.

The CoC does not prevent the participating sites from reporting without the participant's consent, information that would identify the subject as a participant in the study regarding

matters that must be legally reported including: child and elder abuse, sexual abuse, or wanting to harm themselves or others.

14.6 Study Discontinuation

If the trial is discontinued, participants who sign the EIC and are randomized and treated will continue to be followed for safety assessments. No further study product will be administered.

14.7 Future Use of Stored Specimens

Both the screening and enrolled subject populations will be consented to have vaginal swab samples stored for future vaginitis research. Storage of samples is optional and not a requirement for the study. If the participant consents to storage and future use of specimens, specimens will be kept at the UAB Central laboratory and LabCorp. Participants will not be contacted with the results of these future research studies. Future testing on specimens will only occur to the extent authorized in each study site's SIC and EIC or as otherwise authorized under applicable law and after review and approval by the DMID and the IRB of the researcher requesting the specimens.

Archived specimens will be identified only by the participant number which will allow linkage of the specimens to study data but not to any personal identifiers.

There will be no direct benefit to the participant from allowing the specimen to be stored and used for future purposes. However, the results may provide information that will help in the diagnosis or treatment of future patients.

The participant's specimen will be kept until it is used up or destroyed. It may be used to develop new tests or products. In some instances, these may have potential commercial value. If a participant decides at any time that she does not want the specimen stored for future research she must contact the study nurse/doctor who will then notify the laboratory/specimen archive. The laboratory staff will then mark the specimens by adding a "Destroy" label and they will be destroyed at the end of this study or removed from storage and destroyed.

15 DATA HANDLING AND RECORD KEEPING

The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

Data collection forms will be derived from the eCRFs and provided by the SDCC to the sites to record and maintain data for each participant enrolled in the study. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Permanent ink is required to ensure clarity of reproduced copies. When making a change or correction, the original entry should be crossed out with a single line, and the change should be initialed and dated. Do not erase, overwrite, or use correction fluid or tape on the original.

Data reported in the eCRF should be consistent with the data collection form/source documents or the discrepancies should be documented.

The sponsor and/or its designee will provide guidance to investigators on making corrections to the data collection forms and eCRFs.

15.1 Data Management Responsibilities

All data collection forms and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. Adverse events must be graded, assessed for severity and causality, and reviewed by the site PI or designee. Data collection is the responsibility of the clinical trial staff at the site under the supervision of the Site PI. During the study, the investigator must maintain complete and accurate documentation for the study.

The Emmes Corporation will serve as SDCC for this study and will be responsible for data management, quality review, analysis, and reporting of the study data.

15.2 Data Capture Methods

Clinical data (including AEs and concomitant medications) will be entered into a 21 CFR Part 11-compliant internet data entry system provided by The Emmes Corporation. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the data collection forms/source documents.

15.3 Types of Data

Data for this study will include safety, laboratory, and outcome measures.

15.4 Timing/Reports

Safety data will be provided to the DSMB and investigators as per the DSMB Charter.

15.5 Study Records Retention

Records and documents pertaining to the conduct of this study, including data collection forms, source documents, consent forms, laboratory test results, and medication inventory records, must be retained by the investigator for at least 2 years following the completion of this study. No records may be destroyed without written permission from the sponsor.

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP), or Manual of Procedures requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3

5.1 Quality Assurance and Quality Control, section 5.1.1

5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID, via the Emmes Corporation's IDES.

All deviations from the protocol must be addressed in study data collection forms. A completed copy of the DMID approved Protocol Deviation (PD) Form must be maintained in the Regulatory File. Protocol deviations must be submitted to the local IRB/IEC per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements.

16 PUBLICATION POLICY

All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/) an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication.

Refer to:

- NIH Public Access Policy, http://publicaccess.nih.gov/
- NIH Office of Extramural Research (OER) Grants and Funding, http://grants.nih.gov/grants/oer.htm

As of January 2018, all clinical trials supported by the NIH must be registered on ClinicalTrials.gov, no later than 21 days after the enrollment of the first subject. Results of all clinical trials supported by the NIH, generally, need to be submitted no later than 12 months following the primary completion date. A delay of up to 2 years is available for trials that meet certain criteria and have applied for certification of delayed posting.

As part of the result posting a copy of this protocol (and its amendments) and a copy of the Statistical Analysis Plan will be posted on ClincialTrials.gov.

For this trial, the responsible party is DMID which will register the trial and post results.

Following completion of the study, the lead principal investigator (PI) is expected to publish the results of this research in a scientific journal. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov* (http://clinicaltrials.gov/), which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies.

The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics (PK) or major toxicity (e.g., Phase I trials), would be exempt from this policy.

Results of any exploratory immunogenicity analysis will not be published prior to publication of the primary immunogenicity results for this study.

Refer to Public Law 110-85, Section 801, Clinical Trial Databases.

*Journal Citation:
De Angelis C, Drazen JM, Frizelle FA, Haug C, Hoey J, Horton R, et al. Clinical trial registration: a statement from the International Committee of Medical Journal Editors. N Engl J Med. 2004;351:1250-1.

17 LITERATURE REFERENCES

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4. Eschenbach DA, Hillier S, Critchlow C, Stevens C, DeRouen T, Holmes KK. Diagnosis and clinical manifestations of bacterial vaginosis. American journal of obstetrics and gynecology. 1988;158(4):819-28.
5. Ahmed A, Earl J, Retchless A, Hillier SL, Rabe LK, Cherpes TL, et al. Comparative genomic analyses of 17 clinical isolates of Gardnerella vaginalis provide evidence of multiple genetically isolated clades consistent with subspeciation into genovars. Journal of bacteriology. 2012;194(15):3922-37.

APPENDIX A: SCHEDULE OF EVENTS

		Screening	Enrollment	Follow-Up	Unscheduled	Early Termination
		Visit 0 (Day -14 to -7)	Visit 1 (Day 1)	Visit 2 (Day 15 [Window: 15-21])		
Signed Consent Form[1]		X	X			
Assessment of Eligibility Criteria		X	X			
Collection of Demographics		X				
Review of Sexual History		X	X	X	(X)	X
Review of Medical History[2]		X	X	X	(X)	X
Review of Concomitant Medications		X	X	X	(X)	X
Randomization			X			
Dispense memory aid and instructions			X			
Reminder on abstinence or condom use during vaginal intercourse to prevent GV reinfection			X			
Study Intervention			X			
Pelvic Examination		X		X	(X)	(X)
Assessment of Adverse Events				X	X	X
Assessment of study drug compliance (via review of memory aid and drug return)				X	(X)	(X)
Clinical Laboratory	Urine Pregnancy Test	X				
	Trichomonas, NAAT Test	X				
	pH, whiff test, and wet microscopy (Amsel criteria)	X		X	(X)	(X)
Research Laboratory	BV, Nugent score	X		X	(X)	(X)

		Screening	Enrollment	Follow-Up	Unscheduled	Early Termination
		Visit 0 (Day -14 to -7)	Visit 1 (Day 1)	Visit 2 (Day 15 [Window: 15-21])		
Research Laboratory	Future Use[3]	X		X	(X)	(X)
LabCorp	GV, quantitative NAAT	X		X	(X)	(X)

(X) – As indicated/appropriate.
[1]At Visit 0 the participant is signing the Screening Informed Consent Form. At Visit 1 the participant will then sign the Enrollment Informed Consent Form.
[2] Medical history is to be originally collected only on active conditions within one year prior to screening. Then at follow up visits, the active conditions will be reviewed and any new conditions captured.
[3] At Visit 0 Screening and at Visit 2, participants that agree will have an extra swab specimen collected for future use.

APPENDIX B: TOXICOLOGY TABLE

Gastrointestinal	Grade 1	Grade 2	Grade 3
Nausea	No interference with activity	Some interference with activity	Prevents daily activities
Vomiting	No interference with activity or 1 - 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity or requires IV hydration
Diarrhea	2 - 3 loose or watery stools or < 400 gms/24 hours	4 - 5 loose or watery stools or 400 - 800 gms/24 hours	6 or more loose or watery stools or > 800gms/24 hours or requires IV hydration
Abdominal Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity
Systemic Reactions	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Allergic Reaction	Pruritus without rash	Localized urticaria OR requires oral therapy	Generalized urticaria; angioedema OR anaphylaxis OR requires epinephrine
Headache	No interference with activity	Repeated use of non-narcotic pain reliever >24 hours OR some interference with activity	Significant; any use of narcotic pain reliever OR prevents daily activity OR requires triptans
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity