Phase II-b Randomized Double-Blind Placebo-Controlled Trial of *Lactobacillus crispatus* CTV-05 (LACTIN-V) to Prevent the Recurrence of Bacterial Vaginosis

A Study of the Sexually Transmitted Infection Clinical Trials Group (STI CTG)

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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.

Site Investigator:

Signed: _____ Date: _____

Name
Title

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LIST OF ABBREVIATIONS

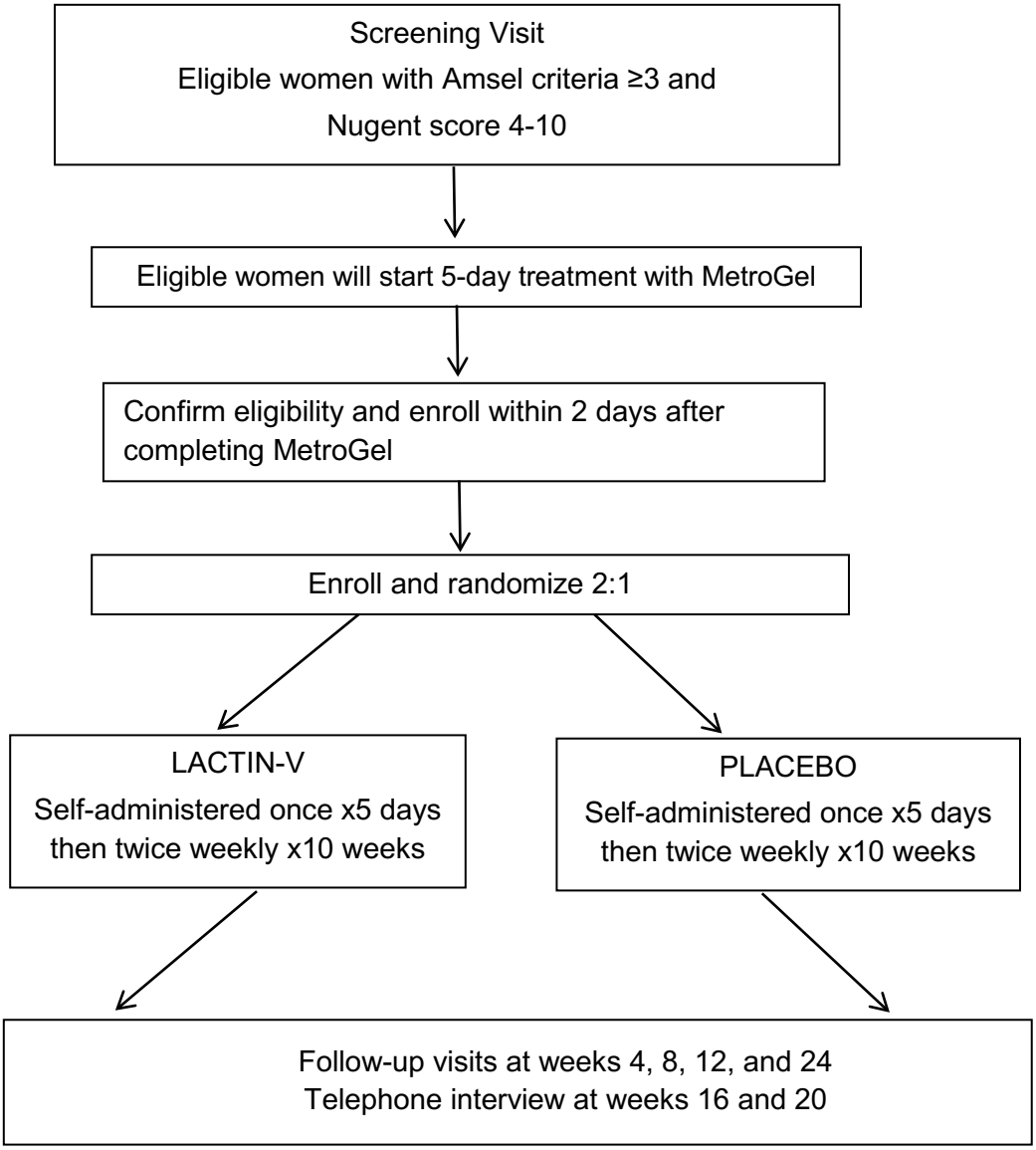
AE	Adverse Event
ATP	According to Protocol
βHCG	Beta Human Chorionic Gonadotropin
BV	Bacterial Vaginosis
CAPRISA	Centre for the AIDS Programme of Research in South Africa
CAR	Clinical Agents Repository
CC	Complete Case
cfu	Colony Forming Units
CMH	Cochran-Mantel-Haenszel
CI	Confidence Interval
CRF	Case Report Form
CROMS	Clinical Research Operations and Management Support
DAIDS	Division of AIDS, NIAID, NIH, DHHS
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DSMB	Data and Safety Monitoring Board
ELISA	Enzyme-linked Immunosorbent Assay
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDES	Internet Data Entry System
IEC	Independent or Institutional Ethics Committee
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ITT	Intent-to-treat
LACTIN-V	*Lactobacillus crispatus* CTV-05
LOCF	Last Observation Carried Forward
MedDRA	Medical Dictionary for Regulatory Activities
MetroGel	MetroGel vaginal gel
mITT	Modified Intent-to-Treat
MOP	Manual of Procedures
N	Number (typically refers to participants)
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
ORA	Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
OSEL	Office of Science and Engineering Laboratories
pH	Potential Hydrogen
PI	Principal Investigator
PP	Per Protocol
qPCR	Quantitative Polymerase Chain Reaction
RR	Rate Ratio
rUTI	Recurrent Urinary Tract Infection
SAE	Serious Adverse Event
SDCC	Statistical and Data Coordinating Center
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
STAR	Sexually Transmitted Infections Treatment and Research
STI	Sexually Transmitted Infection
STI-CTG	Sexually Transmitted Infections Clinical Trials Group
US	United States
UTI	Urinary Tract Infection

PROTOCOL SUMMARY

Title:	Phase II-b Randomized Double-Blind Placebo-Controlled Trial of *Lactobacillus crispatus* CTV-05 (LACTIN-V) to Prevent the Recurrence of Bacterial Vaginosis
Phase:	II-b
Population:	228 non-pregnant, pre-menopausal women age 18 to 45 years diagnosed with bacterial vaginosis (BV)
Number of Sites:	Four U.S. sites
Study Duration:	72 weeks after enrollment of the first participant
Participant Participation Duration:	24 weeks per participant
Description of Agent:	Lactobacillus *(L.) crispatus* CTV-05 (LACTIN-V) contains a naturally occurring vaginal strain of *L. crispatus* CTV-05, preserved as a powder applied by a vaginal applicator. LACTIN-V at 2 x 10^9 cfu/dose or matching placebo will be administered by vaginal applicator once daily for 5 consecutive days, followed by twice weekly for 10 consecutive additional weeks.
Primary Objectives	1) To estimate the efficacy of repeated doses of LACTIN-V (2 x 10^9 cfu/dose) as compared to placebo in preventing BV recurrence by 12 weeks following treatment of BV with MetroGel vaginal gel (MetroGel) 2) To assess the safety of LACTIN-V over 24 weeks by comparing the incidence of AEs between individuals randomized to LACTIN-V or placebo
Secondary Objectives	1) To investigate the colonization of LACTIN-V (presence of *L. crispatus* CTV-05 in the vaginal specimen) and fluctuations over 12 weeks, in relation to menses and sexual intercourse 2) To evaluate user acceptability and tolerability of LACTIN-V over 12 weeks, including perceptions around method of delivery and dosing 3) To measure long-term colonization of LACTIN-V at 24 weeks (12 weeks after last dosing) 4) To estimate the long-term efficacy of repeated doses of LACTIN-V (2 x 10^9 cfu/dose) as compared to placebo in preventing BV recurrence at 24 weeks (12 weeks after last dosing)
Exploratory Objective	To estimate the efficacy of repeated doses of LACTIN-V (2 x 10^9 cfu/dose) as compared to placebo in preventing repeated (more than one) episodes of BV recurrence up to 12 weeks following treatment of BV with MetroGel
Description of Study Design:	See schematic
Time to Complete Enrollment:	48 weeks

SCHEMATIC OF STUDY DESIGN:



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2.0 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Bacterial vaginosis (BV), characterized by an imbalanced vaginal flora deficient in naturally occurring acid-producing lactobacilli, is one of the most frequent vaginal infections and affects about 15–50% of reproductive aged women globally [1]. Many women are unaware of their condition. BV has been associated with significant gynecologic and obstetric complications, such as pelvic inflammatory disease [2], endometritis [3] and post-operative infections, including post-cesarean endometritis [4] and post-hysterectomy vaginal cuff cellulitis [5]. Strong associations have also been reported between BV and pre-term delivery, miscarriage [6], and amniotic fluid infections [7]. Studies have linked BV to both female HIV-1 acquisition, and female-to-male HIV transmission [8-10]. A recent study showed that the presence of lactobacilli decrease the odds for fetal inflammatory responses to placental colonization with pathogens [11]. Following standard antibiotic treatment of BV, 20–75% of women relapse within 1–3 months [12, 13]. The high risk of recurrence and sequelae suggests that investigational studies of new agents like live biotherapeutic products [14] may be effective for the improved treatment and prevention of BV.

Reconstituting a normal, *Lactobacillus*-predominant vaginal flora has been promoted for many years as a microbial defense against pathogens. The vaginal live biotherapeutic product *Lactobacillus (L.) crispatus* CTV-05 (LACTIN-V) was developed by Osel, Inc. in Mountain View, California, and is designed to replenish the vaginal lactobacilli population following conventional antibiotic treatment with MetroGel® (topical metronidazole gel 0.75%). The product contains a naturally occurring vaginal strain of *L. crispatus* CTV-05, preserved as a powder applied by a vaginal applicator. Since the *Lactobacillus* strain used in LACTIN-V is a commensal organism normally present in the vagina associated with vaginal health, the product has an excellent pre-clinical and clinical safety profile.

Early studies of LACTIN-V administered the product in gelatin-coated capsules. After the completion of a Phase I safety trial testing low doses of 10^6 and 10^8 cfu/dose or placebo, a Phase II multisite placebo-controlled trial tested a dose of 5×10^8 cfu of LACTIN-V administered in a gelatin capsule in 149 women with BV following standard antibiotic treatment. The product was dosed for 5 consecutive days followed by a weekly dose over 10 additional weeks. After 4 months, colonization efficiency in the LACTIN-V group reached 42% in the intent-to-treat (ITT) cohort and 59% in the according-to-protocol (ATP) cohort. Although not statistically significant, the time to first BV recurrence was longer in the LACTIN-V arm (118.7 days) compared to the placebo arm (98.7 days, $p = 0.37$). The proportion of women in the LACTIN-V treatment group successfully colonized with *L. crispatus* CTV-05 experienced fewer episodes of recurrent symptomatic BV (12.5%) than those who were not colonized (36%), or those in the placebo group (30%). The product had a good safety profile with mostly mild (Grade 1 severity) adverse events (AEs) including vaginal discharge, odor and pruritus, each affecting less than 40% of participants. AEs were evenly distributed between LACTIN-V and placebo arms (Osel, personal communication).

LACTIN-V has also been evaluated as a therapeutic agent to prevent recurrent urinary tract infections (rUTI). In a Phase II study in 100 women who received antibiotic treatment for cystitis, those who additionally received 10^8 cfu of LACTIN-V administered in a gelatin capsule over 5 consecutive days followed by a weekly dose over 10 additional weeks had a reduction in rUTI from 27% in the placebo arm to 15% in the LACTIN-V arm (response rate [RR] = 0.5, 95% confidence interval [CI] 0.2-1.2) in comparison to the placebo arm. Among women receiving LACTIN-V who had detected high levels of vaginal colonization with *L. crispatus*, the reduction of rUTI reached statistical significance (RR=0.07, 95% CI 0.02-0.3). Interestingly, high levels of pre-existing endogenous *L. crispatus* strains in women receiving placebo did not provide protection against rUTI [15].

Our research team at the University of California, San Francisco (UCSF) and Osel hypothesized that the efficacy of LACTIN-V to prevent BV recurrence could be improved by increasing the vaginal colonization rate of *L. crispatus* CTV-05. In order to achieve this, a vaginal applicator was developed capable of delivering a higher dose of LACTIN-V powder (up to 2 x 10^9 cfu/dose) directly to the vagina without the impediment of a gelatin capsule, which was found to dissolve slowly in the vaginal environment. The new dosage form was first studied in a small Phase I escalating dose trial at UCSF to assess safety, tolerability and acceptability of three doses [5 X 10^8 cfu/dose (150mg), 1 X 10^9 cfu/dose (300mg), 2 x 10^9 cfu/dose (600mg)] of LACTIN-V (IND No. 11363). Four healthy women were randomized 3:1 to receive LACTIN-V or placebo at each dose level. The product was found to be safe and well tolerated at all three doses. All AEs were Grade 1 and 2 severity and evenly distributed between LACTIN-V and placebo groups [16]. This study was followed by a Phase II-a trial of 24 women diagnosed with BV randomized 3:1 to LACTIN-V (2 x 10^9 cfu/dose) vs. an inert placebo administered daily for 5 days, and once weekly for 2 weeks. Participants completed a 5-day treatment course with MetroGel and within 24-72 hours initiated a regimen of high-dose LACTIN-V or inert placebo, administered once daily for 5 consecutive days and then once weekly over 2 additional weeks. After 4 weeks, colonization efficiency in the LACTIN-V group reached 61% (95%CI: 36%-83%) in the ITT cohort and 78% (95%CI: 40%-97%) in the ATP cohort [17]. We also explored the effect of endogenous lactobacilli and BV-associated bacteria on vaginal colonization with the exogenous *L. crispatus* CTV-05 using DNA extraction from vaginal swabs and bacterium-specific quantitative polymerase chain reaction (qPCR) assays. The median vaginal concentrations of BV-associated bacteria (*Megasphaera*, *Atopobium* spp., *Leptotrichia, Sneathia*, *Gardnerella (G.) vaginalis*, *Lactobacillus iners*, *Clostridium*-like bacteria, BVAB1-3) declined after metronidazole treatment. In participants who subsequently colonized with *L. crispatus* CTV-05, this trend was maintained throughout the study. Participants who did not colonize with CTV-05 saw a resurgence of *G. vaginalis*, *Leptotrichia/Sneathia* species, *A. vaginae,* and BVAB2 concentration during the course of the study. This provides supporting evidence that these bacteria are important in the pathogenesis of BV and may impede the success of treatment with both antibiotics and live biotherapeutic products [18].

Collecting a larger body of data on colonization and efficacy of unmodified *Lactobacillus* strains to prevent BV recurrence will greatly contribute to the efforts of developing unmodified as well as genetically modified *Lactobacillus* strains as a multipurpose

technology for the prevention of HIV-1 and other genital tract infections. In the completed Centre for the AIDS Programme of Research in South Africa (CAPRISA)-004 trial that tested vaginally delivered 1% tenofovir gel for HIV-1 prevention [19], participants with recurrent symptoms of vaginal irritation and inflammation had a higher risk of HIV-1 acquisition even in the 1% tenofovir gel arm (Karim SA, personal communication). In addition, a strong link has been shown between high-diversity cervicovaginal microbiota with low *Lactobacillus* levels and genital inflammation in a study of South African women. This genital inflammation was accompanied by increased numbers of activated HIV-infectable CD4+ cells in the cervix, providing a potential cellular link to increased HIV acquisition risk [20]. Thus, normalization of vaginal flora through use of exogenous vaginal *Lactobacillus crispatus*, and subsequent reduction of inflammation in the genital tract, could potentially enhance HIV-1 prevention associated with tenofovir gel use and other topical pre-exposure prophylaxis therapies under development [21].

In the Phase II-a clinical trial, 19 (79%) of 24 participants agreed or strongly agreed that they would use the product again indicating that satisfaction of the product was high. Four women with persistent symptomatic BV at study exit were significantly less satisfied with the product (p=0.03); only two of these four women would use the product again. Efficacy, improved vaginal health, and being a 'natural' alternative to antibiotics were mentioned as positive attributes, and messiness and leakage arose as a negative attribute of the product [17]. In order to address participant concerns for messiness and leakage, Osel has increased the potency of LACTIN-V. Thus, while 600mg of powder was used in the Phase II-a trial, a lesser amount of powder is required to deliver the same dose of CTV-05 (2×10^9 cfu). Overall, these results suggest that women are highly interested in live biotherapeutics and future studies testing these products should include an acceptability component with both quantitative and qualitative components to fully characterize women's satisfaction with the product.

CTV-05 is a strain of *L. crispatus*, a gram-positive rod isolated from the vagina of a healthy woman. *L.crispatus* is found naturally in the vaginas of healthy women and is commonly found as a component of the natural human intestinal flora. It is a facultative anaerobe, homofermentor of lactic acid, fastidious in its growth, and capable of H2O2 production. Unlike most commercially available strains of *Lactobacillus*, CTV-05 adheres well to vaginal epithelial cells and is capable of colonizing the vaginal epithelium.

LACTIN-V administered as a capsule has been tested in four trials (LV 001-004) with a dose level up to 5×10^8 cfu/capsule. Since the dose of 5×10^8 cfu/capsule resulted in colonization rates lower than desired, several changes to the study product were made. The higher dose of 2×10^9 cfu/dose delivered via vaginal applicator as dried powder directly into the upper vaginal vault has been tested in a Phase 1 trial (LV-005) in 12 healthy women and subsequently in a Phase II-a trial (LV-006) in 24 women with BV.

2.2 Rationale

This Phase II-b trial is designed to provide a screening evaluation for the hypothesis that, following a 5-day course of MetroGel to treat BV, LACTIN-V administered at 2×10^9 cfu/dose using a vaginal applicator reduces the 12-week incidence of BV recurrence by

≥50% when compared to placebo. Based on the achieved colonization rate of *L. crispatus* CTV-05 at 2 x 10^9 cfu/dose of LACTIN-V, coupled with prior data from the Phase II-a clinical trial suggesting that vaginal colonization with *L. crispatus* CTV-05 was associated with a reduced risk of BV recurrence, the next logical step is to perform a larger Phase II-b trial.

We plan to enroll 228 women in a 2:1 ratio of LACTIN-V to placebo to estimate the effectiveness of 2 x 10^9 cfu/dose of LACTIN-V to prevent the recurrence of BV following standard antibiotic treatment with MetroGel and establish longer term safety and acceptability data up to 24 weeks.

The study will be conducted at domestic sites with a known track record of recruiting participants in other BV studies to enable enrollment of all 228 women over 48 weeks. This will allow the study to complete study follow up 18 months after the first woman enrolls in the trial. This timeline assumes that all sites initiate enrollment at about the same time.

2.2.1 Rationale for BV Enrollment Criteria

We are planning to enroll women with untreated BV (asymptomatic or symptomatic) as diagnosed during the screening visit defined by at least three of the following four Amsel criteria AND a Nugent score ≥4 on a Gram stain in the laboratory using the Nugent scoring system.

The current 2015 CDC Sexually Transmitted Diseases Treatment Guidelines specify on page 69 that the clinical Amsel criteria can be used for the diagnosis of BV, and require three of the following symptoms or signs [22]:

- Homogeneous, thin, grayish-white discharge that smoothly coats the vaginal walls;
- Vaginal pH >4.5;
- Positive whiff-amine test, defined as the presence of a fishy odor when a drop of 10% potassium hydroxide (KOH) is added to a sample of vaginal discharge;
- Presence of clue cells (>20%) on microscopy.

Detection of three of these criteria has been correlated with results by Gram stain [23, 24].

The recent 2013 Food and Drug Administration (FDA) draft guidance for the use of metronidazole gel suggests all four Amsel criteria for inclusion, and states that a Nugent score of four or higher is recommended for inclusion [25].

Recent studies enrolling participants with BV since 2012 routinely combined Amsel criteria and Nugent scores for BV diagnosis at enrollment and follow up (see Table 2.2.1).

Table 2.2.1:

Author	Year	Enrollment Criteria		
		Amsel Only	Nugent Only	Combination of Amsel (A) and Nugent (N)
Bradshaw [26]	2013			≥3 A and N=4-10
Cruciani [27]	2013			≥3 A and N=4-10
Donders [28]	2013			≥3 A and N=4-10
Bradshaw [29]	2012			N = 7-10 or ≥3 A and N=4-10
Balkus [30]	2012			≥3 A and N=7-10
Weissenbacher [31]	2012	A=4		
Schwebke [32]	2011	A=4		
Marrazzo [33]	2012			≥3 A and N=4-10
Madhivanan [34]	2014			≥3 A and N=7-10

In practice intermediate (N=4-6) and high Nugent scores (N=7-10) are almost exclusively linked to Amsel criteria no lower than three. A study conducted by Schwebke et al [35] showed this and the data of our previous LV-006 Phase II-a study confirms this finding (Hemmerling 2010, subanalysis unpublished). For all 96 BV measurements (24 enrolled participants at four time points), Nugent scores of 4-10 correlated with Amsel criteria of 3 or 4. The same data set showed that whenever Amsel criteria reached 4 (19 of 96 measurements), the corresponding Nugent score was 8 or higher, never corresponding with lower intermediate Nugent score of 4-6.

After weighing recent FDA guidance, CDC guidelines, our data from previous studies, and reviewing recent BV enrollment practices of other studies, we propose to adopt the same enrollment criteria as the vast majority of recent studies: a combined requirement of at least 3 of 4 Amsel criteria and a Nugent score of 4-10.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

2.3.1.1 LACTIN-V and Placebo

The study product contains a naturally occurring strain of *L. crispatus* CTV-05. To date, there has been considerable amount of experience using LACTIN-V at concentrations up to 2×10^9 cfu/dose without any apparent related adverse effects, with the exception of a short duration of asymptomatic vaginal discharge. No serious adverse events have been reported in Protocol No. LV-001, LV-002 and LV-004 using LACTIN-V at 5×10^6 and 5×10^8 cfu/dose, and no subject left the protocol due to an adverse event. No type of severe or severe-related

adverse event has been experienced by more than one subject. In Protocol No. LV-003, using LACTIN-V at 5 x 10^8 cfu/dose, one subject in the placebo control substance group experienced three simultaneous SAEs unrelated to LACTIN-V. Two subjects in the LACTIN-V group discontinued study treatment due to AEs; one subject became pregnant and the other reported nausea and diarrhea, which required treatment.

Safety results of the recently completed Phase II-a trial (LV-006) assessing colonization efficiency, safety, tolerability and acceptability of LACTIN-V at 2 x 10^9 cfu/ dose in 24 women with BV show that no AE Grade 3 or higher and no SAE was observed during the clinical phase of the study. Based on the DAIDS Toxicity Table Addendum for Vaginal Microbicide Studies (November 2007) [36] designed to standardize AE assessment, a total of 120 total AEs were reported, 108 (90%) of which were Grade 1 and 12 (10.0%) were Grade 2 severity. The most common genitourinary AEs included vaginal discharge of study product (46%), abdominal pain (46%) and dysuria (21%). AEs were evenly distributed between LACTIN-V and placebo groups. All enrolled women (n=24) reported at least one AE. A single participant receiving placebo discontinued herself from study product due to a moderate AE (vaginal irritation).

Risks from the administration of LACTIN-V are low since colonization with this type of organism is strongly associated with improved vaginal health. No systemic risks are anticipated since this is an organism that is applied topically and not expected to be absorbed. These risks will be explained in the written informed consent form. No pregnant women, fetuses, prisoners, children, persons with an active STI or humans undergoing in vitro fertilization are included in this study.

Adverse effects that may be associated with LACTIN-V include those seen with other vaginally administered products, and include:
Most likely:

- Vaginal, genital or menstrual symptoms: vaginal discharge

Less likely:

- Gastrointestinal symptoms: abdominal (stomach area) pain, constipation, diarrhea, nausea, vomiting
- Urinary symptoms: needing to urinate urgently, needing to urinate at night and pain with urination
- Vaginal, genital or menstrual symptoms: genital itching, bleeding between menstrual periods, delayed menstrual periods, vaginal odor, vaginal burning sensation, vaginal irritation, vaginal bleeding, vaginal dryness, genital swelling, rash, vaginal candidiasis (yeast infection)
- Other symptoms: lower back pain

Rare but potentially life-threatening:

- Allergic reaction (including anaphylaxis) to the study product

2.3.1.2 MetroGel vaginal gel

MetroGel vaginal gel is indicated in the treatment of bacterial vaginosis. It is contraindicated in patients with a prior history of hypersensitivity to metronidazole, parabens, other ingredients of the formulation, or other nitroimidazole derivatives.

Rare but serious*

- *Convulsive Seizures and Peripheral Neuropathy:* Convulsive seizures and peripheral neuropathy, the latter characterized mainly by numbness or paresthesia of an extremity, have been reported in patients treated with oral or intravenous metronidazole. The appearance of abnormal neurologic signs demands the prompt discontinuation of metronidazole vaginal gel therapy. Metronidazole vaginal gel should be administered with caution to patients with central nervous system diseases.
- *Psychotic Reactions:* Psychotic reactions have been reported in alcoholic patients who were using oral metronidazole and disulfiram concurrently. Metronidazole vaginal gel should not be administered to patients who have taken disulfiram within the last two weeks.

**MetroGel vaginal gel affords minimal peak serum levels and systemic exposure (AUCs) of metronidazole compared to 500 mg oral metronidazole dosing. Although these lower levels of exposure are less likely to produce the common reactions seen with oral metronidazole, the possibility of these and other reactions cannot be excluded presently. Data from well-controlled trials directly comparing metronidazole administered orally to metronidazole administered vaginally are not available.*

2.3.1.3 Study Procedures

Participants may experience discomfort when having pelvic exams and/or undergoing phlebotomy for this study. During phlebotomy, they also may feel dizzy or faint, or develop a bruise, swelling or infection where the needle is inserted.

2.3.1.4 Disclosure of Sexually Transmitted Infections

Participants may become embarrassed, worried, or anxious when receiving HIV and STI counseling. They may become worried or anxious while waiting for their HIV and STI test results.

2.3.1.5 Procedures for Minimizing Potential Risks

Allergic reactions (including anaphylaxis): Participants with a known allergy to components of the study product will be excluded from enrollment. If an allergy or sensitivity occurs during the course of the study, the participant will be advised to immediately discontinue study product use and seek medical attention. This event will be recorded on an AE form.

STI testing: Participants will receive counseling prior to testing. Counselors will prepare them for possible feelings of anxiety and the ramifications of a positive test result. All results will be kept confidential by the study physician/nurse and will be maintained in a limited access database.

2.3.2 Known Potential Benefits

There may be no direct benefits to participants in this study. However, participants and others may benefit in the future from information learned from this study. Information learned in this study may lead to the development of a safe and effective live biotherapeutic product that can prevent the recurrence of BV. Participants will have HIV testing and pelvic exam performed during screening. Participants will be screened for a number of STIs and a prescription will be provided, if applicable, following CDC STD Treatment Guidelines. Participants testing positive for BV during screening will receive study-supplied MetroGel.

3.0 OBJECTIVES

3.1 Study Objectives

3.1.1 Primary Objectives

1) To estimate the efficacy of repeated doses of LACTIN-V (2 x 10^9 cfu/dose) as compared to placebo in preventing BV recurrence by 12 weeks following treatment of BV with MetroGel
2) To assess the safety of LACTIN-V over 24 weeks by comparing the incidence of AEs between individuals randomized to LACTIN-V or placebo

3.1.2 Secondary Objectives

1) To investigate the colonization of LACTIN-V (presence of *L. crispatus* CTV-05 in the vaginal specimen) and fluctuations over 12 weeks, in relation to menses and sexual intercourse
2) To evaluate user acceptability and tolerability of LACTIN-V over 12 weeks, including perceptions around method of delivery and dosing
3) To measure long-term colonization of LACTIN-V at 24 weeks (12 weeks after last dosing)
4) To estimate the long-term efficacy of repeated doses of LACTIN-V (2 x 10^9 cfu/dose) as compared to placebo in preventing BV recurrence at 24 weeks (12 weeks after last dosing)

3.1.3 Exploratory Objective

To estimate the efficacy of repeated doses of LACTIN-V (2 x 10^9 cfu/dose) as compared to placebo in preventing repeated (more than one) episodes of BV recurrence up to 12 weeks following treatment of BV with MetroGel

3.2 Study Outcome Measures

3.2.1 Primary Efficacy Outcome Measure

The proportion of participants with a positive BV diagnosis in each study arm by Visit 4 (Week 12, Day 84)

BV recurrence is defined by Amsel criteria 3-4 and Nugent score 4-10. Following FDA draft guidance all BV diagnoses during follow-up visits are considered incident, as they occur at least 22-30 days after the commencement of MetroGel treatment and consequently are treatment failures or new infections [25]. For the purpose of this trial, treatment failure and new infection will both be considered recurrent BV.

3.2.2 Primary Safety Outcome Measure

The proportion of participants reporting product-related AEs and SAEs in each study arm through Visit 7 (Week 24, Day 168)

3.2.3 Secondary Efficacy Outcome Measures

Secondary Outcome Measure 1
The proportion of participants experiencing successful colonization with *L. crispatus* CTV-05 following dose of study product through Visit 4 (Week 12, Day 84) in the LACTIN-V arm, overall and by occurrence of menses and intercourse

Secondary Outcome Measure 2
The proportion of participants experiencing successful colonization with *L. crispatus* CTV-05 following dose of study product at Visit 7 (Week 24, Day 168) in the LACTIN-V arm, overall and by occurrence of menses and intercourse

Secondary Outcome Measure *3*
Tolerability of LACTIN-V and the applicator will be measured by:
1. The proportion of participants who discontinue study product early in each study arm due to adverse events

Acceptability of LACTIN-V and the applicator will be measured by:
1. The proportion of participants who are compliant with the complete dose regimen in each study arm as assessed by participant reporting and applicator staining
2. Self-administered questionnaires about acceptability of the study product in each study arm

Secondary Outcome Measure 4
The proportion of participants with a positive BV diagnosis in each study arm by Visit 7 (Week 24, Day 168)

Exploratory Outcome Measure
The statistical parameterizations of the exploratory objective will include, but are not limited to, the following:
1. The number of BV recurrences per 12 weeks of follow-up
2. The probability of BV recurrence during the 12 weeks of follow-up

4.0 STUDY DESIGN

This is a Phase II-b multicenter randomized double-blind placebo-controlled trial to assess the efficacy of repeated doses of LACTIN-V compared to placebo in preventing BV recurrence in women diagnosed with BV. The study will also assess the safety of LACTIN-V by comparing the incidence of AEs between women randomized to LACTIN-V or placebo. The study plans to enroll 228 sexually-experienced, pre-menopausal women age 18 to 45 years. Women will be randomized 2:1 to receive LACTIN-V or placebo.

Women will be consented and will have a history and physical examination to include a pelvic exam with STI diagnostics and testing for BV. Potentially eligible women with Amsel criteria ≥3 will have a vaginal smear sent to a central laboratory for Gram stain evaluation by Nugent score. Blood will be obtained for HIV and syphilis serologies, a vaginal swab for gonorrhea, chlamydia and trichomonas molecular testing, and urine for βhCG. Potentially eligible women will start a standard 5-day course of MetroGel.

Women will return to the study clinic within 2 days after completing the 5-day course of MetroGel to re-evaluate eligibility criteria and review the BV test results from the screening visit. Women with Amsel criteria ≥3 and Nugent score 4-10 will be instructed to administer the LACTIN-V or placebo at home for 5 consecutive days and then twice weekly for 10 weeks. LACTIN-V (or placebo) will be administered at 2×10^9 cfu/dose by vaginal applicator.

Women will complete a memory aid to record times of product application, menses, sexual intercourse, concomitant medications, condom use and any AEs. Reminders will be sent to women in order to increase adherence with all doses and prior to scheduled follow-up visits. Women will complete either a paper or electronic memory aid. If the electronic version of the memory aid is utilized by the participant, the study clinic will be alerted of any potential AEs reported by the participant for follow-up and reporting as deemed necessary.

Follow-up visits are scheduled 4, 8, 12 and 24 weeks after enrollment. A memory aid to gauge symptoms and AEs will be reviewed, a questionnaire to collect clinical, including AEs, data will be administered and a pelvic exam will be performed to detect genital tract AEs. Vaginal swabs for wet mount evaluation (Amsel criteria) and Gram stain evaluation (Nugent score) will be collected. A vaginal swab for *L. crispatus* (including CTV-05) identification will be collected. Vaginal applicators will be collected at Visits 2, 3, and 4 and stained with Trypan Blue upon return to the site as a proxy for product use. Telephone interviews will be scheduled between the 12 and 24 week visits, at 16 and 20 weeks after enrollment.

5.0 STUDY ENROLLMENT AND WITHDRAWAL

The study plans to enroll 228 non-pregnant, pre-menopausal women age 18 to 45 years diagnosed with symptomatic or asymptomatic BV. Eligibility criteria for participation in the study are described in detail in sections 5.1 and 5.2. A screening consent form will be used for procedures to determine eligibility and an enrollment consent form will be used for study participation if eligibility is confirmed. Pregnant women, men and children are excluded from study participation (see section 14.4).

5.1 Subject Inclusion Criteria

Participants must meet all of the inclusion criteria in order to be eligible to participate in the study:

1. Capable of reading and writing English and voluntarily provide written informed consent to participate in the study and comply with all study procedures
2. Untreated BV (asymptomatic or symptomatic) as diagnosed during the screening visit defined by ≥3 Amsel criteria

   Note: Amsel criteria include the following:

   - Homogeneous, thin, grayish-white discharge that smoothly coats the vaginal walls;
   - Vaginal pH >4.5;
   - Positive whiff-amine test, defined as the presence of a fishy odor when a drop of 10% potassium hydroxide (KOH) is added to a sample of vaginal discharge;
   - Presence of clue cells (>20% on microscopy).
3. Untreated BV (asymptomatic or symptomatic) as confirmed in the laboratory using the Nugent scoring system (Nugent Score ≥4)
4. Otherwise healthy pre-menopausal women 18–45 years of age on the day of screening
5. Regular predictable menstrual cycles or amenorrheic for at least 3 months due to use of a long-acting progestin or continuous use of oral contraceptives
6. Willing to be asked questions about personal medical health and sexual history
7. Willing to apply study agent vaginally and comply with study examinations
8. Agree to abstain from sexual intercourse during the first 5 consecutive days of study product administration, 12 hours prior to study visits and for 12 hours after each study product application
9. Agree to abstain from the use of any other intravaginal product throughout the trial period from the time of screening through Visit 7 (Week 24, Day 168)

   Note: Intravaginal products include contraceptive creams such as Gynol II, gels, foams, sponges, lubricants not approved by the study investigators, and douches. Limit use of tampons during menstruation to unscented products.
10. Must be of non-childbearing potential or if of childbearing potential, must agree to use a reliable method of birth control for the duration of the study

    Note: Reliable methods of birth control include tubal ligation, male partner with a vasectomy, a steroidal contraceptive (oral, patch, injectable or implantable), IUD, condoms or abstinence.

5.2 Subject Exclusion Criteria

Participants meeting any of the following criteria at screening, or when assessed at the enrollment visit, will be excluded from the study:

1. Urogenital infection at screening
   Note**:** Urogenital infection includes urinary tract infection, *Trichomonas (T.) vaginalis*, *Neisseria (N.) gonorrhoeae*, *Chlamydia (C.) trachomatis*, *Treponema (T.) pallidum*, or vulvo-vaginal candidiasis.
2. Diagnosis of two or more outbreaks of *N. gonorrhoeae*, *C. trachomatis*, *T. pallidum*, *T. vaginalis,* or herpes simplex virus (herpes genitalis) within 6 months prior to screening
3. Positive for syphilis or HIV at screening
4. Current pregnancy or within 2 months of last pregnancy and/or currently breastfeeding**
5. Vaginal or systemic antibiotic or antifungal therapy (other than MetroGel given as part of study procedures) within 21 days of screening or within 30 days of enrollment**
6. Use of disulfiram within past 2 weeks or other contraindication to use of MetroGel**
7. Any condition requiring regular periodic use of systemic antibiotics during participation in the trial
8. Active genital herpes lesion (if not resolved by enrollment)**
9. Investigational drug use other than LACTIN-V within 30 days or 10 half-lives of the drug, whichever is longer, of enrollment visit**
10. Other planned participation in an investigational drug study while participating in this study**
11. Menopause defined as more than 12 consecutive months of amenorrhea without another known cause including pregnancy
12. IUD insertion or removal, pelvic surgery, cervical cryotherapy or cervical laser treatment within the last 2 months prior to screening
13. Use of vaginal ring (eg, NuvaRing) within 3 days of screening or during the course of the study**
14. Failure to complete 5 days of MetroGel with the last dose taken no later than 48 hours prior to randomization***
15. Use of new long-acting hormonal treatments. Participant may be enrolled if stable (>3 months) on existing therapy as determined by the principal investigator**
16. Known allergy to any component of LACTIN-V/placebo or MetroGel or to nitroimidazole derivatives or latex (condoms)
17. Any social, medical, or psychiatric condition including history of drug or alcohol abuse that in the opinion of the investigator would make it unlikely for the participant to comply with the study

** Note: Criteria will be assessed at screening and enrollment.
*** Note: Criteria will be assessed at enrollment.

5.3 Treatment Assignment Procedures

5.3.1 Randomization Procedures

Women will be randomized at the enrollment visit 1 upon completion of the initial standardized antibiotic treatment with 0.75% topical MetroGel.

Women will be randomly assigned to receive LACTIN-V at 2 x 10^9 cfu/dose or placebo in a 2:1 ratio. The 2:1 randomization was selected to increase precision of the estimated effectiveness of LACTIN-V to prevent recurrence of BV 12 weeks after treatment with MetroGel.

The list of randomized treatment assignments will be prepared by statisticians at The Emmes Corporation and provided to Fisher Bioservices. Fisher Bioservices will label each box of study treatment applicators sequentially with a treatment number according to the randomization scheme in a 2:1 (LACTIN-V to placebo) ratio.

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

5.3.2 Masking Procedures

At the time of enrollment, the site pharmacist will select the next available box of study product applicators in sequential order and will distribute to masked study personnel with no labels that identify the product or applicators as LACTIN-V or placebo. No masking procedures are required for the MetroGel.

The site pharmacist, participants, study personnel who perform study assessments, data entry personnel at the sites, and laboratory personnel performing study assays will be masked to treatment assignment. The Data and Safety Monitoring Board (DSMB) may receive data in aggregate and presented by treatment group, but without the treatment group identified. The DSMB may be unmasked to individual study treatment assignments, as needed, to adequately assess safety issues. Refer to the MOP for unmasking procedures, including emergency unblinding procedures.

5.3.3 Reasons for Withdrawal

5.3.3.1 Screening Failures

Participants with the following diagnoses at the screening visit (Visit 0) will be considered screening failures and will be treated or referred for appropriate follow-up. All screening failures will be replaced unless the diagnosis allows for Rescreening.

- A diagnosis of a UTI, yeast vaginitis or an STI (*T. vaginalis*, *N. gonorrhoeae*, *C. Trachomatis, T.pallidum),* or vulvo-vaginal candidiasis*)*
- Any diagnosis other than BV requiring antibiotics

A history of herpes genitalis does not exclude participants from participation as long as no active lesions are present at the enrollment visit and the participant experienced no more than one active outbreak of herpes genitalis within 6 months prior to screening.

Participants diagnosed with a UTI at the screening visit (Visit 0) may choose to return to clinic for rescreening at least 21 days after completing antibiotic treatment. Participants diagnosed with an STI or participants with yeast vaginitis at the screening visit may be re-screened at least 21 days after completing antibiotic treatment. Participants with active genital herpes lesions at enrollment are ineligible but can be re-screened after the outbreak has subsided. All screening procedures must be repeated unless otherwise clinically indicated.

Participants who are eligible after screening but are subsequently deemed ineligible at the enrollment visit or do not return for the enrollment visit are considered screen-failures.

5.3.3.2 Withdrawal after Enrollment

Participants may withdraw or be withdrawn for any of the reasons given below. The reason for withdrawal will be recorded in the data collection form.

- Participant withdraws consent
- Pregnancy or breastfeeding
- Adverse event which requires discontinuation of the treatment regimen or results in inability to comply with study procedures
- Discretionary decision by the site investigator
- At the discretion of the IRB, FDA, NIH, or other government agencies as part of their duties to ensure that research participants are protected, or the industry supporter or its designee
- Study is terminated

5.3.4 Handling of Withdrawals

All randomized participants who are discontinued from receiving further study product will continue to be followed through Visit 7 (Week 24, Day 168). Once a participant is randomized, she will not be replaced. Withdrawals and drop-outs will not be replaced.

If withdrawal of consent or study product discontinuation occurs after study treatment is initiated, the participant will be asked to continue scheduled study procedures including safety 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 withdraw their consent for further participation in the study after their study treatment ends or discontinue study product early will be reminded of the importance of continuing in the study for safety evaluations. Participants will be encouraged to complete the Early Termination evaluations described in section 7.9 if they choose not to complete the remaining study visits.

Participants who enroll in the study but do not return for study visits after a minimum of three attempts to contact them over a 2-week period will be considered lost to follow-up.

5.3.5 Termination of Study

Study closure may occur due to DSMB review, or at the discretion of the Institutional Review Board (IRB), FDA, DMID, and other government agencies as part of their duties to ensure that research participants are protected or the industry supporter or its designee.

6.0 STUDY PRODUCT

6.1 Study Product Description

LACTIN-V *(Lactobacillus crispatus* CTV-05)
LACTIN-V contains a naturally occurring vaginal strain of *Lactobacillus (L.) crispatus* CTV-05, preserved as a powder and applied by a vaginal applicator.

MetroGel vaginal gel (metronidazole gel)
MetroGel vaginal gel is the intravaginal dosage form of the synthetic antibacterial agent, metronidazole, USP at a concentration of 0.75%. Metronidazole is a member of the imidazole class of antibacterial agents and is classified therapeutically as an antiprotozoal and antibacterial agent. Chemically, metronidazole is a 2-methyl-5-nitro imidazole-1-ethanol.

At the Screening visit, eligible participants will receive MetroGel for the treatment of BV as described in section 7.0. Participants who receive MetroGel, but do not complete the full MetroGel treatment, do not return for the Enrollment visit, or are otherwise found ineligible to be randomized to study treatment will not be enrolled into the study and will not be followed for safety or for AEs.

6.1.1 Acquisition

LACTIN-V and placebo
Osel, Inc. will provide LACTIN-V and placebo. Upon request by DMID, LACTIN-V and placebo will be transferred to the following address:

DMID Clinical Agents Repository (CAR)
Fisher BioServices
20439 Seneca Meadows Parkway
Germantown, MD 20876
Tel: (240) 477-1350
Fax: (240) 477-1360
Email: DMID.CAR@ThermoFisher.com

MetroGel vaginal gel
MetroGel vaginal gel will be purchased and supplied by the DMID CAR.

LACTIN-V, placebo and MetroGel vaginal gel will be shipped from the DMID CAR to the study site upon request and approval by DMID.

6.1.2 Formulation, Packaging, and Labeling

LACTIN-V and placebo
Each applicator will contain a LACTIN-V powder formulation containing *L. crispatus* CTV-05 at a potency of 2 x 10^9 cfu/dose. [redacted]. A

matching placebo formulation without *L. crispatus* CTV-05 is supplied in an identical applicator containing placebo powder.

LACTIN-V or placebo pre-filled applicators will be individually packaged in heat-sealed foil pouches, each containing one desiccant packet. Each participant will receive one box containing 25 applicators. Each foil pouch and box will be labeled with the study number and unique participant number.

The foil pouches and boxes will be labeled by the Osel Study Product Supplier. Each label will contain the study number and the FDA-required cautionary statement *"Caution- New drug -Limited by Federal Law to Investigational Use."* The label of each box of study product applicators will also include the unique participant number and the statements: "*Store at home in a cool, dry place out of reach of children (at room temperature)."*

MetroGel vaginal gel
MetroGel vaginal gel is a gelled purified water solution, containing metronidazole at a concentration of 7.5 mg/g (0.75%). The gel is formulated at pH 4.0. The gel also contains carbomer 934P, edetate disodium, methylparaben, propylparaben, propylene gycol and sodium hydroxide.

MetroGel vaginal gel is supplied in a 70 gram tube. Each participant will receive one box of MetroGel vaginal gel sufficient for the 5-day course of treatment.

6.1.3 Product Storage and Stability

LACTIN-V and placebo

LACTIN-V and placebo applicators will be shipped overnight in an appropriate container on frozen gel packs and immediately placed into temperature-controlled conditions (2–8°C) upon arrival.

The study sites will store LACTIN-V and placebo applicators at 2-8°C.

Participants will be instructed to store study product boxes in a cool, dry place, out of reach of children.

MetroGel vaginal gel
Store at controlled room temperature 15° to 30°C (59° to 86°F). Protect from freezing.

6.2 Dosage, Preparation, and Administration of Study Product

LACTIN-V and placebo
Eligible participants will be instructed on how to insert the pre-filled applicator (LACTIN-V at 2 x 10^9 cfu/dose, or the placebo control substance) and will administer the first dose under direct supervision by the study clinician before leaving the clinic (Day 1). Study personnel will verify that each carton contains all 24 remaining applicators and this will be recorded on the appropriate Case Report Form (CRF). Participants will be instructed to administer one dose once a day at bedtime for the next 4 days (beginning on Day 2), and then two times a week for 10 weeks. The remaining 20 applicators will be used for the two doses each week during weeks 2 through 11.

MetroGel vaginal gel
Participants will be instructed to administer one dose once a day at bedtime for 5 days initiated as soon as possible (ideally within 5 days of diagnosis at the screening or follow-up visit for symptomatic participants, or within 24 days of the screening visit for asymptomatic participants (see section 7.0 for additional details).

6.3 Accountability Procedures for the Study Investigational Product

After receipt of the study product, the site Principal Investigator (PI) is responsible for distribution and disposition of these study products, and has ultimate responsibility for drug accountability. The PI may delegate this responsibility to the site pharmacist (or designee). The site pharmacist (or designee) must maintain study product records and document logs of receipt, accountability, and storage temperature conditions. These study product accountability and dispensing logs must be maintained in the study file. Upon completion of the study and after the final monitoring visit, unused applicators will be retained until monitored and released for disposition as per the Sponsor. Used LACTIN-V or placebo applicators will be collected and stained to determine if the participant used the study product and then disposed of after evaluation. For detailed information regarding final disposition of study product see the protocol-specific MOP.

6.4 Assessment of Subject Compliance with Study Product

Compliance with study product will be assessed by participant self-report and applicator staining as described in the MOP.

6.5 Concomitant Medications/Treatments

The study requires documentation of all medications (name, dose, route, frequency of dosing, and reason for use) taken by participants 30 days prior to screening through Visit 7 (Week 24, Day 168) or early termination, whichever occurs first.

The following medications are permitted throughout the study:

- Vitamins and other nutritional supplements
- Hormonal contraceptives

- Cough medicine
- Non-steroidal anti-inflammatory drugs
- Prescription and over-the counter medications for allergies and asthma
- Herbal, naturopathic, and traditional preparations (eg, Chinese traditional medications)
- Externally applied topical medications (except genital area)
- Antibiotics prescribed during the trial, including for MetroGel for BV

The following medications are prohibited throughout the study, and participants will be instructed to not use them throughout the study up to Visit 7 (Week 24, Day 168):

- Immune suppressants
- Investigational drug preparations other than the study product
- Intravaginal medications/preparations and topical medications/preparations other than the study product applied to the external genitalia

Any other medication/treatment not listed as permitted or prohibited is subject to the judgment of the investigator.

7.0 STUDY SCHEDULE

7.1 Screening Visit 0 (Day -30 to Day -5)

The purpose of the screening visit is to identify participants who satisfy all eligibility criteria, including the diagnosis of symptomatic or asymptomatic bacterial vaginosis. Participants who agree to be screened will be asked to sign the screening informed consent form that will describe the purpose of the screening, the procedures to be followed, and the risks and benefits of screening and will include a separate section for consent to store samples for future use. A copy of the consent form will be given to the participant and this fact will be documented in the medical record. A separate consent form will be completed during the enrollment visit after study eligibility is established.

Participants will be asked to provide contact information (ie, address, email address, home/cell phone number, emergency contact), which will be recorded on appropriate form.

At the screening visit (Visit 0) the woman's menstrual cycle history will be evaluated to ensure regular menstrual cycles (or amenorrhea due to long-acting contraceptives). Depending on the timing of her menstrual cycle, the screening procedure will be as follows:

1. If she has just finished her menstrual cycle or is within the first half of her cycle with 12 days or more expected until the onset of the next menstrual cycle, (latest screening visit on the 16th day of a regular 28-day cycle), she will proceed with screening, and will be able to complete screening, the treatment course of BV with 5 days of topical MetroGel, the Enrollment Visit (Visit 1) (in case of eligibility confirmed by the laboratory results of the screening visit) within 48 hours of her last MetroGel administration, and the 5 days of continuous product use starting during the enrollment visit (Visit 1).
2. If she is menstruating at the time of the screening visit she will be asked to return for the screening visit as soon as possible after her menstrual cycle ends.
3. If she is in the second half of her cycle with less than 12 days expected before the onset of the next menstrual bleeding, she will proceed with screening, but will be instructed to wait until after her next period before starting the 5-day treatment course of MetroGel.

The screening visit must also be timed depending on her availability to return for the enrollment visit (visit 1) within 12-48 hours after the termination of the 5-day course of MetroGel. MetroGel treatment for women with symptomatic BV should be initiated as soon as possible (ideally within 5 days of diagnosis at the screening visit). For women diagnosed with asymptomatic BV at the screening visit, MetroGel should be initiated according to her menstrual cycle and within 24 days of the screening visit to ensure the time window between screening and enrollment is not more than 30 days.

Women must start MetroGel treatment on a Tuesday, Wednesday, Thursday, Friday or Saturday (NOT Sunday or Monday) in order to return for enrollment and within 12-48 hours after the 5th dose of her MetroGel treatment on the subsequent weekday Monday – Friday.

Table 7.1

TUE	WED	THU	FRI	SAT	SUN	MON	TUE	WED	THU	FRI	SAT	SUN	MON	TUE
5 day MetroGel					Break 12-48 hours	Enrollment 5 days study product								
	5 day MetroGel					Break 12-48 hours	Enrollment 5 days study product							
		5 day MetroGel					Break 12-48 hours	Enrollment 5 days study product						
			5 day MetroGel					Break 12-48 hours	Enrollment 5 days study product					
				5 day MetroGel					Break 12-48 hours	Enrollment 5 days study product				

Participants who give informed consent will undergo the following procedures and assessments at the Screening Visit:

1. Assessment of eligibility
2. Detailed structured interview to obtain demographic information, medical, gynecological and sexual history to include assessment of STI and HIV risk
3. Review of concomitant medications
4. Acceptability questionnaire to determine participant's experiences with BV and use of vaginal products in the past
5. Complete physical and pelvic examination by a study physician or nurse that will include vital signs, including temperature, height and weight, and examination of respiratory and cardiovascular systems
6. The pelvic examination will be conducted in the following order:
   a. Examination of the external genitalia
   b. Speculum insertion and examination of vagina, fornices and cervix with naked eye. Water will be used to ease speculum insertion. No lubricant should be used as it could contain chlorhexidine which may kill *Lactobacillus*.
   c. Vaginal swab for vaginal pH
   d. Vaginal swab for wet mount taken from the posterior fornix for trichomoniasis, yeast vaginitis, and clue cells and for amine test (whiff test)
   e. Vaginal swab for Gram stain (Nugent scoring system)
   f. Vaginal swabs (no more than two) for storage and future identification of vaginal bacteria, and possible multiomic (e.g. proteomics, metabolomics, etc.) testing
   g. Cervical (or vaginal) swabs for nucleic acid amplification of *N. gonorrhoeae, C. trachomatis*, and *T. vaginalis*
7. Clean catch urine dipstick; urinalysis if abnormal results indicate
8. Rapid urine βhCG pregnancy test. If the test is positive, the screening visit will be terminated as the woman is not eligible.
9. Blood draw for Syphilis serology testing and HIV testing by standardized algorithms
10. If the examiner suspects or confirms a UTI, vulvo-vaginal candidiasis, or an STI (*T.vaginalis*, *N. gonorrhoeae*, *C.trachomatis, T. pallidum),* the woman will be referred to standard of care treatment and may be invited for rescreening (see section 7.1.1).
11. All clinical and laboratory findings at the time of the evaluation will be recorded on the CRF. These findings will include observations present on the pelvic exam, such

as genital findings including location; presence or absence and character of vaginal or cervical discharge or lesions; and results of any on-site tests.

12. Participants diagnosed with BV by Amsel criteria ≥3 who agree to be treated with MetroGel and meet all eligibility criteria will be invited to return for the enrollment visit immediately after the completion of MetroGel, pending confirmation of BV diagnosis by Nugent score 4-10 and laboratory test results for STIs and HIV.
13. Participants with confirmed BV by Amsel criteria ≥3 will be given 0.75% topical MetroGel to apply vaginally once daily in the evening for 5 consecutive days. Should the woman be in the first half of her menstrual cycle and her period is not expected for the next 12 days, she should start MetroGel on the evening of the screening visit. It also needs to be timed in accordance with her availability to return for the enrollment visit (Visit 1) within 12-48 hours after the completion of the 5-day course of MetroGel. See scheduling sequences in Table 7.1. MetroGel treatment for women with symptomatic BV should be initiated as soon as possible (ideally within 5 days of diagnosis at the Screening Visit). For women diagnosed with asymptomatic BV at the screening visit, MetroGel treatment needs to be initiated according to her menstrual cycle and within 24 days of the screening visit to ensure the time window between screening and enrollment is not more than 30 days.
14. Participants will be advised to return for the enrollment visit within 12-48 hours after completion of MetroGel treatment.
15. Non-spermicidal condoms will be dispensed.

At screening, women with evidence of any STI diagnosed according to the study site's protocol will be referred to standard of care treatment in compliance with current CDC STD Treatment Guidelines. If laboratory test results received after the screening visit determine ineligibility, the woman will be managed as clinically indicated for her respective condition and contacted to cancel her scheduled enrollment visit. She will not be eligible for rescreening until 21 days after resolution of the abnormality (see section 7.1.1).

7.1.1 Rescreening Visit

At least 21 days after completion of successful treatment for UTI or STI, participants will be invited for rescreening. At rescreening, the following procedures will be repeated to determine the diagnosis of bacterial vaginosis and eligibility for enrollment into the study.

1. Detailed structure interview to obtain demographic information and medical, gynecological and sexual history to include assessment of STI and HIV risk
2. Review of concomitant medications
3. Complete physical and pelvic examination by a study physician or nurse that will include vital signs, including temperature, height and weight, and examination of respiratory and cardiovascular systems
4. The pelvic examination will be conducted in the following order:
    a. Examination of the external genitalia
    b. Speculum insertion and examination of vagina, fornices and cervix with naked eye. Water will be used to ease speculum insertion. No lubricant

should be used as it could contain chlorhexidine which may kill *Lactobacillus.*

c. Vaginal swab for vaginal pH
d. Vaginal swab for wet mount taken from the posterior fornix for trichomoniasis, yeast vaginitis, and clue cells and for amine test (whiff test)
e. Vaginal swab for Gram stain (Nugent scoring system)
f. Vaginal swabs (no more than two) for storage and future identification of vaginal bacteria, and possible multiomic (e.g. proteomics, metabolomics, etc.) testing
g. Cervical (or vaginal) swabs for nucleic acid amplification of *N. gonorrhoeae, C. trachomatis*, and *T. vaginalis*

5. Clean catch urine dipstick; urinalysis if abnormal results indicate
6. Rapid urine βhCG pregnancy test
7. Blood draw for Syphilis serology testing and HIV testing by standardized algorithms if re-screening occurs more than 90 days after initial screening visit

7.2 Enrollment Visit 1 (Day 1)

Only women who are diagnosed at the screening visit with BV (by Amsel criteria ≥3 and have a Nugent score 4-10 on the vaginal specimen collected at the screening visit) and complete a 5-day course of MetroGel are eligible to enroll in the study. These women will be scheduled to return for the enrollment visit within 12-48 hours after completing the 5th dose of MetroGel treatment.

Once eligibility is determined from screening tests, the woman will be asked to sign the enrollment consent form which will describe the purpose of the study, the procedures to be followed, the risks and benefits of the study, and will include a separate section for consent to store samples for future use. A copy of the consent form will be given to her and this fact will be documented in her medical record.

After signing the enrollment consent form, she will be asked to verify previously reported contact information which will be recorded on the appropriate form.

At the enrollment visit, her menstrual cycle history will be re-evaluated to ensure regular menstrual cycles (or amenorrhea with long-acting contraceptives) and that menstruation will not be expected during the next 5 days when the use of the study product for 5 consecutive days (days 1-5) has to occur. If she is not currently having menstrual bleeding and her period is not to be expected within the next 5 days, she will proceed with the enrollment visit. Before commencing with the enrollment exam, eligibility should be confirmed by reviewing the medical history and ALL results of the screening visit (including all laboratory results which must be complete). In addition, it must be confirmed that she completed MetroGel treatment on 5 consecutive days, and that the 5th dose was administered within 48 hours before the enrollment visit.

If still eligible, participants will undergo the following procedures at the enrollment visit (Day 1):

1. Detailed confirmation of demographic information, medical, gynecological and sexual history obtained to include assessment of STI and HIV risk
2. Review of concomitant medications
3. Symptom-directed physical and pelvic examination by a study physician or nurse that will include vital signs, including temperature, height and weight, and examination of respiratory and cardiovascular systems
4. The pelvic examination will be conducted in the following order:
   a. Examination of the external genitalia
   b. Speculum insertion and examination of vagina, fornices and cervix with naked eye. Water will be used to ease speculum insertion. No lubricant should be used as it could contain chlorhexidine which may kill *Lactobacillus*.
   c. Vaginal pH
   d. Vaginal swab for wet mount taken from the posterior fornix for yeast vaginitis and clue cells and for amine test (whiff test)
   e. Vaginal swab for Gram stain (Nugent scoring system)
   f. Vaginal swabs (no more than two) for storage and future identification of vaginal bacteria, and possible multiomic (e.g. proteomics, metabolomics, etc.) testing
   g. Vaginal swabs for *L. crispatus* identification with qPCR
      If indicated, cervical (or vaginal) swabs for nucleic acid amplification of *N. gonorrhoeae, C. trachomatis*, and *T. vaginalis*

      Women with findings on pelvic exam at the enrollment visit, including active genital herpes lesions or for other reasons will be considered screening failures, will not be enrolled and will be referred for further evaluation.
5. If indicated, clean catch urine dipstick; urinalysis if abnormal results indicate
6. Rapid urine βhCG pregnancy test. If the test is positive, the enrollment visit will be terminated as the woman is not eligible.
7. All clinical and laboratory findings at the time of the evaluation will be recorded on the CRF. These findings will include observations present on the pelvic exam such as genital findings including location; presence or absence, and character of vaginal or cervical discharge or lesions; and results of any on-site tests.
8. If the clinician suspects the participant developed an STI since the screening visit, appropriate tests will be conducted and if needed the woman will be referred to standard of care treatment in compliance with current CDC STD Treatment Guidelines. The participant cannot be enrolled at this time but could be invited for rescreening after completion of treatment, if indicated.
9. Randomization and enrollment into AdvantageEDC^{SM}
10. LACTIN-V/placebo and applicator will be dispensed to participants. Participants will self-administer the first dose of LACTIN-V/placebo in the clinic during this visit. Participants will then be instructed to administer one dose of LACTIN-V/or placebo once a day at bedtime for the next 4 days (beginning on Day 2) and then twice weekly for 10 weeks. Participants will be instructed to transport the pre-filled applicators while avoiding exposure to high temperatures.

11. Participants will be given a memory aid or a web link (with a username and temporary password) and instructed to make an entry following each insertion of a pre-filled applicator beginning with the first application at Visit 1 (Day 1). Any symptoms that occur during the study up until Visit 7 (Week 24, Day168) will be recorded on the memory aid. Participants not using the web link will be reminded to return with the paper memory aid at each study visit where it will be reviewed.
12. Participants will be instructed to abstain from sexual intercourse during the first 5 days of study product administration, for 12 hours after the weekly study product applications and 12 hours before study Visit 2 (Week 4, Day 28), Visit 3 (Week 8, Day 56), Visit 4 (Week 12, Day 84) and Visit 7 (Week 24, Day 168).
13. Participants will receive non-spermicidal lubricated condoms for their male sexual partners who will be encouraged to use condoms from Visit 1 (Day 1) through Visit 7 (Week 24, Day 168) if they engage in vaginal intercourse.
14. Participants will be instructed not to use scented tampons or any other vaginal products through Visit 7 (Week 24, Day 168).
15. Participants will be advised to return for Visit 2 (Week 4, Day 28), Visit 3 (Week 8, Day 56) and Visit 4 (Week 12, Day 84) with allowable scheduling within 7 days of the target date. Depending on the time of the enrollment and the average length of the woman's menstrual cycle, careful attention should be paid to the approximate time of her next menstrual period when scheduling those visits. Later adjustments of those dates may be necessary.
16. Participants will be asked to collect all used applicators and store them as described in the MOP.
17. Participants will be asked to return all used applicators at their next visit.

Participants immediately diagnosed with yeast on the wet mount (with or without discharge or vaginitis symptoms) during the enrollment visit will be referred for standard treatment. They will not be randomized and will not be invited for rescreening.

If available test results at the enrollment visit suggest a UTI, cervicitis, vulvovaginitis, or active genital herpes lesion, the woman will be managed as clinically indicated and not randomized at this time, but invited for rescreening after completion of treatment.

The antibiotics administered to treat BV need time to eliminate pathogens and the vaginal flora needs time to naturally replenish its lactobacilli population. By standard definition, a treatment of BV is only considered to have failed if Amsel criteria or Nugent score on Gram stain diagnose BV to persist at 22-30 days after the initiation of the antibiotic treatment. As a result, women who continue to have BV during the enrollment visit will not be re-treated with MetroGel at the time of enrollment, as additional time is needed for BV to resolve after antibiotic treatment. Thus, they are not considered treatment failures at this time (approximately 2 days after termination of antibiotic treatment).

Participants diagnosed with an STI after randomization (when showing symptoms at subsequent visits that are confirmed with laboratory testing) will be referred for standard treatment and continue to receive study product.

7.3 Follow-up Study Visit 2, Week 4, Day 28 (Allowable Days 21-35)

The following procedures and assessments will be performed at Visit 2, Week 4, Day 28:

1. Participants will return on Day 28. Staff will review the memory aid with the participant. The participant will be asked about symptoms, concomitant medications and any medical problems since the last visit, which will be recorded on the appropriate CRF. The participant will be asked to provide exact dates and times of the study product administration.
2. Medical, gynecological and sexual history to include assessment of STI and HIV risk
3. Symptom-directed physical and pelvic examination by a study physician or nurse that will include vital signs, including temperature, and examination of respiratory and cardiovascular systems, as well as assessment of symptoms and adverse events
4. The pelvic examination will be conducted in the following order:
   a. Examination of the external genitalia
   b. Speculum insertion and examination of vagina, fornices and cervix with naked eye. Water will be used to ease speculum insertion. No lubricant should be used as it could contain chlorhexidine which may kill *Lactobacillus.*
   c. Vaginal pH
   d. Vaginal swab for wet mount taken from the posterior fornix for yeast vaginitis and clue cells and for amine test (whiff test)
   e. Vaginal swab for Gram stain (Nugent scoring system)
   f. Vaginal swabs (no more than two) for storage and future identification of vaginal bacteria, and possible multiomic (e.g. proteomics, metabolomics, etc.) testing
   g. Vaginal swabs for *L. crispatus* identification with qPCR
   h. If indicated, cervical (or vaginal) swab for nucleic acid amplification of *N. gonorrhoeae, C. trachomatis,* and *T. vaginalis*
5. If indicated, clean catch urine dipstick; urinalysis if abnormal results indicate
6. If indicated, urine βhCG pregnancy test
7. All clinical and laboratory findings at the time of the evaluation will be recorded on the CRF. These findings will include observations present on the pelvic exam such as genital findings including location; presence or absence, and character of vaginal or cervical discharge or lesions; and results of any on-site tests.
8. Participants will be reminded not to use scented tampons or any other vaginal products through Visit 7 (Week 24, Day 168).
9. Participants will be reminded to abstain from sexual intercourse for 12 hours after each study product application and for 12 hours before the remaining study visits at Visit 3 (Week 8, Day 56), Visit 4 (Week 12, Day 84) and Visit 7 (Week 24, Day 168).
10. Participants will receive non-spermicidal lubricated condoms for their male sexual partners. Participants will be encouraged to use condoms if they engage in vaginal intercourse through Visit 7 (Week 24, Day 168).
11. Participants will be asked if they still have the remaining applicators for the twice weekly doses stored at home. If not, they will receive additional pre-filled applicators to administer twice weekly through week 11. Participants will be instructed to transport the pre-filled applicators while avoiding exposure to high temperatures.

12. Participants will be instructed to collect all used applicators and store them as described in the MOP and to return all used applicators at their next visit, Visit 3 (Week 8, Day 56).
13. Applicators used since the last study visit will be collected. Applicators will be stained and tested as described in the MOP.
14. An appointment for the follow-up visit will be scheduled. Depending on the time of the enrollment and the average length of the participant's menstrual cycle, careful attention should be paid to the approximate time of the participant's next menstrual period when scheduling those visits. Later adjustments of those dates may be necessary.

Women with vaginal discharge or vaginitis symptoms who were diagnosed with yeast on the wet mount during the follow-up visit will be referred for standard treatment and continue to receive study product.

If the test results at the follow-up visit suggest a urinary tract infection, cervicitis or vulvovaginitis, the participant will be referred for standard treatment and continue to receive study product.

If the clinician suspects the woman to have developed an STI since the last visit, appropriate tests will be conducted and if needed the woman will be referred to standard of care treatment in compliance with current CDC STD Treatment Guidelines. The participant should be treated in case of positive findings and continue to use study product.

Women who develop *symptomatic* BV confirmed by wet mount Amsel criteria ≥3 (with or without confirmatory Gram stain) at least 4 weeks after the last MetroGel treatment will be retreated with MetroGel. This practice follows CDC treatment guidelines and FDA recommendations. The 5-day course of MetroGel will begin as soon as possible. Women will skip their regularly scheduled dose of study product until 24 hours after their last dose of MetroGel when study product dosing will continue twice weekly. Women diagnosed with *asymptomatic* BV during the follow-up visit will not be retreated with MetroGel.

7.4 Follow-up Study Visit 3, Week 8, Day 56 (Allowable Days 49-63)

The same procedures and assessments performed at Study Visit 2, Week 4, Day 28 will also be done at this visit.

7.5 Follow-Up Study Visit 4, Week 12, Day 84 (Allowable Days 77-91)

The following procedures and assessments will be performed at Visit 4, Week 12, Day 84:

1. Participants will return on Day 84. Staff will review the memory aid with the participant. The participant will be asked about symptoms, concomitant medications and any medical problems since the last visit, which will be recorded on the appropriate CRF. The participant will be asked to provide exact dates and times of the study product administration.
2. Medical, gynecological and sexual history to include assessment of STI and HIV risk

3. Symptom-directed physical and pelvic examination by a study physician or nurse that will include vital signs, including temperature, and examination of respiratory and cardiovascular systems, as well as assessment of symptoms and adverse events
4. The pelvic examination will be conducted in the following order:
   a. Examination of the external genitalia
   b. Speculum insertion and examination of vagina, fornices and cervix with naked eye. Water will be used to ease speculum insertion. No lubricant should be used as it could contain chlorhexidine which may kill *Lactobacillus.*
   c. Vaginal pH
   d. Vaginal swab for wet mount taken from the posterior fornix for yeast vaginitis and clue cells and for amine test (whiff test)
   e. Vaginal swab for Gram stain (Nugent scoring system)
   f. Vaginal swabs (no more than two) for storage and future identification of vaginal bacteria, and possible multiomic (e.g. proteomics, metabolomics, etc.) testing
   g. Vaginal swabs for *L. crispatus* identification with qPCR
   h. If indicated, cervical (or vaginal) swab for nucleic acid amplification of *N. gonorrhoeae, C. trachomatis,* and *T. vaginalis*
5. If indicated, clean catch urine dipstick; urinalysis if abnormal results indicate
6. If indicated, urine βhCG pregnancy test
7. All clinical and laboratory findings at the time of the evaluation will be recorded on the CRF. These findings will include observations present on the pelvic exam such as genital findings including location; presence or absence, and character of vaginal or cervical discharge or lesions; and results of any on-site tests.
8. Participants will be asked to answer a detailed self-administered questionnaire assessing the acceptability of the study product and the applicator to evaluate the possible change of attitudes over time.
9. Participants will be reminded not to use scented tampons or any other vaginal products through Visit 7 (Week 24, Day 168).
10. Participants will be reminded to abstain from sexual intercourse for 12 hours before study Visit 7 (Week 24, Day 168).
11. Participants will receive non-spermicidal lubricated condoms for their male sexual partners. Participants will be encouraged to use condoms if they engage in vaginal intercourse through Visit 7 (Week 24, Day 168).
12. Applicators used since the last study visit will be collected. Applicators will be stained and tested as described in the MOP.
13. An appointment for a telephone interview at Visit 5 (Week 16, Day 112) and at Visit 6 (Week 20, Day 140) will be scheduled.
14. An appointment for the next follow-up visit, Visit 7 (Week 24, Day 168) will be scheduled. Depending on the time of the enrollment and the average length of the participant's menstrual cycle, careful attention should be paid to the approximate time of the participant's next menstrual period when scheduling those visits. Later adjustments of those dates may be necessary.

Women with vaginal discharge or vaginitis symptoms who were diagnosed with yeast on the wet mount during the follow-up visit will be referred for standard treatment.

If the test results at the follow-up visit suggest a urinary tract infection, cervicitis or vulvovaginitis, the participant will be referred for standard treatment.

If the clinician suspects the woman to have developed an STI since the last visit, appropriate tests will be conducted and if needed the woman will be referred to standard of care treatment in compliance with current CDC STD Treatment Guidelines. The participant should be treated in case of positive findings.

Women who develop symptomatic BV confirmed by wet mount Amsel criteria ≥3 (with or without confirmatory Gram stain) at least 4 weeks after the last MetroGel treatment will be retreated with MetroGel. This practice follows CDC treatment guidelines and FDA recommendations. The 5-day course of MetroGel will begin as soon as possible. Women will skip their regularly scheduled dose of study product until 24 hours after their last dose of MetroGel when study product dosing will continue twice weekly. Women diagnosed with asymptomatic BV during the follow-up visit will not be retreated with MetroGel.

7.6 Telephone Interview, Visit 5, Week 16, Day 112 (Allowable Days 105-119)

Between Visit 4 (Week 12, Day 84) and Visit 7 (Week 24, Day 168), communication with participants will be continued by monthly telephone interviews.

1. Participants will be called for a prearranged telephone interview at Day 112. Staff will review the memory aid with the participant. The participant will be asked about symptoms, concomitant medications, last menses, sexual activity and any medical problems since the last visit, which will be recorded on the appropriate CRF.
2. Participants are invited to present at the clinic for an unscheduled visit if the site investigator deems it necessary after reviewing any medical problems or symptoms.
3. Participants will be reminded not to use scented tampons or any other vaginal products through Visit 7 (Week 24, Day 168).
4. Participants will be reminded to abstain from sexual intercourse for 12 hours before the last study visit, Visit 7 (Week 24, Day 168).
5. An appointment for the next telephone interview at Visit 6 (Week 20, Day 140) will be confirmed or rescheduled if necessary.
6. An appointment for the final visit, Visit 7 (Week 24, Day 168) will be confirmed. Depending on the time of the enrollment and the average length of the participant's menstrual cycle, careful attention should be paid to the approximate time of the participant's next menstrual period when scheduling those visits. Later adjustments of those dates may be necessary.

If the participant fails to cancel the appointment and reschedule, and is unreachable during the agreed upon time for the telephone interview, the study team will make three attempts to reach the participant to reschedule the telephone interview.

7.7 Telephone Interview, Visit 6, Week 20, Day 140 (Allowable Days 133-146)

The same assessments and instructions at Telephone Interview, Visit 5, Week 16, Day 112 will also be done during this telephone interview.

7.8 Final Study Visit 7, Week 24, Day 168 (Allowable Days 161-175)

The following procedures and assessments will be performed at Visit 7, Week 24, Day 168.

1. Participants will return on Day 168. Staff will review the memory aid with the participant. The participant will be asked about symptoms, concomitant medications and any medical problems since the last visit, which will be recorded on the appropriate CRF.
2. Medical, gynecological and sexual history to include assessment of STI and HIV risk
3. Symptom-directed physical and pelvic examination by a study physician or nurse that will include vital signs, including temperature, and examination of respiratory and cardiovascular systems, as well as assessment of symptoms and adverse events
4. The pelvic examination will be conducted in the following order:
   a. Examination of the external genitalia
   b. Speculum insertion and examination of vagina, fornices and cervix with naked eye. Water will be used to ease speculum insertion. No lubricant should be used as it could contain chlorhexidine which may kill *Lactobacillus.*
   c. Vaginal pH
   d. Vaginal swab for wet mount taken from the posterior fornix for yeast vaginitis and clue cells and for amine test (whiff test)
   e. Vaginal swab for Gram stain (Nugent scoring system)
   f. Vaginal swabs (no more than two) for storage and future identification of vaginal bacteria, and possible multiomic (e.g. proteomics, metabolomics, etc.) testing
   g. Vaginal swabs for *L. crispatus* identification with qPCR
   h. If indicated, cervical (or vaginal) swab for nucleic acid amplification of *N. gonorrhoeae, C. trachomatis,* and *T. vaginalis*
5. If indicated, clean catch urine dipstick; urinalysis if abnormal results indicate urinalysis
6. If indicated, urine βhCG pregnancy test.
7. All clinical and laboratory findings at the time of the evaluation will be recorded on the CRF. These findings will include observations present on the pelvic exam such as genital findings including location; presence or absence, and character of vaginal or cervical discharge or lesions; and results of any on-site tests.

Women with vaginal discharge or vaginitis symptoms who were diagnosed with yeast on the wet mount during the follow-up visit will be referred for standard treatment.

If the test results at the follow-up visit suggest a urinary tract infection, cervicitis or vulvovaginitis, the participant will be referred for standard treatment.

If the clinician suspects the woman to have developed an STI since the last visit, appropriate tests will be conducted and if needed the woman will be referred to standard of care treatment in compliance with current CDC STD Treatment Guidelines. The participant should be treated in case of positive findings.

Women who develop *symptomatic* BV confirmed by wet mount Amsel criteria ≥3 (with or without confirmatory Gram stain) at least 4 weeks after the last MetroGel treatment will be retreated with MetroGel. This practice follows CDC treatment guidelines and FDA recommendations. The 5-day course of MetroGel will begin as soon as possible. Women will skip their regularly scheduled dose of study product until 24 hours after their last dose of MetroGel when study product dosing will continue twice weekly. Women diagnosed with *asymptomatic* BV during the follow-up visit will not be retreated with MetroGel.

7.9 Study Product Discontinuation/Early Termination Visit

Study product discontinuation and early termination evaluations will be performed as described below. Women who must discontinue taking the study product, for reasons described in section 5.3.3.2, before the end of the study-defined treatment period, will be asked to stay in the study to be followed on study/off treatment until study completion. Women who terminate their participation in the study early will be asked to have one final study visit but could return to be followed on study/off treatment until study completion if circumstances of the termination change.

1. Staff will review the memory aid with the participant if the participant declines to return for follow-up visits. The participant will be asked about symptoms, concomitant medications and any medical problems since the last visit, which will be recorded on the appropriate CRF. The participant will be asked to provide exact dates and times of the study product administration.
2. Medical, gynecological and sexual history to include assessment of STI and HIV risk
3. Symptom-directed physical and pelvic examination by a study physician or nurse that will include vital signs, including temperature, and examination of respiratory and cardiovascular systems, as well as assessment of symptoms and adverse events
4. The pelvic examination will be conducted in the following order:
   a. Examination of the external genitalia
   b. Speculum insertion and examination of vagina, fornices and cervix with naked eye. Water will be used to ease speculum insertion. No lubricant should be used as it could contain chlorhexidine which may kill *Lactobacillus*.
   c. Vaginal pH
   d. Vaginal swab for wet mount taken from the posterior fornix for yeast vaginitis and clue cells and for amine test (whiff test)
   e. Vaginal swab for Gram stain (Nugent scoring system)
   f. Vaginal swabs (no more than two) for storage and future identification of vaginal bacteria, and possible multiomic (e.g. proteomics, metabolomics, etc.) testing
   g. If indicated, cervical (or vaginal) swab for nucleic acid amplification of *N. gonorrhoeae, C. trachomatis,* and *T. vaginalis*
   h. Other tests as deemed necessary by the site investigator in response to symptoms presented at this visit
5. If indicated, clean catch urine dipstick; urinalysis if abnormal results indicate
6. If indicated, urine βhCG pregnancy test
7. All clinical and laboratory findings at the time of the evaluation will be recorded on the CRF. These findings will include observations present on the pelvic exam such

as genital findings including location; presence or absence, and character of vaginal or cervical discharge or lesions; and results of any on-site tests.

8. Applicators used since the last study visit will be collected. Any remaining unused applicators will also be collected.
9. Administer acceptability questionnaire
10. Dispense condoms to subjects who discontinue study product but remain in follow up.
11. If the clinician suspects the participant developed an STI since the last visit, appropriate tests will be conducted and if needed the woman will be referred to standard of care treatment in compliance with current CDC STD Treatment Guidelines.

7.10 Unscheduled Visit

An unscheduled visit following a participant's request or the site investigator's recommendation should be recorded on the appropriate CRF. The following procedures and assessments may be performed at the unscheduled visit:

1. Staff will review the memory aid with the participant. The participant will be asked about symptoms, concomitant medications and any medical problems since the last visit, which will be recorded on the appropriate CRF. Additionally, the participant will be asked to provide exact dates and times of the study product administration.
2. Medical, gynecological and sexual history to include assessment of STI and HIV risk
3. Symptom-directed physical and pelvic examination by a study physician or nurse that will include vital signs, including temperature, and examination of respiratory and cardiovascular systems, as well as assessment of symptoms and adverse events
4. The pelvic examination will be conducted in the following order:
    a. Examination of the external genitalia
    b. Speculum insertion and examination of vagina, fornices and cervix with naked eye. Water will be used to ease speculum insertion. No lubricant should be used as it could contain chlorhexidine which may kill *Lactobacillus.*
    c. Vaginal pH
    d. Vaginal swab for wet mount taken from the posterior fornix for yeast vaginitis and clue cells and for amine test (whiff test)
    e. Vaginal swab for Gram stain (Nugent scoring system)
    f. Vaginal swabs (no more than two) for storage and future identification of vaginal bacteria, and possible multiomic (e.g. proteomics, metabolomics, etc.) testing
    g. Vaginal swabs for *L. crispatus* identification with qPCR
    h. If indicated, cervical (or vaginal) swab for nucleic acid amplification of *N. gonorrhoeae, C. trachomatis*, and *T. vaginalis*
    i. Other tests as deemed necessary by the site investigator in response to symptoms presented at this visit
5. If indicated, clean catch urine dipstick; urinalysis if abnormal results indicate
6. If indicated, βhCG pregnancy test
7. All clinical and laboratory findings at the time of the evaluation will be recorded on the CRF. These findings will include observations present on the pelvic exam such

as genital findings including location; presence or absence, and character of vaginal or cervical discharge or lesions; and results of any on-site tests.

8. The applicators used since the last study visit will be collected. Applicators will be stained and tested as described in the MOP.
9. An additional unscheduled visit as deemed necessary by the site investigator to ensure short-term surveillance will be planned.
10. If the site investigator decides together with the participant that study product use can continue, participants will be asked if they still have the remaining applicators for the twice weekly doses stored at home. If not, they will receive additional pre-filled applicators to administer twice weekly through week 11. Participants will be instructed to transport the pre-filled applicators while avoiding exposure to high temperatures.
11. Participants will be reminded not to use scented tampons or any other vaginal products through Visit 7 (Week 24, Day 168).
12. Participants will be reminded to abstain from sexual intercourse for 12 hours after each study product application and 12 hours before the remaining study visits.
13. Participants will receive non-spermicidal lubricated condoms for their male sexual partners. Participants will be encouraged to use condoms if they engage in vaginal intercourse through Visit 7 (Week 24, Day 168).
14. Participants will be instructed to collect all used applicators and store them as described in the MOP and to return all used applicators at their next visit.
15. An appointment for the next regular follow-up visit will be scheduled. Depending on the time of the enrollment and the average length of the participant's menstrual cycle, careful attention should be paid to the approximate time of the participant's next menstrual period when scheduling those visits. Later adjustments of those dates may be necessary.

Women with vaginal discharge or vaginitis symptoms who were diagnosed with yeast on the wet mount during the follow-up visit will be referred for standard treatment and continue to receive study product.

If the test results at the follow-up visit suggest a urinary tract infection, cervicitis or vulvovaginitis, the participant will be referred for standard treatment and continue to receive study product.

If the clinician suspects the woman to have developed an STI since the last visit, appropriate tests will be conducted and if needed the woman will be referred to standard of care treatment in compliance with current CDC STD Treatment Guidelines. The participant should be treated in case of positive findings and continue to use study product.

Women who develop *symptomatic* BV confirmed by wet mount Amsel criteria ≥3 (with or without confirmatory Gram stain) at least 4 weeks after the last MetroGel treatment will be retreated with MetroGel. This practice follows CDC treatment guidelines and FDA recommendations. The 5-day course of MetroGel will begin as soon as possible. Women will skip their regularly scheduled dose of study product until 24 hours after their last dose of MetroGel when study product dosing will continue twice weekly. Women diagnosed with *asymptomatic* BV during the follow-up visit will not be retreated with MetroGel.

8.0 STUDY PROCEDURES/EVALUATIONS

8.1 Clinical Evaluations

The following assessments and procedures will be performed:

- Sociodemographic and participant contact information
- Medical, gynecological and sexual history
- Review of concomitant medications
- Physical exam to include vital signs
- Pelvic exam
- Acceptability questionnaire

8.2 Laboratory Evaluations

8.2.1 Clinical Laboratory Evaluations

At each clinical site, the following tests will be performed using approved standard in-house tests:

- Pregnancy will be tested by standard rapid urine βhCG assays.
- Urine will be tested by urine dipstick for evidence of urinary tract infection, with follow-up urine culture for positives.
- HIV testing will be conducted using standardized algorithms.*
- Syphilis will be tested by rapid plasma reagin with a confirmatory agglutination.*
  *NOTE: The total blood required is approximately 15mL (3 teaspoons).
- Cervical (or vaginal) swabs will be tested for *N. gonorrhoeae*, *C. trachomatis* and *T. vaginalis* by nucleic acid amplification.

The following test will be performed by a central laboratory for all clinical sites:

- Gram-stained vaginal smear by Nugent's scoring system for diagnosis of BV

8.2.2 Special Assays or Procedures

Vaginal specimens will be analyzed for vaginal colonization with *L. crispatus* using qPCR assays and will be performed by a central laboratory at UCSF. At UCSF, and at UCSF expense, a vaginal specimen will be stored for future testing of other bacteria associated with BV (cultivable and uncultivable) by 16S rRNA qPCR pending the availability of non-STI CTG research funds.

8.2.3 Specimen Preparation, Handling, and Shipping

8.2.3.1 Instructions for Specimen Preparation, Handling, and Storage

Instructions for specimen preparation, handling, and storage will be described in the MOP.

8.2.3.2 Specimen Shipment

Instructions for specimen shipment will be described in the MOP.

9.0 ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

All AEs and SAEs will be collected through Study Visit 7, Week 24, Day 168.

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

9.2.1 Adverse Events

ICH (International Conference on Harmonisation) E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject 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 (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study participant 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 and will include:

- Vaginal bleeding other than menstruation
- Abnormal vaginal discharge
- Abnormal vaginal odor
- Genital itching
- Genital burning
- External genital irritation
- External genital swelling
- Nausea
- Vomiting
- Abdominal pain/cramps
- Diarrhea
- Constipation
- Genital rash
- Pain/burning with urination
- Frequent urination
- Blood in urine
- Headache

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), 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 participant 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. The 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: All AEs will be assessed by the clinician using a protocol defined grading system. For events not included in the protocol defined grading system, than the following guidelines will be used to quantify intensity.

- Mild: Events require minimal or no treatment and do not interfere with the participant'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 participant'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 to be performed. AEs 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 test article 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 AE. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the AE.
- Not Related: There is not a reasonable possibility that the administration of the study product caused the event.

9.2.2 Serious Adverse Events

An AE or SAE 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

- 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 AE 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 AE that, 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 physician
- Reviewed and evaluated by the study chairs, relevant IRBs, DMID and the DSMB

9.2.3 Procedures to be followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

The site principal investigator or appropriate sub-investigator is responsible for reporting all AE/SAEs that are observed or reported during the study, regardless of their relationship to study product. AE/SAEs, abnormal laboratory values, or abnormal clinical findings will be documented, reported, and followed appropriately.

For grading abnormal gynecological events, refer to Appendix B, Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, December 2004; Addendum 1: Female Genital Grading Table for Use in Microbicide Studies, November 2007.

For grading abnormal laboratory and clinical events, refer to Appendix C, DAIDS Toxicity Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0 - December 2004 (Clarification dated August 2009).

To meet FDA approved Clinical Data Interchange Standards Consortium (CDISC) data standards, Appendix B and Appendix C were revised to remove Grade 4 toxicities and combine them with Grade 3 toxicities.

9.3 Reporting Procedures

9.3.1 Serious Adverse Events

AEs will be followed until resolution even if this extends beyond the study-reporting period. Resolution of an AE 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 20814, 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 subject 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 Conducted Under DMID-Sponsored IND

Following notification from the site principal investigator or appropriate sub-investigator, DMID, the Investigational New Drug (IND) sponsor, will report any suspected adverse reaction that is both serious and unexpected. DMID will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the AE. DMID will notify FDA and all participating site principal investigators (i.e., all principal investigators to whom the sponsor is providing drug under its IND(s) or under any principal investigator's IND(s) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. DMID will also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. Relevant follow-up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

9.3.3 Reporting of Pregnancy

Pregnancies occurring in study participants will be reported on the Pregnancy Report form. Efforts will be made to follow all pregnancies reported during the course of the study to pregnancy outcome pending the participant's permission.

9.4 Type and Duration of Follow-up of Subjects 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. 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

Study enrollment and dosing will be halted and an ad hoc DSMB review will be performed if any of the following occur at any time during the study:

1) One or more participants experience a treatment-related SAE.
2) Two or more participants experience treatment-related vulvar and/or vaginal ulceration, abscess, or necrosis.
3) Four or more participants experience a treatment-related severe (Grade 3) systemic adverse event.
4) An overall pattern of symptomatic, clinical, or laboratory events that the DMID, medical monitor or DSMB consider associated with study product and that may collectively represent a serious potential concern for safety.

A decision to reinitiate the study and proceed with study treatments will be made based on the recommendation of the DSMB and DMID.

9.6 Safety Oversight (ISM plus DSMB)

An Independent Safety Monitor (ISM) at each clinical research site will oversee the safety of research subjects 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 subject-related issues. The ISM will communicate with the site PI and study PIs to resolve any issues.

Safety oversight will be under the direction of a DSMB, consisting of at least 3 voting members. The DSMB will meet after a third of the 228 participants complete Study Visit 2 (Week 4), and then annually to assess safety and efficacy data on each arm of the study. The DSMB will review aggregate safety data for increased rate of occurrence of serious suspected adverse reactions. If halting rules are initiated, more frequent meetings may be held. The DMSB will operate under the rules of a DMID-approved charter that will be written at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The DSMB will advise DMID of its findings.

10.0 CLINICAL MONITORING

10.1 Site Monitoring Plan

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 MOP and applicable sponsor standard operating procedures. DMID, the sponsoring agency, or its designee will conduct site-monitoring visits as detailed in the clinical monitoring plan.

Site visits will be made at standard intervals as defined by DMID and may be made more frequently as directed by DMID. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, CRFs or eCRFs, informed consent forms, medical and laboratory reports, 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 site principal investigators to discuss any problems and actions to be taken and document visit findings and discussions.

11.0 STATISTICAL CONSIDERATIONS

11.1 Introduction

This is a Phase II-b multicenter randomized double-blind placebo-controlled trial to assess the safety and efficacy of repeated doses of LACTIN-V in comparison with placebo in preventing BV recurrence in women diagnosed with BV. The study plans to enroll 228 sexually-experienced, pre-menopausal women age 18 to 45 years who are using a reliable method of birth control. Potentially eligible women will start a standard 5-day course of MetroGel. After completing the 5-day course of MetroGel, eligible participants will be randomized 2:1 to receive LACTIN-V or matching placebo. There will be five clinic visits and two telephone interviews over 24 weeks.

11.2 Study Objectives and Outcome Measures

As described in section 3.1 and 3.2, the primary study objectives are to compare LACTIN-V with placebo following a 5-day treatment course with MetroGel to treat BV with respect to safety and efficacy. The primary safety outcome measure is the proportion of participants reporting AEs and SAEs considered product-related following dose of study product in each study arm through Visit 7 (Week 24, Day 168). The primary efficacy outcome measure is the proportion of participants who experience BV recurrence (i.e. positive BV diagnosis) in each study arm by Visit 4 (Week 12, Day 84).

Following FDA guidelines [25], all BV diagnoses during follow-up visits are considered incident, as they occur at least 22-30 days after the commencement of MetroGel treatment and consequently are treatment failures or new infections. For the purpose of this trial, treatment failure and new infection will both be considered recurrent BV.

11.3 Study Hypotheses

There are two planned formal tests of hypotheses which compare LACTIN-V to placebo, with respect to the primary safety and efficacy outcome measures. The first hypothesis test compares the proportion of participants in each study arm who experience AEs or SAEs considered product related following the first dose of study product through Visit 7 (Week 24, Day 168). The second hypothesis test compares the proportion of participants in each study arm who experience BV recurrence by Visit 4 (Week 12, Day 84).

The null hypothesis for each comparison is that there is no difference in proportions between study arms, with a two-sided alternative. The test for the primary safety hypothesis will be conducted using the Fisher's exact test at the 5% two-sided level of significance without adjustment for multiplicity. The test for the primary efficacy hypothesis will be conducted using the Pearson's chi-square test at the 5% two-sided level of significance without adjustment for multiplicity. No adjustments for multiplicity will be used as this is a Phase II-b trial which will provide screening evidence about safety and efficacy for a subsequent Phase III trial.

The primary safety hypothesis will be tested in the safety data analysis population. The primary efficacy analysis will be conducted in the Complete Case analysis population, and repeated as a secondary analysis in the modified intent-to-treat and Per Protocol analysis populations. See Section 11.6.2 for definitions of and rationales for use of the analysis populations.

11.4 Sample Size Considerations

Sample size calculations are based on the assumption of detecting a 50% reduction in the cumulative proportion of participants who experience BV recurrence by 12 weeks post-administration of study product among participants receiving LACTIN-V compared to those receiving placebo in a complete case analysis at the 0.05 significance level. The proportion of participants among controls who will experience BV recurrence at 12 weeks is assumed to be 30%. We assume a LACTIN-V to placebo allocation ratio of 2:1 and 10% of subjects drop-out from the Complete Case population. Given logistical and feasibility constraints on the sample size and that this is a Phase II-b trial which will provide screening evidence about safety and efficacy for a subsequent Phase III trial, this trial is powered at the 70% level. Given the assumptions above, a sample size of 228 participants is required to detect a 50% reduction in the cumulative proportion of participants experiencing recurrence in the Complete Case population with 70% power using the Pearson Chi-Square test.

11.5 Planned Interim Analyses

11.5.1 Safety Review

As described in Section 9.6, the DSMB will meet and review safety data and enrollment data after one-third (N = 76) enrolled participants have completed Visit 2 (Day 28) and then annually thereafter. 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.

11.5.2 Efficacy Review

There is no planned interim analysis of efficacy data for this study.

11.6 Final Analysis Plan

11.6.1 General Principles

Tabulations will be used extensively to summarize the data. Summary statistics displayed for continuous data will include measures of central tendency such as the mean or median, and measures of dispersion such as the standard deviation or inter-quartile range. Summary statistics for discrete data will include contingency tables, odds ratios, and/or associated confidence intervals.

In presenting statistical inferences, the statistical claim (i.e. null and alternative hypotheses) will be clearly stated, the method used for hypothesis testing or confidence interval estimation will be described and appropriately referenced, and the assumptions underlying the methods will be validated.

Analytical and/or graphical methods for assessing goodness of model fit may be performed.

More details of the analyses to be performed will be included in a separate Statistical Analysis Plan.

11.6.2 Data Analysis Populations

The safety population will include all randomized participants who received study treatment.

The Modified Intent-To-Treat (mITT) population will include all randomized participants who met all inclusion/exclusion criteria, received study product, and returned for at least one post-baseline visit.

The Complete Case (CC) population will include all participants in the mITT population who were followed up until the first diagnosis of BV or through Visit 4 (Week 12, Day 84), or discontinued from the study as a treatment failure. The CC population will be used as the primary efficacy analysis population for the test of LACTIN-V to placebo.

The Per-Protocol (PP) population will include all randomized participants who met all inclusion/exclusion criteria, complied with the assigned study product, were followed up until the first diagnosis of BV or through Visit 4, or discontinued from the study as a treatment failure.

A participant is compliant with the assigned study product if she takes at least 75% of the scheduled doses prior to the first diagnosis of BV or through Visit 4 (Week 12, Day 84).

Prior to unblinding, a blinded case review committee will review participants with a reported concomitant infection/disease/procedure that may interfere with study product, use of concomitant medications or products that may interfere with study product, significant protocol deviations, and other events that may impact study product effectiveness or study analyses. On a case-by-case basis, the case review committee will determine if each participant will be included in PP population, if and when a participant should be censored or removed from PP analyses, and/or any other analytical requirements for the participant.

For the primary efficacy analysis participants who discontinue for reasons other than lack of treatment effect, as well as participants with a missing or non-evaluable Amsel or Nugent result with no positive result at another visit, are

excluded from the CC and PP populations, but included in the mITT population using imputation methods noted in Section 11.6.4.1.

11.6.3 Safety Analyses

Solicited AEs will be analyzed by taking the most severe response over the follow-up period, dichotomizing into a binary variable (none versus mild, moderate, or severe) and using standard techniques, such as exact confidence intervals, to summarize the event rates.

Standard summary statistics, including 95% confidence intervals will be computed. Additionally, graded results may either be analyzed as a binary variable (normal, abnormal) or multinomial variable (Grade 0, Grade 1, Grade 2, Grade 3) using either logistic regression or ordinal logistic regression respectively to make treatment group comparisons.

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 in aggregate, as well as by MedDRA® categories, will be computed. 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.

11.6.3.1 Analysis of Primary Safety Outcome Measure

The primary safety outcome measure is the proportion of participants reporting product-related AEs and SAEs in each study arm through Visit 7 (Week 24, Day 168).

The outcome will be assessed in the safety analysis population. The outcome will be reported by study arm. The point estimates for the arm-specific proportions and difference in proportions along with the corresponding standard errors will be reported. Fisher's exact test at the 5% two-sided level of significance level without adjustment for multiplicity will be used for the comparison of proportions between study arms.

As described in Section 11.6.3, additional descriptive analyses of adverse event data and clinical safety laboratory values will be performed.

11.6.3.2 Analysis of Secondary Safety Outcome Measure

The secondary safety outcome measure is the tolerability of LACTIN-V measured by the proportion of participants who discontinue study product due to adverse events in each study arm.

The outcome will be assessed in the safety analysis population. The outcome will be reported by study arm. The point estimates for the

arm-specific proportions and difference in proportions along with the corresponding standard errors will be reported. Fisher's exact test at the 5% two-sided level of significance level without adjustment for multiplicity will be used for the comparison of proportions between study arms.

11.6.4 Efficacy Analyses

11.6.4.1 Analysis of Primary Efficacy Outcome Measure

The primary efficacy outcome measure is the proportion of participants with a positive BV diagnosis in each study arm by Visit 4 (Week 12, Day 84) after study product administration.

As described in Section 3.1.2, the Amsel Criteria and Nugent criteria will be used to determine diagnosis of BV recurrence. Cumulative proportion of BV recurrence by Visit 4 is defined as follows: the numerator is the number of participants who experience recurrence at least once through Visit 4 in the particular analysis population and the denominator is the number of participants included in the particular analysis population.

The primary efficacy outcome measure will be assessed in the CC population. Use of the mITT population has previously been recommended by the FDA where missing values from participants who drop out early are imputed using Last Observation Carried Forward (LOCF) [25]. However, this includes participants who dropped out after completing at least one-follow-up visit, but before their first positive diagnosis. LOCF would classify such participants as 'not recurrent.' Thus, it is possible that the estimated proportions of treatment successes in a study arm could be larger than they are in reality. Thus LOCF could result in an over-optimistic estimate of treatment effect, conflicting with the following rational for using an ITT analysis population stated in ICH E9 [37]: "[ITT analysis] tends to avoid over-optimistic estimates of efficacy resulting from a per protocol analysis, since the non-compliers included in the [ITT] analysis set will generally diminish the estimated treatment effect." To assess the effect of the choice of analysis population on the primary analysis, the analysis will be repeated as a secondary analysis in the mITT and PP populations. The mITT population includes participants who discontinue for reasons other than lack of treatment effect, as well as participants with a missing or non-evaluable Amsel or Nugent result with no positive result at another visit. The missing results will be imputed using LOCF. To assess the effect of missing data and the method of imputation on the primary analysis, a sensitivity analysis will be performed. The details of the sensitivity analysis will be detailed in the Statistical Analysis Plan.

As described in Section 11.3, a test of hypothesis will be performed. The analysis will be based on comparisons of cumulative proportion of BV recurrence between study groups by the Visit 4 using the Pearson Chi-Square test (or, if more appropriate, Fisher's Exact Test) for differences in proportions between two independent groups. The point estimates for the within-arm proportions, odds ratio, and corresponding two-sided level 95% confidence interval will be reported. A secondary time-to-event analysis of the primary efficacy outcome measure will be performed. Details of the analysis will be included in the Statistical Analysis Plan.

11.6.4.2 Analysis of Secondary Efficacy Outcome Measures

All secondary efficacy outcomes measures will be assessed in the mITT, CC, and PP populations separately.

The secondary efficacy outcome measures are as follows:

1. The proportion of participants experiencing successful colonization with *L. crispatus* CTV-05 following dose of study product through Visit 4 (Week 12, Day 84) in the LACTIN-V arm, overall and stratified by occurrence of menses and intercourse.
2. The proportion of participants experiencing successful colonization with *L. crispatus* CTV-05 following dose of study product at Visit 7 (Week 24,Day 168) in the LACTIN-V arm, overall and stratified by occurrence of menses and intercourse
3. A) The proportion of participants compliant with the dose regimen in each study arm as assessed by participant reporting and applicator staining
   B) Summaries from a self-administered questionnaire about acceptability of the study product in each study arm
4. The proportion of participants with a positive BV diagnosis in each study arm by Visit 7 (Week 24, Day 168)

Secondary outcome measures 1 and 2 will be reported within the LACTIN-V arm and across time. The point estimates for the overall proportions and stratified proportions, along with the corresponding standard errors, will be reported. A secondary time-to-event analysis of secondary outcome measure 1 will also be performed, which will be described in the Statistical Analysis Plan.

Secondary Outcome 3A will be reported by study arm. The point estimates for the arm-specific proportions and difference in proportions along with the corresponding standard errors will be reported. Pearson's chi-square test or, if more appropriate, Fisher's exact test, at the 5% two-sided level of significance level without adjustment for multiplicity will be used for the comparisons of proportions between study arms.

For Secondary Outcome 3B, responses to the self-administered questionnaire will be summarized by study arm. Categorical responses will be summarized using contingency tables and/or odds ratios, while continuous responses will be summarized using measures of central tendency such as the mean or median, and measures of dispersion such as the standard deviation or inter-quartile range.

The analysis of Secondary Outcome 4 will be based on comparisons of cumulative proportion of BV recurrence between study groups by the Visit 7 using the Pearson Chi-Square test or Fisher's Exact Test for differences in proportions between two independent groups. The point estimates for the within-arm proportions, odds ratio, and corresponding two-sided level 95% confidence interval will be reported. A secondary time-to-event analysis of secondary outcome measure 4 will also be performed, which will be described in the Statistical Analysis Plan.

11.6.4.3 Analysis of Exploratory Efficacy Outcome

The statistical parameterizations of the exploratory objective will include, but are not limited to, the following:

- The number of positive BV diagnoses per 12 weeks of follow-up
- The probability of positive BV diagnoses during the 12 weeks of follow-up

For the first parameterization, a Poisson regression model will be fit to estimate the 12-week incidence of positive BV diagnoses in each of the treatment arms and to compare the incidence in the LACTIN-V arm to that in the placebo arm.

For the second parameterization, a repeated measures logistic regression model will be fit to estimate the probability of positive BV diagnoses in each of the treatment arms and to compare the probability of recurrence in the LACTIN-V arm to that in the placebo arm.

As the exploratory analyses include participants who received additional MetroGel treatment after experiencing symptomatic BV at a particular follow-up visit, a sensitivity analysis will be performed to assess the effect of the additional treatment on the outcome. Details of the sensitivity analysis will be provided in the Statistical Analysis Plan.

12.0 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).

13.0 QUALITY CONTROL AND QUALITY ASSURANCE

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 prompt clarification and resolution.

The investigational sites are responsible for conducting routine quality control (QC) and quality assurance (QA) activities to internally monitor study progress and protocol compliance. All sites will follow Sexually Transmitted Infections Treatment and Research (STAR) STI-CTG Standard Operating Procedures. A Clinical Quality Management Plan (CQMP) is in place for ensuring compliance with the protocol, applicable federal regulations, and Good Clinical Practice guidelines.

The Principal Investigator will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

The Principal Investigator will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

14.0 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with the principles set forth in the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6; 62 Federal Regulations 25691 (1997).

14.2 Institutional Review Board

Each participating institution will provide for the review and approval of this protocol and the associated informed consent documents and recruitment material by an appropriate independent ethics committee (IEC) or IRB registered with the OHRP. Any amendments to the protocol or consent materials will also be approved before they are placed into use.

14.3 Informed Consent Process

Informed consent is a process that is initiated prior to the participant's agreeing to participate in the study and continuing throughout the participant's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided to the participant and their families. For this study, separate screening and enrollment consent forms will be used. Consent forms describing in detail the study interventions, products, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention or administering study product. Consent forms will be IRB-approved and the participant will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the participant and answer any questions that may arise. The participants will sign the informed consent document prior to any procedures being done specifically for the study. The participants should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

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

This trial will enroll adult women who meet the participant inclusion criteria regardless of religion or ethnic background.

The following populations will be excluded from study participation:

- *Pregnant women*
  Pregnant women are not eligible for this study because there are no current recommendations for the use of LACTIN-V during pregnancy.

- *Men*
  Men are not eligible for this study as the study evaluates the uterine cervix and vagina for changes associated with the use of LACTIN-V.
- *Children*
  The NIH has mandated that children (defined as anyone under the age of 21) be included in research trials when appropriate. This study will enroll children aged 18 to 20 who are able to give informed consent. This study meets "Justifications for Exclusion" criteria for younger children as set forth by the NIH. Specifically, "insufficient data are available in adults to judge potential risk in children" and "children should not be the initial group to be involved in research studies."

14.5 Subject Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participating participants.

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

The study monitor or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

14.6 Study Discontinuation

If the trial is discontinued, participants who sign the informed consent form, 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

Vaginal swabs (no more than two) will be collected at each visit and stored for future use. These swabs will be in addition to the swabs being collected to test for sexually transmitted infections and bacterial vaginosis. The additional swabs may be used for future testing and identification of vaginal bacteria. Samples will be kept confidential and coded with a unique ID. Upon request and ethical approval, these samples may also be made available to other researchers to test for the bacteria, and possible multiomic (e.g. proteomics, metabolomics, etc.) testing. Storage of samples is optional and not a requirement for the study. Participants will be asked to consent separately to have their specimens stored. Consent can be withdrawn at any time and refusal or withdrawal of consent will not affect study participation.

15.0 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 source documents 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 principal investigator (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 the Statistical and Data Coordinating Center 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, but not limited to, AE/SAEs, concomitant medications, and reactogenicity data) and clinical laboratory data will be entered into a 21 CFR Part 11-compliant Internet Data Entry System, AdvantageEDC^{SM}, 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 source documents.

15.3 Types of Data

Data for this study will include clinical, efficacy, safety, laboratory and outcome measures (eg, AEs, SAEs, and reduction of BV reoccurrence).

15.4 Timing/Reports

A final report will be prepared following the availability of all the safety and laboratory data. Interim statistical reports may be generated as deemed necessary and appropriate by DMID. Safety and laboratory data summary reports may be generated for the DSMB.

15.5 Study Records Retention

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP), or MOP 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. I

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 SDCC's AdvantageEDC^{SM}.

All deviations from the protocol must be addressed in study subject data collection forms. A completed copy of the DMID-approved Protocol Deviation Form must be maintained in the regulatory file, as well as in the participant's source document. Protocol deviations must be sent to the local IRB/IEC per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB/IEC requirements

16.0 PUBLICATION POLICY

Following completion of the study, the Investigator is expected to publish the results of this research in a scientific journal. Publication of the results of this study will be governed by STICTG policies and the International Committee of Medical Journal Editors (ICMJE) member journals, which 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 which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. It is the responsibility of DMID to register this trial in an acceptable registry. Any clinical trial starting enrollment after 01 July 2005 must be registered on or before patient enrollment. For trials that began enrollment prior to this date, the ICMJE member journals will require registration by 13 September 2005, before considering the results of the trial for publication

The ICMJE defines a clinical trial as any research project that prospectively assigns human participants 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 or major toxicity (eg, Phase 1 trials), would be exempt from this policy.

Any presentation, abstract, or manuscript will be made available by the Investigator to the STI-CTG Manuscript Review Committee, DMID, and Osel Inc. for review prior to submission.

17.0 LITERATURE REFERENCES

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APPENDICES

Appendix A	Schedule of Events
Appendix B	Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, December 2004; Addendum 1: Female Genital Grading Table for Use in Microbicide Studies, November 2007
Appendix C	DAIDS Toxicity Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0 - December 2004 (Clarification dated August 2009

Appendix A
Schedule of Events

Evaluation		Screening Visit 0 Day -30 to -5	Study Treatment: Enrollment Visit 1 Day 1	Study Visit 2 Day 28 (Week 4)	Study Visit 3 Day 56 (Week 8)	Study Visit 4 Day 84 (Week 12)	Phone Visit 5 Day 112 (Week 16)	Study Follow Up: Phone Visit 6 Day 140 (Week 20)	Study Visit 7 Day 168 (Week 24)	Unscheduled Visit	Study Product Discontinuation	Early Termination Visit
Visit window					± 1 week							
Signed consent form		X	X									
Assessment of eligibility criteria		X	X									
Demographics		X	X									
Randomization			X									
Detailed medical, gynecological and sexual history		X										
Dispense MetroGel		X		(X)	(X)	(X)						
Dispense LACTIN-V/placebo and applicators			X	(X)	(X)							
Dispense condoms		X	X	X	X	X				X	X	
Dispense memory aid or instructions on using web based memory aid			X									
Brief medical, gynecological and sexual history			X	X	X	X			X	X	X	X
Review of concomitant medications		X	X	X	X	X	X	X	X	X	X	X
Study intervention			X	X	X	X						
Physical Examination	Complete	X										
	Symptom-directed		X	X	X	X			X	X	X	X
	Vital signs	X	X	X	X	X			X	X	X	X
	Pelvic examination	X	X	X	X	X			X	X	X	X
Review memory aid and symptoms				X	X	X	X	X	X	X	X	X
Reminder to abstain from sexual intercourse			X	X	X	X	X	X		X		

Evaluation		Screening Visit 0 Day -30 to -5	Study Treatment: Enrollment Visit 1 Day 1	Study Visit 2 Day 28 (Week 4)	Study Visit 3 Day 56 (Week 8)	Study Visit 4 Day 84 (Week 12)	Phone Visit 5 Day 112 (Week 16)	Study Follow Up: Phone Visit 6 Day 140 (Week 20)	Study Visit 7 Day 168 (Week 24)	Unscheduled Visit	Study Product Discontinuation	Early Termination Visit
Reminder not to use vaginal products			X	X	X	X	X	X		X		
Reminder to collect and return used applicators			X	X	X	X	X	X		X	X	X
Assessment of adverse events				X	X	X	X	X	X	X	X	X
Clinical Laboratory	Clean catch urine dipstick	X	(X)	(X)	(X)	(X)			(X)	(X)	(X)	(X)
	Urinalysis	(X)	(X)	(X)	(X)	(X)			(X)	(X)	(X)	(X)
	Rapid urine βhCG pregnancy test	X	X	(X)	(X)	(X)			(X)	(X)	(X)	(X)
	HIV and syphilis serology (total blood required is approximately 15mL (3 teaspoons)	X										
	Vaginal swab for *N. gonorrhoeae, C. trachomatis, T. vaginalis*	X	(X)	(X)	(X)	(X)			(X)	(X)	(X)	(X)
Research Laboratory	Vaginal swab for pH	X	X	X	X	X			X	X	X	X
	Vaginal swab for wet mount, amine test	X	X	X	X	X			X	X	X	X
	Vaginal swab for Gram stain	X	X	X	X	X			X	X	X	X
	Vaginal swab for storage and future identification of vaginal bacteria	X	X	X	X	X			X	X	X	X
	Vaginal swab for qPCR (*L. crispatus* identification)		X	X	X	X			X	X	X	X
Other Procedures	Acceptability questionnaire	X				X					X	X
	Staining of used applicators			X*								
	Telephone interview						X	X				

(X) if indicated
X* per site discretion

APPENDIX B
DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS PUBLISH DATE: DECEMBER 2004
Addendum 1
Female Genital Grading Table for Use in Microbicide Studies

INDIVIDUAL SIGNS/SYMPTOMS				
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**
GENERAL				
Odor	No complaint	Mild-moderate unpleasant odor	Severe unpleasant odor	NA
PAIN AND TENDERNESS (Specify Area: Vulvar/Perineum, Vagina, Cervix (including cervical motion tenderness), Uterus, Adnexae, Pelvic/Lower Abdominal, or Ovulatory) ***Note – if both pain and tenderness are present, only report the one with the most severe grade**				
Pain* [1]	None	Pain causing no or minimal interference with usual social & functional activities	Pain causing greater than minimal interference with usual social & functional activities or the need for non-narcotic medication	Pain causing inability to perform usual social & functional activities or the need for narcotic medication OR hospitalization (other than emergency room visit) indicated
Tenderness* [1]	None	Mild tenderness	Moderate tenderness	Severe tenderness
Dyspareunia (pain with sexual activity)	None	Pain causing no or minimal interference with sexual function	Pain causing greater than minimal interference with sexual function	NA
Dysmenorrhea/cramping with menses	None	Pain causing no or minimal interference with usual social & functional activities	Pain causing greater than minimal interference with usual social & functional activities or the need for non-narcotic medication	Pain causing inability to perform usual social or functional activities or the need for narcotic medication

[1] If pain or tenderness is included in the grading of another category (e.g., PID), it should not be graded again in the pain or tenderness category

NOTE: For protocols utilizing this Addendum, when the same parameter appears in both this Female Genital Grading Table and the main DAIDS AE Grading Table, use the grading scheme in this table.

APPENDIX B (Cont'd)
DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS PUBLISH DATE: DECEMBER 2004
Addendum 1
Female Genital Grading Table for Use in Microbicide Studies

INDIVIDUAL SIGNS/SYMPTOMS				
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**
GENITOURINARY SIGNS/SYMPTOMS – VULVA				
Vulvar/vaginal itching	None	Itching causing no, mild, or moderate interference with usual social & functional activities	Itching causing inability to perform usual social & functional activities; may require intervention such as antihistamine or bathing to provide relief	NA
Vulvar edema	None	Mild, non-pitting edema	Moderate, 1-2+ pitting edema	3+ pitting edema, severe enough to require urinary drainage, or weeping edema ± skin breakdown
Vulvar erythema	None	Erythema covering < 50% of vulvar surface	Erythema covering ≥ 50% of vulvar surface	NA
Vulvar lesions (findings seen only by colposcopy should not be included here)	Normal variants including skin tags, moles, scars, etc.	Blisters, ulcerations, or pustules - no treatment indicated	Blisters, ulcerations or pustules, with treatment indicated	Severe epithelial disruption with hospitalization indicated (other than emergency room visit)
Vulvar rash	None	Rash covering < 50% of vulvar surface	Rash covering ≥ 50% of vulvar surface	Severe epithelial disruption with hospitalization indicated (other than emergency room visit)
Bartholin's or Skene's gland	No findings	Cyst with no inflammation	Cyst or abscess with outpatient intervention indicated	Cyst or abscess with hospitalization indicated, (other than emergency room visit) including necrotizing fasciitis from Bartholin's abscess
GENITOURINARY SIGNS/SYMPTOMS – VAGINA **** Note – if vaginal discharge is present both by history and on examination, only report the one with the most severe grade**				
Vaginal edema	None	Mild-moderate engorgement	Loss of ruggae and friability	NA
Vaginal erythema	None	Erythema covering < 50% of vaginal surface	Erythema covering ≥ 50% of vaginal surface	NA
Vaginal dryness	No complaint	Dryness causing no or minimal interference with usual sexual, social, & functional activities	Dryness causing greater than minimal interference with usual sexual, social, & functional activities	NA

NOTE: For protocols utilizing this Addendum, when the same parameter appears in both this Female Genital Grading Table and the main DAIDS AE Grading Table, use the grading scheme in this table.

APPENDIX B (Cont'd)
DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS PUBLISH DATE: DECEMBER 2004
Addendum 1
Female Genital Grading Table for Use in Microbicide Studies

INDIVIDUAL SIGNS/SYMPTOMS				
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**
Vaginal discharge by participant report **	Participant's usual amount of discharge, regardless of color or quantity	Mild-moderate increase in amount above participant baseline - no sanitary protection required	Profuse increase in discharge requiring pad use or other hygienic intervention	NA
Vaginal discharge as observed by clinician ** (red or brown discharge should be reported under bleeding, not discharge)	Slight amount of discharge, any color	Mild-moderate increase in amount	Significant increase in amount with pooling in vagina on examination	NA
Vaginal abrasions or lacerations (including probable applicator injuries)	None	Superficial disruptions and disruptions extending through the mucosa with minimal impact on life	Large disruptions extending through the mucosa or large superficial disruptions, hospitalization not indicated	Large disruptions extending through the mucosa or large superficial disruptions including lacerations, hospitalization indicated(other than emergency room visit)
Vaginal lesions (findings seen only by colposcopy should not be included here)	Normal variants including skin tags, moles, scars, etc.	Blisters, ulcerations, or pustules, no treatment indicated	Blisters, ulcerations, or pustules with treatment indicated	Severe epithelial disruption requiring hospitalization (other than emergency room visit)
Vaginal and Cervical masses (polyps, myomas, or possible malignancy)	None or normal variants such as Nabothian cyst or Gartner duct cyst	Polyp or myoma or undiagnosed mass without symptoms	Polyp, myoma, or undiagnosed mass causing mild symptoms, e.g., bleeding/pain not requiring more than mild analgesia	Polyp, myoma, or undiagnosed mass causing severe symptoms, e.g., bleeding/pain affecting bladder and bowel function, or visible cervical cancer
GENITOURINARY SIGNS/SYMPTOMS – CERVIX				
Cervical edema and friability	None	Edema without friability	Friable cervix	NA
Cervical erythema	None	Erythema covering < 50% of cervix	Erythema covering ≥ 50% of cervix	NA
Cervical discharge	White or clear discharge	Small amount of purulent discharge at	Purulent discharge extending onto cervix or	NA

NOTE: For protocols utilizing this Addendum, when the same parameter appears in both this Female Genital Grading Table and the main DAIDS AE Grading Table, use the grading scheme in this table.

APPENDIX B (Cont'd)
DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS PUBLISH DATE: DECEMBER 2004
Addendum 1
Female Genital Grading Table for Use in Microbicide Studies

Visible cervical lesions (findings seen only by colposcopy should not be included here)	Normal variants including skin tags, moles, scars, etc.	Blisters, ulcerations, or pustules, no treatment indicated	Blisters, ulcerations, or pustules with treatment indicated	NA

INDIVIDUAL SIGNS/SYMPTOMS				
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**
GENITOURINARY SIGNS/SYMPTOMS – UTERUS				
Uterine masses/enlargement based on bimanual examination	Normal to 8 week size, no palpable myomas	Enlarged uterus and mild symptoms, e.g., bleeding/pain requiring mild analgesics	Enlarged uterus/myoma with moderate pain or symptoms, e.g., bleeding	Mass causing severe bleeding/pain or with impact on bowel/bladder function or requires transfusion or surgery
Polyp, submucosal fibroid, or thickened endometrium detected by transvaginal ultrasound (new or increasing in size from prior exam)	None or unchanged/reduced in size from prior exam	New myomas < 6 cm diameter (single or multiple) or diameter increased < 6 cm since prior exam	New myomas ≥ 6 cm diameter (single or multiple) or diameter increased ≥ 6 cm since prior exam	Hospitalization (other than emergency room visit) and/or surgery indicated
GENITOURINARY SIGNS/SYMPTOMS – ADNEXA				
Not pregnancy- or infection-related adnexal masses based on bimanual exam (use if no ultrasound done; if ultrasound done, use ultrasound categories below)	None, ≤ 4 cm, normal size ovary	> 4 cm with minimal or no symptoms	> 4 cm with severe symptoms, e.g., pain, but hospitalization not indicated (see footnote #1)	> 4 cm with severe symptoms, e.g., pain and hospitalization indicated (other than emergency room visit) (see footnote #1)
Hydrosalpinx based on ultrasound	None	Asymptomatic, suspected hydrosalpinx	Hydrosalpinx with pain, but without evidence of infection or ectopic pregnancy	Signs/symptoms of infection with hospitalization (other than emergency room visit) and/or surgery indicated
Adnexal mass based on ultrasound	None	Simple cyst, asymptomatic	Simple cyst, symptomatic	Malignant mass suspicious for malignancy

NOTE: For protocols utilizing this Addendum, when the same parameter appears in both this Female Genital Grading Table and the main DAIDS AE Grading Table, use the grading scheme in this table.

APPENDIX B (Cont'd)
DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS PUBLISH DATE: DECEMBER 2004
Addendum 1
Female Genital Grading Table for Use in Microbicide Studies

INDIVIDUAL SIGNS/SYMPTOMS				
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**
GENITOURINARY SIGNS/SYMPTOMS – ABDOMEN				
Abdominal mass not palpable on pelvic exam of unknown diagnosis	None or known (pre-existing) mass unchanged in size	New mass or increased size of known mass requiring mild analgesia with minimal impact	New mass or increased size of known mass with moderate symptoms	Mass causing severe bleeding/ pain with impact on bladder/bowel function or with hospitalization (other than emergency room visit) indicated or malignancy
GENITOURINARY SIGNS/SYMPTOMS – URINARY TRACT				
Urinary frequency	None	Up to 2 times participant's normal frequency	> 2 times participant's normal frequency	NA
Dysuria	None	Superficial only	Deep ± superficial	Inability to void due to pain
Hematuria	None	Microscopic, no intervention indicated (beyond evaluation for infection)	Gross blood in urine or medical intervention/ evaluation indicated (beyond evaluation for infection)	Persistent bleeding with transfusion, hospitalization (other than emergency room visit) or intervention indicated to obtain hemostasis (endoscopy, interventional radiology, or operative)

NOTE: For protocols utilizing this Addendum, when the same parameter appears in both this Female Genital Grading Table and the main DAIDS AE Grading Table, use the grading scheme in this table.

APPENDIX B (Cont'd)
DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS PUBLISH DATE: DECEMBER 2004
Addendum 1
Female Genital Grading Table for Use in Microbicide Studies

COMPOSITE SIGNS/SYMPTOMS (Use instead of individual categories if 2 or more signs/symptoms are present)				
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD** (Use if all signs/ symptoms would individually be Grade 0 or 1)	**GRADE 2 MODERATE** (Use if one or more signs/symptoms would individually be Grade 2 and all others Grade 0 or 1)	**GRADE 3 SEVERE** (Use if one or more signs/symptoms would individually be Grade 3)
NO ORGANISM IDENTIFIED BUT INADEQUATE TESTING PERFORMED				
Vulvovaginitis (combinations of pain, itching, erythema, edema, rash, tenderness, or discharge)	None	Mild signs/symptoms	Moderate signs/symptoms	Severe signs/ symptoms
Cervicitis (combinations of dyspareunia, erythema, edema, tenderness, and discharge)	None	Mild signs/ symptoms	Moderate signs/ symptoms	Severe signs/ symptoms
PID (if Gonorrhea or Chlamydia identified use that category)	None	NA	Cervicitis with mild uterine tenderness, ± mild cervical motion tenderness, no signs of peritoneal irritation	More diffuse tenderness, any signs of peritoneal irritation, or indications for hospitalization (other than emergency room visit) including tubo-ovarian
NO ORGANISM IDENTIFIED AFTER APPROPRIATE TESTING PERFORMED				
Vulvovaginitis (combinations of pain, itching, erythema, edema, rash, tenderness, or discharge)	None	Mild signs/ symptoms	Moderate signs/ symptoms	Severe signs/ symptoms
Cervicitis (combinations of dyspareunia, erythema, edema, tenderness, and discharge)	None	Mild signs/ symptoms	Moderate signs/ symptoms	Severe signs/ symptoms
PID (if Gonorrhea or Chlamydia identified use that category)	None	NA	Cervicitis with mild uterine tenderness, ± mild cervical motion tenderness, no signs of peritoneal irritation	More diffuse tenderness, any signs of peritoneal irritation, or indications for hospitalization (other than emergency room visit) including tubo-ovarian abscess

NOTE: For protocols utilizing this Addendum, when the same parameter appears in both this Female Genital Grading Table and the main DAIDS AE Grading Table, use the grading scheme in this table.

APPENDIX B (Cont'd)
DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS PUBLISH DATE: DECEMBER 2004
Addendum 1
Female Genital Grading Table for Use in Microbicide Studies

INFECTIONS AND DYSPLASIA				
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**
GENITOURINARY INFECTIONS				
Genital herpes	No lesions	Characteristic ulcerative or vesicular lesions confirmed by culture, PCR, Tzanck prep or other diagnostic test of lesion or previous type-specific serology, covering < 25% of vulva, vagina, or cervix	Same criteria as mild but covering 25-50% of vulvar, vaginal, or cervical surface	Same criteria as mild but covering > 50% of vulvar, vaginal, or cervical surface and/or symptoms of significant systemic involvement, e.g., encephalitis, hepatitis
Candida	Absence of symptoms regardless of candida test results	Positive culture, wet mount, or other laboratory test for yeast, with mild symptoms	Positive culture, wet mount, or other laboratory test for yeast, with moderate to severe symptoms	NA
Trichomonas	Negative	NA	Positive wet mount, culture, PCR or other licensed test, excluding pap smear, showing T. vaginalis, regardless of symptoms	NA
Bacterial Vaginosis (BV)	Negative	Asymptomatic BV diagnosed by Amsel criteria, wet mount, Gram stain, or licensed diagnostic test	Symptomatic confirmed by wet mount, Gram stain, or any licensed diagnostic test	NA
Chlamydia	Negative	NA	Positive culture or other diagnostic test for Chlamydia, asymptomatic or with mild uterine or cervical motion tenderness (no signs of peritoneal irritation)	Positive test for Chlamydia with abdominal or uterine or adnexal tenderness on examination, with or without adnexal mass, diffuse tenderness, any signs of peritoneal irritation, or indications for hospitalization (other than emergency room visit) including tubo-ovarian abscess

NOTE: For protocols utilizing this Addendum, when the same parameter appears in both this Female Genital Grading Table and the main DAIDS AE Grading Table, use the grading scheme in this table.

APPENDIX B (Cont'd)
DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS PUBLISH DATE: DECEMBER 2004
Addendum 1
Female Genital Grading Table for Use in Microbicide Studies

INFECTIONS AND DYSPLASIA				
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**
Gonorrhea	Negative	NA	Positive culture or other diagnostic test for Gonorrhea, asymptomatic or with mild uterine or cervical motion tenderness (no signs of peritoneal irritation)	Positive test for Gonorrhea with abdominal or uterine or adnexal tenderness on examination, with or without adnexal mass, diffuse tenderness, any signs of peritoneal irritation, or indications for hospitalization (other than emergency room visit) including tubo-ovarian abscess or disseminated gonococcal infection
Urinary tract infection (by urinalysis and urine culture)	Negative	5-10 WBC/hpf on urinalysis with a negative culture per protocol definition (with or without symptoms)	> 10 WBC/hpf on urinalysis OR a positive culture per protocol definition (with or without symptoms)	Pyelonephritis or sepsis (septicemia) due to urinary tract infection
Syphilis	Negative treponemal or non- treponemal test or both positive with known treatment and stable titers (< 4 fold increase)	NA	Syphilis diagnosed by a positive treponemal test along with a positive non-treponemal test and no previous treatment or a four- fold rise in titer on	Criteria for Grade 2 Syphilis in the presence of neurologic symptoms or a positive CSF VDRL or FTA-ABS
GENITAL DYSPLASIA				
Condyloma (specify site: cervical, vaginal, vulvar, perianal)	None	Condylomata causing no or mild interference with daily function	Condylomata causing moderate interference with daily function	Condylomata causing severe interference with daily function, secondary infection, or hospitalization (other than emergency room visit) indicated
Condyloma (specify site: cervical, vaginal, vulvar, perianal)	None	Condylomata causing no or mild interference with daily function	Condylomata causing moderate interference with daily function	Condylomata causing severe interference with daily function, secondary infection, or hospitalization (other than emergency room visit) indicated

NOTE: For protocols utilizing this Addendum, when the same parameter appears in both this Female Genital Grading Table and the main DAIDS AE Grading Table, use the grading scheme in this table.

APPENDIX B (Cont'd)
DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS PUBLISH DATE: DECEMBER 2004
Addendum 1
Female Genital Grading Table for Use in Microbicide Studies

INFECTIONS AND DYSPLASIA				
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**
Intraepithelial Neoplasia by biopsy (VIN, CIN, VAIN)	None	Intraepithelial Neoplasia 1 (IN1)	Intraepithelial Neoplasia 2 (IN2)	Carcinoma in situ (CIS) or Invasive carcinoma
Pap (use this category only if treatment performed without diagnostic testing, otherwise use biopsy category above)	nl PAP	ASCUS or LSIL	HSIL	Carcinoma in situ or carcinoma

NOTE: For protocols utilizing this Addendum, when the same parameter appears in both this Female Genital Grading Table and the main DAIDS AE Grading Table, use the grading scheme in this table.

APPENDIX B (Cont'd)
DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS PUBLISH DATE: DECEMBER 2004
Addendum 1
Female Genital Grading Table for Use in Microbicide Studies

UTERINE BLEEDING AND PREGNANCY COMPLICATIONS				
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**
ABNORMAL UTERINE BLEEDING UNRELATED TO PREGNANCY				
Menorrhagia [2] (prolonged and/or heavy menstrual bleeding)	Participant report of normal bleeding relative to her baseline	Increase from usual with no or minimal interference with usual social & functional activities (including sexual functioning)	Increase from usual with moderate interference with usual social & functional activities (including sexual)	Incapacitating or severe interference with usual social & functional activities (including sexual functioning), transfusion indicated
Metrorrhagia [2] (intermenstrual or frequent bleeding)	None or any expected nonmenstrual bleeding	Increase from usual with no or minimal interference with usual social & functional activities (including sexual functioning)	Increase from usual with moderate interference with usual social & functional activities (including sexual)	Incapacitating or severe interference with usual social & functional activities (including sexual functioning), transfusion indicated
Unexplained infrequent bleeding (excludes expected absence of menses due to hormonal contraception or pregnancy/ postpartum)	Participant report of normal or expected bleeding frequency	No menses for 1-3 months (missed menses)	No menses for > 3 months (oligomenorrhea/ amenorrhea)	NA
Postcoital bleeding	None	Occasional (< 25% of coital acts) OR Increase from usual with no or minimal interference with usual social functioning (including sexual functioning)	Frequent (25-75% of coital acts) OR Increase from usual with moderate interference with usual social functioning (including sexual)	Consistent (> 75% of coital acts) OR Incapacitating or severe interference with usual social functioning (including sexual functioning), transfusion indicated

[2] If both Menorrhagia and Metrorrhagia are present, a single adverse event should be reported as "Menometrorrhagia" and graded per the Menorrhagia grading scale.

NOTE: For protocols utilizing this Addendum, when the same parameter appears in both this Female Genital Grading Table and the main DAIDS AE Grading Table, use the grading scheme in this table.

APPENDIX B (Cont'd)
DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS PUBLISH DATE: DECEMBER 2004
Addendum 1
Female Genital Grading Table for Use in Microbicide Studies

UTERINE BLEEDING AND PREGNANCY COMPLICATIONS				
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**
COMPLICATIONS OF PREGNANCY				
First trimester bleeding	None	Spotting or bleeding less than menses with continuation of pregnancy	Bleeding like menses or heavier with continuation of pregnancy	Spontaneous abortion, or profuse bleeding with dizziness or orthostatic hypotension, transfusion indicated
Postabortal endometritis/salpingitis	None	Low grade fever and uterine tenderness, resolved with oral antibiotics	Moderate symptoms, requiring ≤ 3 days of parenteral antibiotics	Severe symptoms requiring > 3 days of IV antibiotics or development of tubo-ovarian abscess or diffuse peritonitis or severe uterine infection for which operative intervention indicated
Postpartum hemorrhage	EBL < 500 cc for vaginal delivery or < 1000 cc after CS or reported as normal	EBL 500-1000 for vaginal delivery or 1000-1500 for CS or reported as slightly increased	EBL > 1000 for vaginal delivery or > 1500 for CS, with or without mild dizziness, no transfusion required	Hemorrhage at a level for which transfusion of packed cells or any amount of other blood components is indicated
Postpartum endometritis	None	Low grade fever and uterine tenderness, resolved with oral antibiotics	Moderate symptoms, treated by ≤ 3 days of parenteral antibiotics	Severe symptoms treated with > 3 days of IV antibiotics or addition of heparin or infection for which operative intervention is indicated
Chorioamnionitis	None	Fever (38°C –38.4°C or 100.4°F– 100.9°F) with two or more: FHR> 160 BPM, maternal HR> 120, uterine tenderness between contractions or purulent AF or preterm labor	Same as Grade 1 plus fever 38.5°C –40°C or 101°F –104°F	Criteria for Grade 2 plus fetal distress or fever > 40°C or 104°F or either fetal demise or maternal symptoms of shock

NOTE: For protocols utilizing this Addendum, when the same parameter appears in both this Female Genital Grading Table and the main DAIDS AE Grading Table, use the grading scheme in this table.

APPENDIX B (Cont'd)
DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS PUBLISH DATE: DECEMBER 2004
Addendum 1
Female Genital Grading Table for Use in Microbicide Studies

UTERINE BLEEDING AND PREGNANCY COMPLICATIONS				
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**
Episiotomy infection	None	Mild erythema, edema, and tenderness of wound	Fever > 38°C or 100.4°F with erythema, edema, and tenderness of wound	Fever with wound dehiscence or debridement required or signs shock or necrotizing fasciitis
Second/third trimester bleeding	None	Bleeding less than menses	Bleeding like menses or greater, but not requiring intervention	Bleeding or coagulopathy requiring delivery or other intervention, e.g., transfusion, or fetal demise
Preterm rupture of membranes	None	NA	Preterm rupture with hospitalization but not resulting in delivery at less than 37 weeks' gestation	Delivery at < 37 weeks gestation or ≤ 2500 grams birth weight
Preterm contractions	None	Preterm contractions which resolve without medical intervention	Preterm contractions with cervical change which result in medical intervention but not resulting in preterm delivery	Delivery at < 37 weeks gestation or ≤ 2500 grams birth weight
Poor fetal growth	At or above 10th percentile	Fetal growth < 10th percentile but ≥ 3rd percentile for gestational age by ultrasound or newborn exam	NA	Fetal growth < 3rd percentile for gestational age by ultrasound or newborn exam

NOTE: For protocols utilizing this Addendum, when the same parameter appears in both this Female Genital Grading Table and the main DAIDS AE Grading Table, use the grading scheme in this table.

APPENDIX C
DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS
VERSION 1.0, DECEMBER, 2004; CLARIFICATION AUGUST 2009

The Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events ("DAIDS AE Grading Table") is a descriptive terminology which can be utilized for Adverse Event (AE) reporting. A grading (severity) scale is provided for each AE term.

This clarification of the DAIDS Table for Grading the Severity of Adult and Pediatric AE's provides additional explanation of the DAIDS AE Grading Table and clarifies some of the parameters.

I. Instructions and Clarifications

Grading Adult and Pediatric AEs

The DAIDS AE Grading Table includes parameters for grading both Adult and Pediatric AEs. When a single set of parameters is not appropriate for grading specific types of AEs for both Adult and Pediatric populations, separate sets of parameters for Adult and/or Pediatric populations (with specified respective age ranges) are given in the Table. If there is no distinction in the Table between Adult and Pediatric values for a type of AE, then the single set of parameters listed is to be used for grading the severity of both Adult and Pediatric events of that type.

> **Note:** In the classification of adverse events, the term "**severe**" is not the same as "**serious**." Severity is an indication of the intensity of a specific event (as in mild, moderate, or severe chest pain). The term "**serious**" relates to a participant/event outcome or action criteria, usually associated with events that pose a threat to a participant's life or functioning.

Addenda 1-3 Grading Tables for Microbicide Studies

For protocols involving topical application of products to the female genital tract, male genital area or rectum, strong consideration should be given to using Appendices I-III as the primary grading scales for these areas. The protocol would need to specifically state that one or more of the Appendices would be primary (and thus take precedence over the main Grading Table) for items that are listed in both the Appendix and the main Grading Table.

- Addendum 1 - Female Genital Grading Table for Use in Microbicide Studies - PDF
- Addendum 2 - Male Genital Grading Table for Use in Microbicide Studies - PDF
- Addendum 3 - Rectal Grading Table for Use in Microbicide Studies - PDF

Grade 5

For any AE where the outcome is death, the severity of the AE is classified as Grade 5.

Estimating Severity Grade for Parameters Not Identified in the Table

In order to grade a clinical AE that is not identified in the DAIDS AE grading table, use the category "Estimating Severity Grade" located on Page 3.

Determining Severity Grade for Parameters "Between Grades"

If the severity of a clinical AE could fall under either one of two grades (e.g., the severity of an AE could be either Grade 2 or Grade 3), select the higher of the two grades for the AE. If a laboratory value that is graded as a multiple of the ULN or LLN falls between two grades, select the higher of the two grades for the AE. For example, Grade 1 is 2.5 x ULN and Grade 2 is 2.6 x ULN for a parameter. If the lab value is 2.53 x ULN (which is between the two grades), the severity of this AE would be Grade 2, the higher of the two grades.

Values Below Grade 1

Any laboratory value that is between either the LLN or ULN and Grade 1 should not be graded.

APPENDIX C
DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS
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Determining Severity Grade when Local Laboratory Normal Values Overlap with Grade 1 Ranges

In these situations, the severity grading is based on the ranges in the DAIDS AE Grading Table, even when there is a reference to the local lab LLN.

For example: Phosphate, Serum, Low, Adult and Pediatric > 14 years (Page 20) Grade 1 range is 2.50 mg/dL - < LLN. A particular laboratory's normal range for Phosphate is 2.1 – 3.8 mg/dL. A participant's actual lab value is 2.5. In this case, the value of 2.5 exceeds the LLN for the local lab, but will be graded as Grade 1 per DAIDS AE Grading Table.

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II. Definitions of terms used in the Table:

Basic Self-care Functions	Adult Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding. Young Children Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).
LLN	Lower limit of normal
Medical Intervention	Use of pharmacologic or biologic agent(s) for treatment of an AE.
NA	Not Applicable
Operative Intervention	Surgical OR other invasive mechanical procedures.
ULN	Upper limit of normal
Usual Social & Functional Activities	Adult Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc. Young Children Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

APPENDIX C
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PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE
ESTIMATING SEVERITY GRADE			
Clinical adverse event NOT identified elsewhere in this DAIDS AE Grading Table	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities OR Medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death
SYSTEMIC			
Acute systemic allergic reaction	Localized urticaria (wheals) with no medical intervention indicated	Localized urticaria with medical intervention indicated OR Mild angioedema with no medical intervention indicated	Generalized urticaria OR Angioedema with medical intervention indicated OR Symptomatic mild Bronchospasm OR Acute anaphylaxis OR Life-threatening bronchospasm OR Laryngeal edema
Chills	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities
Fatigue Malaise	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities
Fever (nonaxillary)	37.7 – 38.6˚ C	38.7 – 39.3˚ C	$\geq$ 39.4
Pain (indicate body site) DO NOT use for pain due to injection (See Injection Site Reactions: Injection site pain) See also Headache, Arthralgia, and Myalgia	Pain causing no or minimal interference with usual social & functional activities	Pain causing greater than minimal interference with usual social & functional activities	Pain causing inability to perform usual social & functional activities OR Hospitalization (other than emergency room visit) indicated

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

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PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE
Unintentional weight loss	NA	5 – 9% loss in body weight from baseline	≥ 10% loss in body weight from baseline OR Aggressive intervention indicated [e.g., tube feeding or total parenteral nutrition (TPN)]
INFECTION			
Infection (any other than HIV infection)	Localized, no systemic antimicrobial treatment indicated AND Symptoms causing no or minimal interference with usual social & functional activities	Systemic antimicrobial treatment indicated OR Symptoms causing greater than minimal interference with usual social & functional activities	Systemic antimicrobial treatment indicated AND Symptoms causing inability to perform usual social & functional activities OR Operative intervention (other than simple incision and drainage) indicated
INJECTION SITE REACTIONS			
Injection site pain (pain without touching) Or Tenderness (pain when area is touched)	Pain/tenderness causing no or minimal limitation of use of limb	Pain/tenderness limiting use of limb OR Pain/tenderness causing greater than minimal interference with usual social & functional activities	Pain/tenderness causing inability to perform usual social & functional activities OR Hospitalization (other than emergency room visit) indicated for management of pain/tenderness
Injection site reaction (localized)			
Adult > 15 years	Erythema OR Induration of 5x5 cm – 9x9 cm (or 25 cm^2 – 81cm^2)	Erythema OR Induration OR Edema > 9 cm any diameter (or > 81 cm^2)	Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage OR Necrosis (involving dermis and deeper tissue)
Pediatric ≤ 15 years	Erythema OR Induration OR Edema present but ≤ 2.5 cm diameter	Erythema OR Induration OR Edema > 2.5 cm diameter but < 50% surface area of the extremity segment (e.g., upper arm/thigh)	Erythema OR Induration OR Edema involving ≥ 50% surface area of the extremity segment (e.g., upper arm/thigh) OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage OR Necrosis (involving dermis and deeper tissue)

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

APPENDIX C
DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS
VERSION 1.0, DECEMBER, 2004; CLARIFICATION AUGUST 2009

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE
Pruritis associated with injection See also Skin: Pruritis (itching - no skin lesions)	Itching localized to injection site AND Relieved spontaneously or with < 48 hours treatment	Itching beyond the injection site but not generalized OR Itching localized to injection site requiring48 hours treatment	Generalized itching causing inability to perform usual social & functional activities
SKIN – DERMATOLOGICAL			
Alopecia	Thinning detectable by study participant (or by caregiver for young children and disabled adults)	Thinning or patchy hair loss detectable by health care provider	Complete hair loss
Cutaneous reaction – rash	Localized macular rash	Diffuse macular, maculopapular, or morbilliform rash OR Target lesions	Diffuse macular, maculopapular, or morbilliform rash with vesicles or limited number of bullae OR Superficial ulcerations of mucous membrane limited to one site
Hyperpigmentation	Slight or localized	Marked or generalized	NA
Hypopigmentation	Slight or localized	Marked or generalized	NA
Pruritis (itching – no skin lesions) (See also Injection Site Reactions: Pruritis associated with injection)	Itching causing no or minimal interference with usual social & functional activities	Itching causing greater than minimal interference with usual social & functional activities	Itching causing inability to perform usual social & functional activities
CARDIOVASCULAR			
Cardiac arrhythmia (general) (By ECG or physical exam)	Asymptomatic AND No intervention indicated	Asymptomatic AND Non-urgent medical intervention indicated	Symptomatic AND medical intervention indicated
Cardiac- ischemia/infarction	NA	NA	Symptomatic ischemia (stable angina) OR Testing consistent with ischemia OR Unstable angina OR Acute myocardial infarction

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

APPENDIX C
DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS
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PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE
Hemorrhage (significant acute blood loss)	NA	Symptomatic AND No transfusion indicated	Symptomatic AND Transfusion of packed RBCs indicated
Hypertension			
Adult > 17 years (with repeat testing at same visit)	140 – 159 mmHg systolic OR 90 – 99 mmHg diastolic	160 – 179 mmHg systolic OR 100 – 109 mmHg diastolic	≥ 180 mmHg systolic OR ≥ 110 mmHg diastolic OR Hospitalization indicated (other than emergency room visit)
Correction: in Grade 2 to 160 - 179 from > 160-179 (systolic) and to ≥ 100 -109 from > 100-109 (diastolic) and in Grade 3 to ≥ 180 from > 180 (systolic) and to ≥ 110 from > 110 (diastolic).			
Pediatric ≤ 17 years (with repeat testing at same visit)	NA	91st – 94th percentile adjusted for age, height, and gender (systolic and/or diastolic)	≥ 95th percentile adjusted for age, height, and gender (systolic and/or diastolic) OR Hospitalization indicated (other than emergency room visit)
Hypotension	NA	Symptomatic, corrected with oral fluid replacement	Symptomatic, IV fluids indicated OR Use of vasopressors or mechanical assistance to maintain blood pressure
Pericardial effusion	Asymptomatic, small effusion requiring no intervention	Asymptomatic, moderate or larger effusion requiring no intervention	Effusion with non-life threatening physiologic consequences OR Effusion with non-urgent intervention indicated OR Life-threatening consequences (e.g., tamponade) OR Urgent intervention indicated)
Prolonged PR interval			
Adult > 16 years	PR interval 0.21 – 0.25 sec	PR interval > 0.25 sec	Type II 2nd degree AV block OR Ventricular pause > 3.0 sec OR Complete AV block
Pediatric ≤ 16 years	1st degree AV block (PR > normal for age and rate)	Type I 2nd degree AV block	Type II 2nd degree AV block OR Complete AV block

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

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DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS
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PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE
Prolonged QTc			
Adult > 16 years	Asymptomatic, QTc interval 0.45 – 0.47 sec OR Increase interval < 0.03 sec above baseline	Asymptomatic, QTc interval 0.48 – 0.49 sec OR Increase in interval 0.03 – 0.05 sec above baseline	Asymptomatic, QTc interval ≥ 0.50 sec OR Increase in interval ≥ 0.06 sec above baseline OR Life-threatening consequences (e.g., Torsade de pointes or other associated serious ventricular dysrhythmia)
Pediatric ≤ 16 years	Asymptomatic, QTc interval 0.450 – 0.464 sec	Asymptomatic, QTc interval 0.465 – 0.479 sec	Asymptomatic, QTc interval ≥ 0.480 sec OR Life-threatening consequences, (e.g., Torsade de pointes or other associated serious ventricular dysrhythmia)
Thrombosis/embolism	NA	Deep vein thrombosis AND No intervention indicated (e.g., anticoagulation, lysis filter, invasive procedure)	Deep vein thrombosis AND Intervention indicated (e.g., anticoagulation, lysis filter, invasive procedure) OR Embolic event (e.g., pulmonary embolism, life-threatening thrombus)
Vasovagal episode (associated with a procedure of any kind)	Present without loss of consciousness	Present with transient loss of consciousness	NA
Ventricular dysfunction (congestive heart failure)	NA	Asymptomatic diagnostic finding AND intervention indicated	New onset with symptoms OR Worsening symptomatic congestive heart failure OR Life-threatening congestive heart failure
GASTROINTESTINAL			
Anorexia	Loss of appetite without decreased oral intake	Loss of appetite associated with decreased oral intake without significant weight loss	Loss of appetite associated with significant weight loss OR Life-threatening consequences OR Aggressive intervention indicated [e.g., tube feeding or total parenteral nutrition (TPN)]
Comment: Please note that, while the grading scale provided for Unintentional Weight Loss may be used as a guideline when grading anorexia, this is not a requirement and should not be used as a substitute for clinical judgment.			
Ascites	Asymptomatic	Symptomatic AND Intervention indicated (e.g., diuretics or therapeutic paracentesis)	Symptomatic despite intervention OR Life-threatening consequences

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

APPENDIX C
DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS
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PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE
Cholecystitis	NA	Symptomatic AND Medical intervention indicated	Radiologic, endoscopic, or operative intervention indicated OR Life-threatening consequences (e.g., sepsis or perforation)
Constipation	NA	Persistent constipation requiring regular use of dietary modifications, laxatives, or enemas	Obstipation with manual evacuation indicated OR Life-threatening consequences (e.g., obstruction)
Diarrhea			
Adult and Pediatric ≥ 1 year	Transient or intermittent episodes of unformed stools OR Increase of ≤ 3 stools over baseline per 24-hour period	Persistent episodes of unformed to watery stools OR Increase of 4 – 6 stools over baseline per 24-hour period	Bloody diarrhea OR Increase of ≥ 7 stools per 24-hour period OR IV fluid replacement indicated OR Life-threatening consequences (e.g., hypotensive shock)
Pediatric < 1 year	Liquid stools (more unformed than usual) but usual number of stools	Liquid stools with increased number of stools OR Mild dehydration	Liquid stools with moderate dehydration OR Liquid stools resulting in severe dehydration with aggressive rehydration indicated OR Hypotensive shock
Dysphagia-Odynophagia	Symptomatic but able to eat usual diet	Symptoms causing altered dietary intake without medical intervention indicated	Symptoms causing severely altered dietary intake with medical intervention indicated OR Life-threatening reduction in oral intake
Mucositis/stomatitis (clinical exam) Indicate site (e.g., larynx, oral) See Genitourinary for Vulvovaginitis. See also Dysphagia-Odynophagia and Proctitis	Erythema of the mucosa	Patchy pseudomembranes or ulcerations	Confluent pseudomembranes or ulcerations OR Mucosal bleeding with minor trauma OR Tissue necrosis OR Diffuse spontaneous mucosal bleeding OR Life-threatening consequences

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

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DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS
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PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE
Nausea	Transient (< 24 hours) or intermittent nausea with no or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24 – 48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours OR Aggressive rehydration indicated (e.g., IV fluids) OR Life-threatening consequences (e.g., hypotensive shock)
Pancreatitis	NA	Symptomatic AND Hospitalization not indicated (other than emergency room visit)	Symptomatic AND Hospitalization indicated (other than emergency room visit) OR Life-threatening consequences (e.g., circulatory failure, hemorrhage, sepsis)
Proctitis (functional-symptomatic) Also see Mucositis/stomatitis for clinical exam	Rectal discomfort AND No intervention indicated	Symptoms causing greater than minimal interference with usual social & functional activities OR Medical intervention indicated	Symptoms causing inability to perform usual social & functional activities OR Operative intervention indicated OR Life-threatening consequences (e.g., perforation)
Vomiting	Transient or intermittent vomiting with no or minimal interference with oral intake	Frequent episodes of vomiting with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR Aggressive rehydration indicated (e.g., IV fluids) or Life-threatening consequences (e.g., hypotensive shock)

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

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DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS
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PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE
NEUROLOGIC			
Alteration in personality-behavior or in mood (e.g., agitation, anxiety, depression, mania, psychosis)	Alteration causing no or minimal interference with usual social & functional activities	Alteration causing greater than minimal interference with usual social & functional activities	Alteration causing inability to perform usual social & functional Activities OR Behavior potentially harmful to self or others.
Altered Mental Status For Dementia, see Cognitive and behavioral/attentional disturbance (including dementia and attention deficit disorder)	Changes causing no or minimal interference with usual social & functional activities	Mild lethargy or somnolence causing greater than minimal interference with usual social & functional activities	Confusion, memory impairment, lethargy, or somnolence causing inability to perform usual social & functional activities OR Delirium OR obtundation, OR coma
Ataxia	Asymptomatic ataxia detectable on exam OR Minimal ataxia causing no or minimal interference with usual social & functional activities	Symptomatic ataxia causing greater than minimal interference with usual social & functional activities	Symptomatic ataxia causing inability to perform usual social & functional activities OR Disabling ataxia causing inability to perform basic self-care functions

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

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DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS
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PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE
Cognitive and behavioral/attentional disturbance (including dementia and attention deficit disorder)	Disability causing no or minimal interference with usual social & functional activities OR Specialized resources not indicated	Disability causing greater than minimal interference with usual social & functional activities OR Specialized resources on part-time basis	Disability causing inability to perform usual social & functional activities OR Specialized resources on a full-time basis indicated OR Disability causing inability to perform basic self-care functions OR Institutionalization indicated
CNS ischemia (acute)	NA	NA	Transient ischemic attack OR Cerebral vascular accident (CVA, stroke) with neurological deficit
Developmental delay – **Pediatric ≤ 16 years**	Mild developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Moderate developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Severe developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting OR Developmental regression, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting
Headache	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities OR Symptoms causing inability to perform basic self-care functions OR Hospitalization indicated (other than emergency room visit) OR Headache with significant impairment of alertness or other neurologic function
Insomnia	NA	Difficulty sleeping causing greater than minimal interference with usual social & functional activities	Difficulty sleeping causing inability to perform usual social & functional activities OR Disabling insomnia causing inability to perform basic self-care functions
Neuromuscular weakness (including myopathy & neuropathy)	Asymptomatic with decreased strength on exam OR Minimal muscle weakness causing no or minimal interference with usual social & functional activities	Muscle weakness causing greater than minimal interference with usual social & functional activities	Muscle weakness causing inability to perform usual social & functional activities OR Disabling muscle weakness causing inability to perform basic self-care functions OR Respiratory muscle weakness impairing ventilation

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

APPENDIX C
DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS
VERSION 1.0, DECEMBER, 2004; CLARIFICATION AUGUST 2009

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE
Neurosensory alteration (including paresthesia and painful neuropathy)	Asymptomatic with sensory alteration on exam or minimal paresthesia causing no or minimal interference with usual social & functional activities	Sensory alteration or paresthesia causing greater than minimal interference with usual social & functional activities	Sensory alteration or paresthesia causing inability to perform usual social & functional activities OR Disabling sensory alteration or paresthesia causing inability to perform basic self-care functions
Seizure: (new onset) **– Adult ≥ 18 years** See also Seizure: (known pre-existing seizure disorder)	NA	1 seizure	> 1 seizure
Seizure: (known pre-existing seizure disorder) **– Adult ≥ 18 years** For worsening of existing epilepsy the grades should be based on an increase from previous level of control to any of these levels.	NA	Increased frequency of pre-existing seizures (non-repetitive) without change in seizure character OR Infrequent break- through seizures while on stable medication in a previously controlled seizure disorder	Change in seizure character from baseline either in duration or quality (e.g., severity or focality) OR Seizures of any kind which are prolonged, repetitive (e.g., status epilepticus), or difficult to control (e.g., refractory epilepsy)
Seizure **– Pediatric < 18 years**	Seizure, generalized onset with or without secondary generalization, lasting < 5 minutes with < 24 hours post ictal state	Seizure, generalized onset with or without secondary generalization, lasting 5 – 20 minutes with < 24 hours post ictal state	Seizure, generalized onset with or without secondary generalization, lasting > 20 minutes OR Seizure, generalized onset with or without secondary generalization, requiring intubation and sedation
Syncope (not associated with a procedure)	NA	Present	NA
Vertigo	Vertigo causing no or minimal interference with usual social & functional activities	Vertigo causing greater than minimal interference with usual social & functional activities	Vertigo causing inability to perform usual social & functional activities OR Disabling vertigo causing inability to perform basic self-care functions

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

APPENDIX C
DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS
VERSION 1.0, DECEMBER, 2004; CLARIFICATION AUGUST 2009

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE
RESPIRATORY			
Bronchospasm (acute)	FEV1 or peak flow reduced to 70 – 80%	FEV1 or peak flow 50 – 69%	FEV1 or peak flow 25 – 49% OR Cyanosis OR FEV1 or peak flow < 25% OR Intubation
Dyspnea or respiratory distress			
Adult ≥ 14 years	Dyspnea on exertion with no or minimal interference with usual social & functional activities	Dyspnea on exertion causing greater than minimal interference with usual social & functional activities	Dyspnea at rest causing inability to perform usual social & functional activities OR Respiratory failure with ventilatory support indicated
Pediatric < 14 years	Wheezing OR minimal increase in respiratory rate for age	Nasal flaring OR Intercostal retractions OR Pulse oximetry 90 – 95%	Dyspnea at rest causing inability to perform usual social & functional activities OR Pulse oximetry < 90% OR Respiratory failure with ventilatory support indicated
MUSCULOSKELETAL			
Arthralgia See also Arthritis	Joint pain causing no or minimal interference with usual social & functional activities	Joint pain causing greater than minimal interference with usual social & functional activities	Joint pain causing inability to perform usual social & functional activities OR Disabling joint pain causing inability to perform basic self-care functions
Arthritis See also Arthralgia	Stiffness or joint swelling causing no or minimal interference with usual social & functional activities	Stiffness or joint swelling causing greater than minimal interference with usual social & functional activities	Stiffness or joint swelling causing inability to perform usual social & functional activities OR Disabling joint stiffness or swelling causing inability to perform basic self-care functions
Bone Mineral Loss			
Adult ≥ 21 years	BMD t-score -2.5 to -1.0	BMD t-score < -2.5	Pathological fracture (including loss of vertebral height) OR Pathologic fracture causing life-threatening consequences
Pediatric < 21 years	BMD z-score -2.5 to -1.0	BMD z-score < -2.5	Pathological fracture (including loss of vertebral height) OR Pathologic fracture causing life-threatening consequences

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

APPENDIX C
DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS
VERSION 1.0, DECEMBER, 2004; CLARIFICATION AUGUST 2009

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE
Myalgia (non-injection site)	Muscle pain causing no or minimal interference with usual social & functional activities	Muscle pain causing greater than minimal interference with usual social & functional activities	Muscle pain causing inability to perform usual social & functional activities OR Disabling muscle pain causing inability to perform basic self-care functions
Osteonecrosis	NA	Asymptomatic with radiographic findings AND No operative intervention indicated	Symptomatic bone pain with radiographic findings OR Operative intervention indicated OR Disabling bone pain with radiographic findings causing inability to perform basic self-care functions
GENITOURINARY			
Cervicitis (symptoms) (For use in studies evaluating topical study agents) For other cervicitis see Infection: Infection (any other than HIV infection)	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities and basic self-care functions.
Cervicitis (clinical exam) (For use in studies evaluating topical study agents) For other cervicitis see Infection: Infection (any other than HIV infection)	Minimal cervical abnormalities on examination (erythema, mucopurulent discharge, or friability) OR Epithelial disruption < 25% of total surface	Moderate cervical abnormalities on examination (erythema, mucopurulent discharge, or friability) OR Epithelial disruption of 25 – 49% total surface	Severe cervical abnormalities on examination (erythema, mucopurulent discharge, or friability) OR Epithelial disruption > 50% total surface
Inter-menstrual bleeding (IMB)	Spotting observed by participant OR Minimal blood observed during clinical or colposcopic examination	Inter-menstrual bleeding not greater in duration or amount than usual menstrual cycle	Inter-menstrual bleeding greater in duration or amount than usual menstrual cycle OR Hemorrhage with life-threatening hypotension OR Operative intervention indicated
Urinary tract obstruction (e.g., stone)	NA	Signs or symptoms of urinary tract obstruction without hydronephrosis or renal dysfunction	Signs or symptoms of urinary tract obstruction with hydronephrosis or renal dysfunction OR Obstruction causing life-threatening consequences

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

APPENDIX C
DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS
VERSION 1.0, DECEMBER, 2004; CLARIFICATION AUGUST 2009

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE
Vulvovaginitis (symptoms) (Use in studies evaluating topical study agents) For other vulvovaginitis see Infection: Infection (any other than HIV infection)	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities OR basic self-care functions
Vulvovaginitis (clinical exam) (Use in studies evaluating topical study agents) For other vulvovaginitis see Infection: Infection (any other than HIV infection)	Minimal vaginal abnormalities on examination OR Epithelial disruption < 25% of total surface	Moderate vaginal abnormalities on examination OR Epithelial disruption of 25 - 49% total surface	Severe vaginal abnormalities on examination OR Epithelial disruption ≥50% total surface
OCULAR/VISUAL			
Uveitis	Asymptomatic but detectable on exam	Symptomatic anterior uveitis OR Medical intervention indicated	Posterior or pan-uveitis OR Operative intervention indicated OR Disabling visual loss in affected eye(s)
Visual changes (from baseline)	Visual changes causing no or minimal interference with usual social & functional activities	Visual changes causing greater than minimal interference with usual social & functional activities	Visual changes causing inability to perform usual social & functional activities OR Disabling visual loss in affected eye(s)
ENDOCRINE/METABOLIC			
Abnormal fat accumulation (e.g., back of neck, breasts, abdomen)	Detectable by study participant (or by caregiver for young children and disabled adults)	Detectable on physical exam by health care provider	Disfiguring OR Obvious changes on casual visual inspection
Diabetes mellitus	NA	New onset without need to initiate medication OR Modification of current medications to regain glucose control	New onset with initiation of medication indicated OR Diabetes uncontrolled despite treatment modification OR Life-threatening consequences (e.g., ketoacidosis, hyperosmolar non-ketotic coma)

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

APPENDIX C
DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS
VERSION 1.0, DECEMBER, 2004; CLARIFICATION AUGUST 2009

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE
Gynecomastia	Detectable by study participant or caregiver (for young children and disabled adults)	Detectable on physical exam by health care provider	Disfiguring OR Obvious on casual visual inspection
Hyperthyroidism	Asymptomatic	Symptomatic causing greater than minimal interference with usual social & functional activities OR Thyroid suppression therapy indicated	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment modification OR Life-threatening consequences (e.g., thyroid storm)
Hypothyroidism	Asymptomatic	Symptomatic causing greater than minimal interference with usual social & functional activities OR Thyroid replacement therapy indicated	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment modification OR Life-threatening consequences (e.g., myxedema coma)
Lipoatrophy (e.g., fat loss from the face, extremities, buttocks)	Detectable by study participant (or by caregiver for young children and disabled adults)	Detectable on physical exam by health care provider	Disfiguring OR Obvious on casual visual inspection

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

APPENDIX C

DAIDS Toxicity Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0 - December 2004 (Clarification dated August 2009)

LABORATORY			
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**
HEMATOLOGY ***Standard International Units are listed in italics***			
Absolute CD4+ count **– Adult and Pediatric > 13 years** (HIV NEGATIVE ONLY)	300 – 400/mm^3 *300 – 400/µL*	200 – 299/mm^3 *200 – 299/µL*	≤ 199/mm^3 ≤ *199/µL*
Absolute lymphocyte count **– Adult and Pediatric > 13 years** (HIV NEGATIVE ONLY)	600 – 650/mm^3 *0.600 x 10^9 – 0.650 x 10^9/L*	500 – 599/mm^3 *0.500 x 10^9 – 0.599 x 10^9/L*	≤ 499/mm^3 ≤ *0.499 x 10^9/L*
Comment: Values in children ≤ 13 years are not given for the two parameters above because the absolute counts are variable.			
Absolute neutrophil count (ANC)			
Adult and Pediatric, > 7 days	1,000 – 1,300/mm^3 *1.000 x 10^9 – 1.300 x 10^9/L*	750 – 999/mm^3 *0.750 x 10^9 – 0.999 x 10^9/L*	≤ 749/mm^3 ≤ *0.749 x 10^9/L*
Infant*†, 2 – ≤ 7 days	1,250 – 1,500/mm^3 *1.250 x 10^9 – 1.500 x 10^9/L*	1,000 – 1,249/mm^3 *1.000 x 10^9 – 1.249 x 10^9/L*	≤ 999/mm^3 ≤ *0.999 x 10^9/L*
Infant*†, ≤1 day	4,000 – 5,000/mm^3 *4.000 x 10^9 – 5.000 x 10^9/L*	3,000 – 3,999/mm^3 *3.000 x 10^9 – 3.999 x10^9/L*	≤ 2,999/mm^3 ≤ *2.999 x 10^9/L*
Comment: Parameter changed from "Infant, < 1 day" to "Infant, ≤1 day"			
Fibrinogen, decreased	100 – 200 mg/dL *1.00 – 2.00 g/L* OR 0.75 – 0.99 x LLN	75 – 99 mg/dL *0.75 – 0.99 g/L* OR 0.50 – 0.74 x LLN	≤ 74 mg/dL ≤ *0.74 g/L* OR ≤ 0.49 x LLN OR Associated with gross bleeding

NOTE: For protocols utilizing this Addendum, when the same parameter appears in both this Female Genital Grading Table and the main DAIDS AE Grading Table, use the grading scheme in this table.

APPENDIX C (Cont'd)

DAIDS Toxicity Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0 - December 2004 (Clarification dated August 2009)

LABORATORY			
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**
Hemoglobin (Hgb)			
Comment: The Hgb values in mmol/L have changed because the conversion factor used to convert g/dL to mmol/L has been changed from 0.155 to 0.6206 (the most commonly used conversion factor). For grading Hgb results obtained by an analytic method with a conversion factor other than 0.6206, the result must be converted to g/dL using the appropriate conversion factor for that lab.			
Adult and Pediatric ≥ 57 days (HIV POSITIVE ONLY)	8.5 – 10.0 g/dL *5.24 – 6.23 mmol/L*	7.5 – 8.4 g/dL *4.62–5.23 mmol/L*	≤ 7.4 g/dL ≤ *4.61 mmol/L*
Adult and Pediatric ≥ 57 days (HIV NEGATIVE ONLY)	10.0 – 10.9 g/dL *6.18 – 6.79 mmol/L* OR Any decrease 2.5 – 3.4 g/dL *1.58 – 2.13 mmol/L*	9.0 – 9.9 g/dL *5.55 - 6.17 mmol/L* OR Any decrease 3.5 – 4.4 g/dL *2.14 – 2.78 mmol/L*	≤ 8.9 g/dL ≤ *5.54 mmol/L* OR Any decrease ≥ 4.5 g/dL ≥ *2.79 mmol/L*
Comment: The decrease is a decrease from baseline			
Infant*†, 36 – 56 days (HIV POSITIVE OR NEGATIVE)	8.5 – 9.4 g/dL *5.24 – 5.86 mmol/L*	7.0 – 8.4 g/dL *4.31 – 5.23 mmol/L*	≤ 6.9 g/dL ≤ *4.30 mmol/L*
Infant*†, 22 – 35 days (HIV POSITIVE OR NEGATIVE)	9.5 – 10.5 g/dL *5.87 - 6.54 mmol/L*	8.0 – 9.4 g/dL *4.93 – 5.86 mmol/L*	≤ 7.9 g/dL ≤ *4.92 mmol/L*
Infant*†, ≤ 21 days (HIV POSITIVE OR NEGATIVE)	12.0 – 13.0 g/dL *7.42 – 8.09 mmol/L*	10.0 – 11.9 g/dL *6.18 – 7.41 mmol/L*	≤ 9.9 g/dL ≤ *6.17 mmol/L*
Correction: Parameter changed from "Infant < 21 days" to "Infant ≤ 21 days"			
International Normalized Ratio of prothrombin time (INR)	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	≥ 2.1 x ULN
Methemoglobin	5.0 – 10.0%	10.1 – 15.0%	≥ 15.1%
Prothrombin Time (PT)	1.1 – 1.25 x ULN	1.26 – 1.50 x ULN	≥ 1.51 x ULN
Partial Thromboplastin Time (PTT)	1.1 – 1.66 x ULN	1.67 – 2.33 x ULN	≥ 2.34 x ULN
Platelets, decreased	100,000 – 124,999/mm^3 *100.000 x 10^9 – 124.999 x 10^9/L*	50,000 – 99,999/mm^3 *50.000 x 10^9 – 99.999 x 10^9/L*	≤ 49,999/mm^3 ≤ *49.999 x 10^9/L*
WBC, decreased	2,000 – 2,500/mm^3 *2.000 x 10^9 – 2.500 x 10^9/L*	1,500 – 1,999/mm^3 *1.500 x 10^9 – 1.999 x 10^9/L*	≤ 1,499/mm^3 ≤ *1.499 x 10^9/L*

APPENDIX C (Cont'd)

DAIDS Toxicity Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0 - December 2004 (Clarification dated August 2009)

LABORATORY				
PARAMETER		**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**
CHEMISTRIES	***Standard International Units are listed in italics***			
Acidosis		NA	pH < normal, but ≥ 7.3	pH < 7.3
Albumin, serum, low		3.0 g/dL – < LLN *30 g/L – < LLN*	2.0 – 2.9 g/dL *20 – 29 g/L*	< 2.0 g/dL *< 20 g/L*
Alkaline Phosphatase		1.25 – 2.5 x ULN[†]	2.6 – 5.0 x ULN[†]	≥ 5.1 – 10.0 x ULN[†]
Alkalosis		NA	pH > normal, but ≤ 7.5	pH > 7.5
ALT (SGPT)		1.25 – 2.5 x ULN	2.6 – 5.0 x ULN	≥ 5.1 x ULN
AST (SGOT)		1.25 – 2.5 x ULN	2.6 – 5.0 x ULN	≥ 5.1 x ULN
Bicarbonate, serum, low		16.0 mEq/L – < LLN *16.0 mmol/L – < LLN*	11.0 – 15.9 mEq/L *11.0 – 15.9 mmol/L*	≥ 8.0 mEq/L *≥ 8.0 mmol/L*
Comment: Some laboratories will report this value as Bicarbonate (HCO_3) and others as Total Carbon Dioxide (CO_2). These are the same tests; values should be graded according to the ranges for Bicarbonate as listed above.				
Bilirubin (Total)				
	Adult and Pediatric > 14 days	1.1 – 1.5 x ULN	1.6 – 2.5 x ULN	≥ 2.6 x ULN
	Infant[*†], ≤ 14 days (non-hemolytic)	NA	20.0 – 25.0 mg/dL *342 – 428 µmol/L*	≥ 25.1 mg/dL *≥ 429 µmol/L*
	Infant[*†], ≤ 14 days (hemolytic)	NA	NA	≥ 20.0 mg/dL *≥ 342 µmol/L*
Calcium, serum, high				
	Adult and Pediatric ≥ 7 days	10.6 – 11.5 mg/dL *2.65 – 2.88 mmol/L*	11.6 – 12.5 mg/dL *2.89 – 3.13 mmol/L*	≥ 12.6 mg/dL *≥ 3.14 mmol/L*
	Infant[*†], < 7 days	11.5 – 12.4 mg/dL *2.88 – 3.10 mmol/L*	12.5 – 12.9 mg/dL *3.11 – 3.23 mmol/L*	≥ 13.0 mg/dL *≥ 3.245 mmol/L*
Calcium, serum, low				
	Adult and Pediatric ≥ 7 days	7.8 – 8.4 mg/dL *1.95 – 2.10 mmol/L*	7.0 – 7.7 mg/dL *1.75 – 1.94 mmol/L*	≤ 6.1 mg/dL *≤ 1.53 mmol/L*
	Infant[*†], < 7 days	6.5 – 7.5 mg/dL *1.63 – 1.88 mmol/L*	6.0 – 6.4 mg/dL *1.50 – 1.62 mmol/L*	≤ 5.50 mg/dL *≤ 1.38 mmol/L*
Comment: Do not adjust Calcium, serum, low or Calcium, serum, high for albumin				

APPENDIX C (Cont'd)

DAIDS Toxicity Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0 - December 2004 (Clarification dated August 2009)

LABORATORY			
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**
Cardiac troponin I (cTnI)	NA	NA	Levels consistent with myocardial infarction or unstable angina as defined by the manufacturer
Cardiac troponin T (cTnT)	NA	NA	≥ 0.20 ng/mL OR Levels consistent with myocardial infarction or unstable angina as defined by the manufacturer
Cholesterol (fasting)			
Adult ≥ 18 years	200 – 239 mg/dL *5.18 – 6.19 mmol/L*	240 – 300 mg/dL *6.20 – 7.77 mmol/L*	> 300 mg/dL *> 7.77 mmol/L*
Pediatric < 18 years	170 – 199 mg/dL *4.40 – 5.15 mmol/L*	200 – 300 mg/dL *5.16 – 7.77 mmol/L*	> 300 mg/dL *> 7.77 mmol/L*
Creatine Kinase	3.0 – 5.9 x ULN[†]	6.0 – 9.9 x ULN[†]	≥ 10.0 x ULN[†]
Creatinine	1.1 – 1.3 x ULN[†]	1.4 – 1.8 x ULN[†]	≥ 1.9 x ULN[†]
Glucose, serum, high			
Nonfasting	116 – 160 mg/dL *6.44 – 8.88 mmol/L*	161 – 250 mg/dL *8.89 – 13.88 mmol/L*	≥ 251 mg/dL *≥ 13.89 mmol/L*
Fasting	110 – 125 mg/dL *6.11 – 6.94 mmol/L*	126 – 250 mg/dL *6.95 – 13.88 mmol/L*	≥ 251 mg/dL *≥ 13.89 mmol/L*
Glucose, serum, low			
Adult and Pediatric ≥ 1 month	55 – 64 mg/dL *3.05 – 3.55 mmol/L*	40 – 54 mg/dL *2.22 – 3.06 mmol/L*	< 40 mg/dL *≤ 2.21 mmol/L*
Infant[*†], < 1 month	50 – 54 mg/dL *2.78 – 3.00 mmol/L*	40 – 49 mg/dL *2.22 – 2.77 mmol/L*	< 40 mg/dL *≤ 2.21 mmol/L*
Lactate	ULN - < 2.0 x ULN without acidosis	≥ 2.0 x ULN without acidosis	Increased lactate with pH < 7.3

APPENDIX C (Cont'd)

DAIDS Toxicity Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0 - December 2004 (Clarification dated August 2009)

LABORATORY			
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**
Comment: Added ULN to Grade 1 parameter			
LDL cholesterol (fasting)			
Adult ≥ 18 years	130 – 159 mg/dL *3.37 – 4.12 mmol/L*	160 – 190 mg/dL *4.13 – 4.90 mmol/L*	>190 mg/dL > 4.91 mmol/L
Pediatric > 2 - < 18 years	110 – 129 mg/dL *2.85 – 3.34 mmol/L*	130 – 189 mg/dL *3.35 – 4.90 mmol/L*	≥ 190 mg/dL *≥ 4.91 mmol/L*
Lipase	1.1 – 1.5 x ULN OR Refers to ULN	1.6 – 3.0 x ULN	≥ 3.1 x ULN
Magnesium, serum, low	1.2 – 1.4 mEq/L *0.60 – 0.70 mmol/L*	0.9 – 1.1 mEq/L *0.45 – 0.59 mmol/L*	≤ 0.89 mEq/L *≤ 0.44 mmol/L*
Pancreatic amylase	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	≥ 2.1 x ULN
Phosphate, serum, low			
Adult and Pediatric > 14 years	2.5 mg/dL – < LLN *0.81 mmol/L – < LLN*	2.0 – 2.4 mg/dL *0.65 – 0.80 mmol/L*	≤ 1.9 mg/dL *≤ 0.64 mmol/L*
Pediatric 1 year – 14 years	3.0 – 3.5 mg/dL *0.97 – 1.13 mmol/L*	2.5 – 2.9 mg/dL *0.81 – 0.96 mmol/L*	≤ 2.4 mg/dL ≤ 0.80 mg/dL *≤ 2.21 mmol/L 0.80 mmol/L*
Pediatric < 1 year	3.5 – 4.5 mg/dL *1.13 – 1.45 mmol/L*	2.5 – 3.4 mg/dL *0.81 – 1.12 mmol/L*	≤ 2.4 mg/dL *≤ 0.80 mmol/L*
Potassium, serum, high	5.6 – 6.0 mEq/L *5.6 – 6.0 mmol/L*	6.1 – 6.5 mEq/L *6.1 – 6.5 mmol/L*	≥ 6.6 mEq/L *≥ 6.6 mmol/L*
Potassium, serum, low	3.0 – 3.4 mEq/L *3.0 – 3.4 mmol/L*	2.5 – 2.9 mEq/L *2.5 – 2.9 mmol/L*	≤ 2.4 mEq/L *≤ 2.4 mmol/L*
Sodium, serum, high	146 – 150 mEq/L *146 – 150 mmol/L*	151 – 154 mEq/L *151 – 154 mmol/L*	≥ 155 mEq/L *≥ 155 mmol/L*
Sodium, serum, low	130 – 135 mEq/L *130 – 135 mmol/L*	125 – 129 mEq/L *125 – 129 mmol/L*	≤ 121 mEq/L *≤ 121 mmol/L*
Triglycerides (fasting)	NA	500 – 750 mg/dL *5.65 – 8.48 mmol/L*	≥ 751 mg/dL *≥ 8.49 mmol/L*

APPENDIX C (Cont'd)

DAIDS Toxicity Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0 - December 2004 (Clarification dated August 2009)

LABORATORY			
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**
Uric acid	7.5 – 10.0 mg/dL *0.45 – 0.59 mmol/L*	10.1 – 12.0 mg/dL *0.60 – 0.71 mmol/L*	≥ 12.1 mg/dL *≥ 0.72 mmol/L*
URINALYSIS	***Standard International Units are listed in italics***		
Hematuria (microscopic)	6 – 10 RBC/HPF	> 10 RBC/HPF	Gross, with or without clots OR with RBC casts
Proteinuria, random collection	1 +	2 – 3 +	4 +
Proteinuria, 24 hour collection			
Adult and Pediatric ≥ 10 years	200 – 999 mg/24 h *0.200 – 0.999 g/d*	1,000 – 1,999 mg/24 h *1.000 – 1.999 g/d*	≥ 2,000 mg/24 h *≥ 2.000 g/d*
Pediatric > 3 mo - < 10 years	201 – 499 mg/m^2/24 h *0.201 – 0.499 g/d*	500 – 799 mg/m^2/24 h *0.500 – 0.799 g/d*	≥ 800 mg/m^2/24 h *≥ 0.800 g/d*