Protocol Title	An exploratory Phase 1 mechanism-of-action study of ZB-06, a vaginal film containing HC4-N, an anti-sperm monoclonal antibody
Protocol Number	ZB-06-01
US IND/EudraCT Number	PIND 147498
Development Phase	1
Version	4.0
Date	October 28, 2021
Sponsor	ZabBio, Inc. 6160 Lusk Blvd., Suite 105 San Diego, CA 92121

SPONSOR PROTOCOL APPROVAL PAGE

Name and title of the person(s) authorized to sign the protocol and the protocol amendment(s) for the ZabBio, Inc.

Clinical	**Date**

Regulatory	**Date**

INVESTIGATOR AGREEMENT PAGE

I agree:

- To assume responsibility for the proper conduct of the study at this site and to conduct the study in compliance with this protocol, any future amendments and with any other study conduct procedures provided by ZabBio, Inc.
- Not to implement any deviations from or changes to the protocol, including protocol amendments, without agreement from ZabBio, Inc. and prior review and written approval from the Institutional Review Board/Ethics Committee, except where necessary to eliminate an immediate hazard to the subjects or for administrative aspects of the study (where permitted by all applicable regulatory requirements).
- That I am familiar with the appropriate use of the investigational drug, as described in this protocol and any other information provided by ZabBio, Inc. including, but not limited to, the current Investigator's Brochure provided by ZabBio, Inc.
- To ensure that all persons assisting me with the study are adequately informed about the investigational drug and of their study-related duties and functions.
- That I have been informed that certain regulatory authorities require ZabBio, Inc. to obtain and supply details about the investigator's ownership interest in ZabBio, Inc. or the study drug, and more generally about his or her financial ties with ZabBio, Inc. ZabBio, Inc. will use and disclose this financial information solely for the purpose of complying with regulatory requirements.
- To provide ZabBio, Inc. with such information regarding ownership interest and financial ties (including those of my spouse and dependent children).
- To provide a prompt update to this financial information if any relevant changes occur during the course of the study and for 1 year following completion of the study.
- That ZabBio, Inc. may disclose information about such ownership interests and financial ties to the appropriate regulatory authorities.

Eastern Virginia Medical School Clinical Research Center, Department of Obstetrics and Gynecology, 601 Colley Avenue, Norfolk, VA 23507

Site Name and Location

Andrea R Thurman MD, Professor of Obstetrics and Gynecology, EVMS

Principal Investigator Name and Title

Principal Investigator Signature **Date**

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LIST OF ABBREVIATIONS

AE(s)	Adverse event(s)
BV	Bacterial Vaginosis
CMC	Cervical mucus check
CRF	Case Report Form
CV	Cervicovaginal
CVL	Cervicovaginal Lavage
GCP	Good Clinical Practice
HIV	Human Immunodeficiency Virus
HPF	High Powered Field
ICF	Informed Consent Form
IRB	Institutional Review Board
IQR	Interquartile Range
IUD	Intra-Uterine Device
mAb	Monoclonal Antibody
NAAT	Nucleic Acid Amplification Test
NOAEL	No Observed Adverse Effect Level
OPK	Ovulation Predictor Kit
PCT	Post-Coital Test
PBS	Phosphate Buffered Saline
PI	Principal Investigator
PK	Pharmacokinetic
PMS	Progressively Motile Sperm
PSRT	Protocol Safety Review Team
PVA	Polyvinyl Alcohol
SAE	Serious Adverse Event
STI	Sexually Transmitted Infection
TEAE	Treatment Emergent Adverse Events
TOST	Two One-Sided T-Test Procedure
VCF	Vaginal Contraceptive Film

1 PROTOCOL SUMMARY

Protocol Title	An exploratory Phase 1 mechanism-of-action study of ZB-06, a vaginal film containing HC4-N, an anti-sperm monoclonal antibody
Protocol Number	ZB-06-01
Short Title	*In vivo* anti-sperm activity of ZB-06 film
Sample Size	Screen ~ 60 couples to complete 15 evaluable couples
Population	Healthy women 18 – 50 years of age, who have normal menstrual cycles and are not at risk of pregnancy due to prior surgical sterilization, and their male partners, with whom they are in a monogamous sexual relationship
Centers	One: Eastern Virginia Medical School
Study rationale	This study is intended to make a preliminary assessment of the potential contraceptive effectiveness and safety of ZB-06 by observing its ability to reduce motile sperm access to the endocervix.
Study Design	This is an exploratory Phase 1, single center, open-label, crossover, mechanism of action study using a surrogate marker for contraceptive efficacy (the Standardized Postcoital Test, PCT) to assess the contraceptive potential of the ZB-06 film. The study will enroll up to 35 couples to reach a total of 15 fully evaluable women who have completed the baseline and the active-product post-coital visits. The expected duration of study participation for each participant will be approximately three months, including the screening period.
Schedule of Procedures/Evaluations	Visit 1: Screening Visit 2: Enrollment Visit 3: Cervical mucus check at midcycle Visit 4; Baseline (no product) postcoital visit Visit 5: 24-hour follow-up Visit 6: Cervical mucus check at midcycle Visit 7: Postcoital visit after intercourse using ZB-06 film Visit 8: 24-hour follow-up Visit 9: Cervical mucus check at midcycle Visit 10: No-product postcoital visit 1 month after ZB-06 film use

Study Duration	Estimated total study duration, 18 months; individual participation, three months
Test product, dose, and mode of administration	ZB-06 film: The ZB-06 film contains 20 mg of the HC4-N sperm-agglutinating antibody which will be digitally inserted into the vagina by the participant 30 min before intercourse timed to midcycle, after documentation of sperm-receptive endocervical mucus in the preceding mucus check visit.
Reference therapy, dose, and mode of administration	Baseline PCT cycle, conducted without use of ZB-06 film
Primary Objective	To assess the potential contraceptive activity of the ZB-06 film using a surrogate assessment for contraceptive efficacy: the degree to which progressively motile sperm can be excluded from the endocervical mucus after intercourse preceded by a single administration of the ZB-06 film, compared to the same evaluation performed after intercourse without film.
Secondary Objectives	1. To assess the safety of a single dose of ZB-06 film preceding a single act of vaginal intercourse
Exploratory Objectives	1. To determine antisperm antibody concentrations and agglutination potency in vaginal fluids, endocervical mucus, and serum at baseline, and at 2-3 and 24 hours after intercourse with and without product use, and after intercourse without product, 1 month after product use 2. To assess the effect of the film on cervicovaginal immune mediators
Primary Endpoint	Number of progressively motile sperm in aspirated endocervical mucus at Visit 7, 2-3 hours after sexual intercourse using ZB-06 film.
Secondary Endpoints	Incidence of Grade 2 or greater adverse events in the participant and/or her male partner in the interval between Visit 4 (the baseline intercourse without product) and Visit 6, in comparison to the incidence of Grade 2 or greater adverse events in the interval between Visit 7 (intercourse with product) and Visit 9.

Exploratory Endpoints	1. Concentrations of anti-sperm-antibody in *vaginal fluid* measured by ELISA at baseline, at 2-3 hours, and at 24 hours after intercourse with and without product use, and at 1 month after product use 2. Sperm agglutination titer in *vaginal fluid* measured by endpoint dilution at baseline, and at 2-3 hours and at 24 hours after intercourse with and without product use, and at 1 month after product use 3. Concentrations of anti-sperm-antibody in *endocervical mucus* measured by ELISA at baseline, and at 2-3 hours and at 24 hours after intercourse with and without product use, and at 1 month after product use 4. Sperm agglutination titer in *endocervical mucus* by endpoint dilution measured at baseline, and at 2-3 hours and at 24 hours after intercourse with and without product use, and at 1 month after product use 5. Concentrations of anti-sperm antibodies in *serum* measured by ELISA at baseline, at 24 hours, and at 1 month after exposure to ZB-06 film 6. Sperm agglutination titer in *serum* by endpoint dilution measured at baseline, and 24 hours and 1 month after exposure to ZB-06 film 7. Concentrations of immune mediators in cervicovaginal lavage, measured via multiplex assays before and 24 hours after intercourse with vs. without study product. 8. Vaginal pH at baseline and 24 hours after intercourse with vs. without study product. 9. Nugent Score at Visit 6 baseline and 24 hours after intercourse with study product.

2 INTRODUCTION

2.1 Background

2.1.1 Product description

ZB-06 is an intravaginal film containing 20 mg of the HC4-N drug substance, an IgG1 monoclonal antibody that binds to CD52g, a glycoform expressed on the surface of human sperm. Note that HC4-N targets a unique glycan (CD52g) attached to a 12 amino acid peptide sequence designated CD52 (Diekman *et al.,* 2000). The CD52 peptide is abundant on the surface of human lymphocytes and other immune cells, but the molecule on immunocytes lacks the male-tract specific glycan CD52g. HC4-N does not bind to the CD52 peptide, and thus does not bind immune cells, but binds only to the male-tract specific glycan CD52g expressed on sperm. When HC4-N binds to sperm, it cross-links them causing them to agglutinate. The additional constituents of the film are a film-forming polymer (polyvinyl alcohol, PVA), maltitol, histidine, and polysorbate 20. The film is approximately 2" x 2".

2.1.2 Study rational

The primary objective of the study is to determine whether the ZB-06 film's novel mechanism of action, antibody mediated sperm agglutination, shows promise in a surrogate-efficacy proof-of-concept assessment.

Two lines of *in vivo* evidence suggest that human anti-sperm antibodies may be effective. First, an agglutinating anti-sperm antibody was shown to be effective as a contraceptive in rabbits (Castle *et al.,* 1997). And second, anti-sperm antibodies are observed in women suffering from immune infertility (Ustay, 1967; Bronson 1999). Indeed, the HC4-N antibody was cloned from circulating B-cells of an infertile woman with high-titer anti-sperm antibodies in her blood and genital tract, and without other explanation for her infertility (Isojima S, 1989). However, while *in vitro* and *in vivo* data suggest that an anti-sperm antibody might be efficacious, a demonstration of sperm inhibition in women would be an essential demonstration to justify the continued development of anti-sperm antibodies as nonhormonal contraceptives. This Phase 1 exploratory proof-of-concept study of ZB-06 will provide the evidence on mechanism of action of this HCA, HC4-N.

The assessment that will be used to assess the mechanism of action and potential efficacy of the ZB-06 film is termed the Standardized Postcoital Test (PCT). This protocol, developed by CONRAD and Eastern Virginia Medical School in 1995, has become recognized as the appropriate early assessment of intravaginally-active contraceptive methods, including vaginal spermicides and cervical barrier devices (Archer *et al.,* 1995; Mauck *et al.,* 1997; Mauck *et al.,* 2004; Burke *et al.,* 2010; Mauck *et al.,* 2017). We propose using the PCT to evaluate the highly novel mechanism of the ZB-06 film, namely sperm agglutination via antibody-mediated crosslinking. The PCT is an appropriate test at this exploratory stage of product development because a) multiple vaginally-active contraceptives that have been judged promising during PCT evaluations have gone on to show contraceptive effectiveness in human contraceptive

trials (Shihata and Trussell, 1991; Mauck *et al.,* 1996; Mauck *et al.,* 1999; Barnhart *et al.,* 2007; Burke *et al.,* 2010; Schwartz *et al.,* 2015; Barnhart *et al.,* 2016), and b) the PCT allows testing in women without putting them at risk of pregnancy because it is conducted in women who have been surgically sterilized. The latter consideration is particularly important because since the HC4-N target (the CD52g antigen) is not present on the sperm of other species (other than chimpanzee), preceding animal contraceptive testing of HC4-N has not been possible. Moreover, the novelty of this approach (there are no other contraceptives based on sperm-agglutinating antibodies) makes it difficult to determine its likely contraceptive efficacy. Thus, a mechanism-of-action study using the PCT protocol in sterilized women, is an appropriate early step in the assessment of the viability of this novel concept.

Conducting this exploratory study in sterilized women is also appropriate in light of the potential risk of sperm-antibody immune complexes inducing an active anti-sperm immune response that might prolong the contraceptive effect beyond the duration of the HC4-N antibody product in the female reproductive tract. We judge this to be an unlikely outcome but have nevertheless designed the study to investigate this possibility by including an additional study PCT cycle one month after the PCT cycle using the ZB-06 film. We will thus be able to make a preliminary assessment of a potential actively induced anti-sperm antibody response to the use of the ZB-06 film during semen exposure after unprotected intercourse.

Finally, this study will make preliminary assessments of safety and pharmacokinetics of the ZB-06 film.

2.1.3 Dose rationale

The amount of HC4-N mAb, 20 mg per ZB-06 DP film, matches the total mAb concentration for the similar MB66 film studied previously, containing anti-viral antibodies intended for prevention of HIV and HSV. Three lines of evidence suggest that the dose of HN4-N in ZB-06, inserted intravaginally 30 minutes to 4 hours post ZB-06 insertion, will be sufficient to prevent the large majority of sperm from reaching the upper region of the cervix post coitus:

1. Concentration of HC4-N needed for sperm agglutination *in vitro*: The concentration of HC4-N needed to agglutinate sperm within ~100 seconds is 40 µg/mL. The resting volume of intravaginal fluid has been estimated to be approximately 1 mL (Owen and Katz, 1999), with coital increases due to sexual excitement and semen deposition, typically raising the total volume to between 3 and 10 mL. A 20 mg dose of HC4-N would be expected to result in an antibody concentration of ~ 2,000 ug/mL.

2. Concentration of HC4-N needed for sperm agglutination in a surrogate sheep study: As a preliminary test of *in vivo* vaginal sperm agglutination in an animal model with similar vaginal dimensions to humans, 1 mL of HC4-N (333 ug/mL and 33 ug/mL; n = 3 for each group) in PBS, or PBS alone was instilled into the vaginas of sheep, with the investigators blinded as to the presence or absence and concentration of the antibody. Coital stirring was mimicked by stirring with a vaginal dilator for 10 seconds. Five minutes after dosing, 1 mL of fresh, liquified, pooled human semen was instilled, and

an additional five thrusts with the vaginal dilator were performed. Two minutes after instillation of semen, vaginal fluid was aspirated and immediately assessed for the number of progressively motile sperm. Expressed as a percentage reduction in progressively motile sperm from that observed in the PBS control, the reduction was 100% after instillation of 333 ug/mL (333 ug total dose), and 44% after instillation of 33 ug/mL (33 ug total dose).

3. Monoclonal antibody concentration *in vivo* from the MB66 clinical study: From the pharmacokinetic (PK) data from the MB66-01 clinical trial (Politch et al., submitted), the concentration of VRC01 at 1 and 4 hours after application of MB66 is ~500 microgram/mL at the cervical os. As the amount of VRC01 is 10 mg/ MB66 film, and the amount of HC4-N in a ZB-06 film is 20 mg, the anticipated concentration of HC4-N mAb is twice this, thus ~1,000 ug/mL.

Dose selection summary: As the concentration of HC4-N needed for complete sperm agglutination is ~40 microgram/mL *in vitro* and 33-333 microgram/mL in the sheep model, we anticipate that the concentration predicted by the VRC01 PK data at 1 and 4 hr post application (~1,000 ug/mL at the cervical os) will be sufficient to agglutinate all or nearly all sperm in the ejaculate. The dose of 20 mg of HC4-N per film is near the maximal dose that can be formulated into a film of this size and composition. We have chosen to use this near maximum Mab dose in order to improve the probability of robust protection. We have a high expectation of safety for these antibodies, which are fully human amino acid sequences with glycosylation patterns very similar to those of human antibodies. We believe such antibodies justify a strategy of choosing a dose near the practical limit in order to improve potency and duration of action.

2.1.4 Nonclinical Studies:

A brief summary of the nonclinical safety studies performed with ZB-06 is provided below. Additional details can be found in the Investigator's Brochure for ZB-06.

2.1.4.1 Tissue Cross Reactivity

A tissue cross reactivity study was performed to determine the potential cross reactivity of HC4-N with cryosections from a full panel of normal human, non-human primate (cynomolgus monkey), and rat (Sprague-Dawley) tissues. Epithelial cells in the human epididymis were selected as an ancillary control tissue due to the reported expression of CD52g in this tissue element (Johnston, *et al.,* 2020; Norton, *et al.,* 2002). In order to detect binding, HC4-N was applied to cryosections of tissues at two concentrations (10 and 2 µg/mL).

HC4-N stained the membrane and cytoplasm of epithelial cells in human epididymis consistent with the reported expression of CD52g in this cell type. In addition, membrane and cytoplasmic staining was observed with HC4-N in mesothelial cells in several tissues and ovarian surface epithelial cells in all three species, as well as in mononuclear cells in the rat spleen, although no literature was located describing CD52g expression by any of these cell types. HC4-N

produced cytoplasmic reactivity in retinal cells in all species, mononuclear cells in humans, lymphocytes in humans, and epithelial cells in a few tissues in all species. The reactivity in these cell types was restricted to the cytoplasmic compartment, and monoclonal antibody binding to cytoplasmic sites in tissue cross-reactivity studies generally is considered of little to no toxicologic significance due to the limited ability of antibody drugs to access the cytoplasmic compartment in vivo (Hall, *et al.,* 2008; Leach, *et al.,* 2010).

2.1.4.2 Rat toxicology test

An *in vivo* toxicology study was performed to evaluate the potential effects, identify target organs of toxicity, and to estimate the no observed adverse effect level (NOAEL) and the maximum tolerated dose (MTD) of ZB-06 after 9 consecutive intravaginal administrations to adult, female Sprague Dawley rats followed by 14 days of recovery.

All animals survived to the end of the study, and there were no test-article related body weight changes, clinical observations, clinical chemistry, urinalysis, gross necropsy, or histopathology findings that were considered to be toxicologically significant. Minimal, reversible changes in hematology were observed in the high-dose group compared with the control group, however there were no correlating macroscopic and microscopic findings or irritation associated with ZB-06 treatment, therefore these changes were not considered to be adverse.

Based on the absence of any irritation or any toxicologically significant adverse effects in any of the parameters evaluated following daily intravaginal administration of ZB-06 for 9 days to adult female rats, the NOAEL for ZB-06 was considered to be 5 mg/day. A maximum tolerated dose could not be determined in the study.

2.1.4.3 Rabbit vaginal irritation test

A rabbit vaginal irritation study was performed to evaluate the potential for vaginal irritation following intravaginal administration of ZB-06. The study evaluated once daily intravaginal administration of suspensions of ZB-06 at 1, 4, and 16 mg/mL for 10 consecutive days to New Zealand White Rabbits. Repeat administration of ZB-06 did not result in any meaningful effects on survival, detailed clinical observations, body weight/body weight change, hematology, coagulation, clinical chemistry, macroscopic, or organ weight evaluations. Test article-related microscopic findings were present in the vagina at ≥4 mg/mL but were not considered adverse due to their lack of severity.

Based on the absence of any adverse effects following once daily intravaginal administration of suspensions of ZB-06 to New Zealand White Rabbits at 1, 4, and 16 mg/mL for 10 consecutive days, the NOAEL for ZB-06 was considered to be >16 mg.

2.1.5 Clinical studies

There have been no human studies of the HC4-N drug substance or the ZB-06 film drug product.

2.1.6 Risks and benefits

This is the first study of ZB-06 in humans, and therefore its risks are not known. The animal studies done to date do not raise significant concerns. Prior clinical experience with similar PVA-based vaginal films such as a marketed nonoxynol-9 based contraceptive film (Vaginal Contraceptive Film, Apothecus Pharmaceutical Corporation), have side effects including vaginal irritation/inflammation, vaginal burning, vaginal discharge, and penile irritation for male sexual partners. Rare but serious side effects may include severe allergic reactions with rash, hives, and itching. There are no expected benefits to study participants, other than the possible satisfaction of having aided in the evaluation of a novel contraceptive method.

3 STUDY OBJECTIVES AND MEASUREMENTS

3.1 Study Objectives

3.1.1 Primary Objective:

To assess the potential contraceptive activity of the ZB-06 film using a surrogate assessment for likely contraceptive efficacy (the degree to which progressively motile sperm can be excluded from the endocervical mucus).

3.1.2 Secondary Objective:

To assess the safety of a single dose of ZB-06 film used with a single act of vaginal intercourse among female participants and their male partners.

3.1.3 Exploratory Objectives:

- To determine antisperm antibody concentrations and agglutination potency in vaginal fluids, endocervical mucus, and serum at baseline, and at 2-3 and 24 hours after intercourse with and without product use, and 1 month after product use
- To assess the effect of the film on cervicovaginal immune mediators

3.2 Study Endpoints

3.2.1 Primary endpoint:

Number of progressively motile sperm in aspirated endocervical mucus 2-3 hours after sexual intercourse using ZB-06.

3.2.2 Secondary endpoint:

Incidence of Grade 2 or greater adverse events in the participant and/or her male partner in the interval between Visit 4 (the baseline intercourse without product) and Visit 6, in comparison to the incidence of Grade 2 or greater adverse events in the interval between Visit 7 (intercourse with product) and Visit 9.

3.2.3 Exploratory endpoints:

1. Concentrations of anti-sperm-antibody in *vaginal fluid*, measured by ELISA at baseline, at 2-3 hours, and at 24 hours after intercourse with and without product use, and at 1 month after product use
2. Sperm agglutination titer in *vaginal fluid* measured by endpoint dilution at baseline, and at 2-3 hours and at 24 hours after intercourse with and without product use, and at 1 month after product use
3. Concentrations of anti-sperm-antibody in *endocervical mucus,* measured by ELISA at baseline, and at 2-3 hours and at 24 hours after intercourse with and without product use, and at 1 month after product use
4. Sperm agglutination titer in *endocervical mucus*, by endpoint dilution measured at baseline, and at 2-3 hours and at 24 hours after intercourse with and without product use, and at 1 month after product use
5. Concentrations of anti-sperm antibodies in *serum*, measured by ELISA at baseline, at 24 hours, and at 1 month after exposure to ZB-06 film
6. Sperm agglutination titer in *serum* by endpoint dilution measured at baseline, and 24 hours and 1 month after exposure to ZB-06 film
7. Concentrations of immune mediators in cervicovaginal lavage, measured via multiplex assays before and 24 hours after intercourse with and without study product.
8. Vaginal pH at baseline and 24 hours after intercourse with vs. without study product.
9. Nugent Score at Visit 6 baseline and 24 hours after intercourse with study product.

4 INVESTIGATIONAL PLAN

4.1 Study Design

This is a Phase 1, single center, open label, non-randomized, crossover, mechanism of action study, using a surrogate marker for contraceptive efficacy (the Standardized Postcoital Test, PCT) to assess the mechanism of action and contraceptive potential of the ZB-06 film.

The product to be tested is the ZB-06 vaginal film, containing 20 mg of the HC4-N anti-sperm antibody. Participants will be women who are not at risk for pregnancy due to surgical sterilization, and their male sexual partners. The individual evaluating the cervical mucus for midcycle characteristics and presence of sperm will be blinded as to the treatment cycle. Each participant will be seen in approximately ten visits, over a period of 3 - 6 months. Volunteers will be consented at Visit 1 and undergo procedures to confirm they are eligible to continue in the study. Each participant will undergo three PCT cycles. The first PCT cycle will be a baseline cycle, done without the use of any product, in order to demonstrate the participant's ability to undergo normal ovulatory events and to produce receptive, midcycle cervical mucus. The male partner's ability to produce motile sperm capable of penetrating the cervical mucus is also evaluated in this baseline cycle. A product use PCT cycle will be carried out during the subsequent menstrual cycle using the ZB-06 film. An additional PCT cycle without product will be carried out during the subsequent menstrual cycle. Cycles may need to be repeated depending on the characteristics of the cervical mucus and the number of sperm found in the vaginal pool and endocervical specimens (see Appendix B).

The primary endpoint will be an assessment of the potential contraceptive activity of the ZB-06 film using a surrogate assessment for contraceptive efficacy, i.e., observing the degree to which progressively motile sperm can be excluded from the endocervical mucus.

At ovulation, the participant's cervical mucus will be assessed by Insler scoring. Once adequate cervical mucus is documented to be present, the couple will have unprotected intercourse within approximately 24 hours of the cervical mucus check. Two to three hours after intercourse, the participant's vaginal fluid and cervical mucus will be evaluated for the presence of sperm (motile, immotile). The expectation is that at baseline, participants will have an average of greater than 5 motile sperm per high power field (HPF), averaged over 9 HPFs, and that with the use of the ZB-06 film, participants will on average have at least a 10-fold reduction in motile sperm per HPF, averaged over 9 HPFs.

The secondary objective will be assessed at each visit, by querying participants regarding any treatment-emergent adverse events (TEAEs). TEAEs from the male sexual partner will be assessed at each visit, per the female participant's report or from the male directly. The male partner will be interviewed for TEAEs by phone within 72 hours of intercourse with product. Safety will also be assessed by comparing the concentrations of cervicovaginal (CV) mucosal cytokines and chemokines with and without ZB-06 film use.

Finally, an estimation of the *in vivo* pharmacokinetics of HC4-N will be described in serum cervical mucus, and CV fluid. Our expectation is that serum levels of ZB-06 will be very low to unquantifiable, while CV fluid levels will be high, supporting local activity of the product.

A listing of all study procedures and sampling is provided in the Schedule of Events Table in Appendix A.

4.2 Selection and Withdrawal of Subjects

4.2.1 Inclusion Criteria:

- Age 18 - 50 years, inclusive
- General good health, by volunteer history and per investigator judgment
- History of regular menstrual cycles of 21 - 35 days (inclusive), by volunteer report
- History of a normal PAP smear or ASCUS with negative HPV testing within the previous 12 months
- Willing to abstain from intercourse and use of vaginal medications, lubricants, and other products as required in the protocol
- Willing to use non-spermicidal, lubricated condoms for any vaginal intercourse from the first day of each menstrual cycle until 72 hours before expected midcycle
- In a mutually monogamous relationship with a male partner who:
    - Is at least 18 years old
    - Has no known risk for sexually transmitted infections (STIs)
    - Is willing and able to comply with protocol requirements including sexual activity/ abstinence and condom use requirements
    - Can engage in vaginal intercourse with the participant, with and without condoms, as specified in protocol
    - Is not taking exogenous hormones (e.g. testosterone)
- Protected from pregnancy by female surgical sterilization
- Vaginal and cervical anatomy that, in the opinion of the investigator, lends itself to easy genital tract sample collection
- Willing to give voluntary consent, sign an informed consent form and comply with study procedures as required by the protocol

4.2.2 Exclusion Criteria:

- History of hysterectomy
- Surgical sterility or known history of infertility in male partner

- Sterility or known history of sperm dysfunction in male partner
- Currently pregnant by urine pregnancy test at the enrollment visit, or within two calendar months from the last pregnancy outcome. *Note: If recently pregnant must have had at least two spontaneous menses since pregnancy outcome*
- Current use of any hormonal contraceptive or a copper or hormonal IUD, or use of Depo-Provera in the last 120 days
- Currently breastfeeding or having breastfed an infant in the last two months, or planning to breastfeed during the course of the study
- Significant gynecological abnormalities (including abnormal vaginal bleeding, excessive vaginal discharge, or vulvar/vaginal pain or irritation)
- Current UTI, vaginal candidiasis, or symptomatic bacterial vaginosis
- History of sensitivity/allergy to ZB-06 film components, for either the volunteer or her male partner
- In the last three months, either the volunteer or her male partner diagnosed with or treated for any STI or pelvic inflammatory disease, or either partner with known risk factors for sexually transmitted infections. *Note: Women with a history of genital herpes or condylomata who have been asymptomatic for at least six months may be considered for eligibility*
- Positive test for *Trichomonas vaginalis, Neisseria gonorrhea*, *Chlamydia trachomatis,* or HIV at the screening visit
- Deep epithelial genital findings such as abrasions, ulcerations, and lacerations, or vesicles suspicious for an STI
- Known current drug or alcohol abuse which could impact study compliance
- Participation in any other investigational trial within the last 30 days or planned participation in any other investigational trial during the study
- History of gynecological procedures (including genital piercing) on the external genitalia, vagina or cervix within the last 14 days
- Abnormal finding on laboratory or physical examination or a social or medical condition in either the volunteer or her male partner which, in the opinion of the investigator, would make participation in the study unsafe or would complicate interpretation of data

4.2.3 Withdrawal of Subjects

Participants who sign the informed consent and agree to participate in the study, but do not meet eligibility criteria prior to undergoing the initial cervical mucus check (Visit 2) will not continue in the study. No electronic case report forms (eCRFs) will be completed for participants who do not undergo the cervical mucus check at Visit 2.

Once a participant undergoes Visit 2 procedures, she may be withdrawn from the study for the following reasons:

- Inability to achieve adequate cervical mucus in two attempts at the baseline cycle
- Inadequate sperm in endocervical aspirate during baseline testing, despite adequate mucus and presence of sperm in vaginal pool
- Medical reasons, including diagnosis of an STI (in the participant or her partner) or any intercurrent illness that would jeopardize the participant's health or the interpretation of the results of the study
- Failure to follow protocol requirements that is judged severe enough by the investigator to significantly affect study outcomes
- Pregnancy
- Any AE, laboratory abnormality, or intercurrent illness which, in the opinion of the investigator, indicates that continued participation in the study is not in the best interest of the subject
- Personal reasons (participant request)
- Discontinuation of entire study

4.2.4 Replacement of Subjects and or Repeat of Product Use Cycle

Approximately 15 women will complete the study. Participants who discontinue early may not re-enroll. Participants who discontinue may be replaced as needed to achieve the desired full 15 women completing both a baseline and a product PCT cycle. Participants who have a failed product use cycle, due to factors such as but not limited to: erectile dysfunction, inability to ejaculate, illness, etc., may repeat the product use cycle with a second dose of the 20 mg film, in a subsequent menstrual cycle

4.3 Randomization and Blinding

This is an open-label cross-over study, and neither randomization nor blinding of the participant or the investigator will be employed. The individual evaluating the cervical mucus for quality (Insler score) and the presence or absence of motile sperm in cervical mucus and the vaginal pool will be blinded to the study cycle and use or non-use of the study product.

4.4 Prior and Concomitant Medications and illnesses

Prohibited medications include use of intravaginal products within 72 hours of the expected midcycle date, as well as all hormonal contraceptives and presence of or treatment for STIs. All concomitant medications will be recorded on CRFs.

5 STUDY DRUG MANAGEMENT

5.1 Study Drug Formulation

The ZB-06 drug product is a vaginal film containing 20 mg of the HC4-N drug substance and additional excipients as described in Table 1.

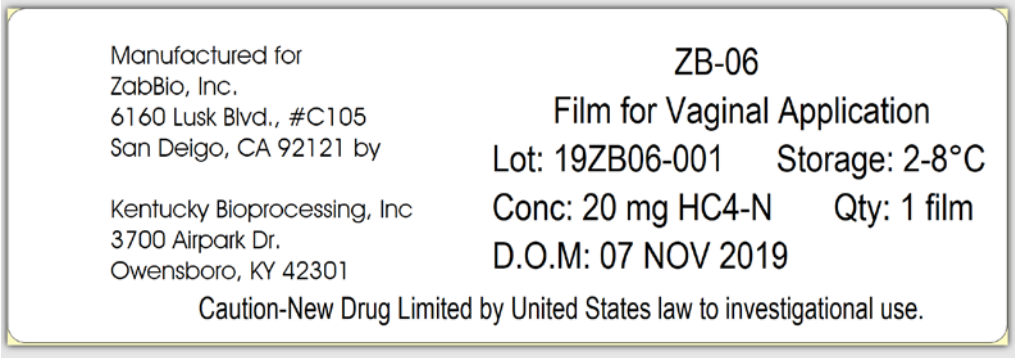
Table 1: ZB-06 Product Composition Unit Dosage Form

Component	Function	Target Quantity per film
Antibody HC4-N Drug Substance	Active Ingredient	20 mg
Maltitol	Stabilizer	54 mg
Histidine	Stabilizer	4.2 mg
Polysorbate 20	Surfactant	13 µg
PVA 8-88	Polymer Base	126 mg

5.2 Study Drug Packaging and Labeling

The ZB-06 film is packaged in a sealed foil laminate pouch. A representative version of the label affixed to the pouch is reproduced below as Figure 1:

Figure 1: ZB-06 Film Package Label



5.3 Study Drug Handling and Storage

The packaged ZB-06 drug product will be shipped and stored at 2-8° C until dispensed to the study participant.

5.4 Study Drug Administration

Participants will insert one ZB-06 film intravaginally approximately 30 minutes prior to sexual intercourse during the product PCT cycle. The film is inserted digitally high in the vagina after folding it the participant's finger.

5.5 Study Drug Accountability Procedures

The ZB-06 film will be stored in a temperature-monitored, access-controlled refrigerator at 2-8 ° C until dispensed by the study staff to the participant at the product cycle cervical mucus check (CMC) visit (Visit 6). A log of receipt and dispensing will be kept by the study staff. Unused films will be returned to the Sponsor after study completion.

6 MEASUREMENTS AND EVALUATIONS

6.1 Efficacy Assessments

The primary method of evaluating preliminary effectiveness (primary objective) for the ZB-06 film will be the average number of progressively motile sperm (PMS) in aspirated endocervical mucus examined across 9 high powered fields (HPFs) at 400X magnification at 100 um sample chamber depth, in the ZB-06 treatment cycles comparted to the no product treatment cycles.

The mean, median, standard deviation, and interquartile range (IQR) of each woman's average number (across 9 HPFs) of progressively motile sperm per HPF will be calculated separately for baseline and each test PCT. Qualitative assessments of change from baseline, if any, will be based on the median and IQR, because of expected non-normality of test cycle data.

6.2 Safety Assessments

Tables and listings will be created for all TEAEs, defined as those occurring on or after first product use. However, the primary evaluation of safety will be the subpopulation of safety AEs that are urogenital, product-related, and/or serious. Data will be presented by presence of ZB-06 film according to relatedness of the finding to study product, whether AEs had onset before or after intercourse, and noted by gender, and the severity/seriousness of the AE.

In addition, concentrations of cytokines and chemokines will be described at baseline and after ZB-06 film use, as a surrogate of subclinical safety endpoints. Safety bloods (hematology, and metabolic panel) will be obtained at screening at Visit 8. Urinalysis will be done at the times indicated in the Schedule of Events (Appendix A)

6.3 Pharmacokinetic Assessments

The concentration of HC4-N by ELISA, and sperm agglutination titer in serum and endocervical mucus, and CV fluid will both be determined after each no-product baseline and product PCT cycle.

6.4 Other Assessments

An acceptability questionnaire will be administered to women and to their male partners at or within 72 hours after their product PCT cycle.

6.5 Study Visits and Procedures

Prospective participants may be pre-screened; the study will be explained, the inclusion/ exclusion criteria reviewed, questions answered, and Visit 1 scheduled.

6.5.1 Visit 1 (Screening)

- The study and informed consent form will be reviewed and all volunteer questions will be answered. If the volunteer wishes to participate and meets the preliminary study criteria, she will be asked to review and sign an informed consent form. The PI or designee will sign the form and provide a copy to the participant.
- The participant will receive an informed consent form to give to her male sexual partner. Study staff will contact him via telephone or videoconference to go over his consent form with him and answer any questions that he has. If he wants to go over the consent form in person, we will make arrangements for him to come to the clinic. The participant will bring his signed consent form in with her once he has been counseled and agrees to sign the consent. The coordinator who counseled the partner and the witness will then sign and date the consent. Visit 2 procedures will be performed after both partners have provided signatures.
- An interview will be conducted to obtain medical history, concomitant medications, and demographic information.
- A urine specimen will be obtained for a urine pregnancy test to confirm that the participant is not pregnant and to perform a urinalysis with reflex urine culture. Participants with asymptomatic bacteruria do not require antibiotic treatment, as per standard clinical practice guidelines.
- An HIV test, chemistry panel, and blood count will be performed.
- A physical exam with vital signs will be performed.
- A pelvic exam will be performed, including:
    - Signs of irritation of the external genitalia, cervix, and vagina, as observed with the naked eye will be noted.
    - A sample will be taken for wet mount and vaginal pH if indicated.
    - Specimens for *Trichomonas vaginalis, Neisseria gonorrhea* and *Chlamydia trachomatis* will be collected.
    - A Pap smear will be performed, if necessary, as consistent with standard medical practice as outlined in the study manual and inclusion criteria.
- Film insertion training:
    - The investigator or designee will explain to the participant how to insert a marketed vaginal film with similar dimensions and physical character to the ZB-06 study film (Vaginal Contraceptive Film (VCF), Apothecus Pharmaceutical Corporation). The participant will then attempt to insert a VCF film without assistance.
    - If insertion by the participant is unsuccessful, the investigator will attempt to determine what is causing the problem and will reinstruct and assist as needed. The participant may be asked to try different positions (standing, squatting,

standing with one leg raised, etc.) for insertion. If the participant can insert the film, the investigator will remove the film once proper placement is confirmed.

- If the investigator determines that the participant cannot insert the surrogate VCF correctly, then the participant will not continue in the study.

- UTI, vaginal candidiasis, and symptomatic bacterial vaginosis (BV) infections will be treated, preferably with oral medication. If the participant is otherwise eligible, she will continue in the study.
- Provided no abnormalities are found on pelvic examination, no information contained in the medical history excludes the volunteer from participating in the study, and she has demonstrated the ability to insert a surrogate film, the participant will be continued in the study.
- Participants will be given a supply of non-spermicidal, lubricated condoms for use during certain times of the study.
- The participant will be instructed to call the coordinator at the start of her next menses. At that time, the participant will be informed of the results of any laboratory tests that were not available at the enrollment visit. She will be instructed that her male partner will need to use non-lubricated condoms for any acts of intercourse after her next menses, up until approximately 72 hours prior to the cervical mucus check visit. The couple should abstain from intercourse for 72 hours prior to the predicted midcycle point.

6.5.2 Visit 2 (Enrollment)

- This visit will occur in the follicular phase of the menstrual cycle, preferably after menses ceases, on or before menstrual cycle day 10.
- Screening laboratory tests will be reviewed, along with the results of other inclusion and exclusion criteria, and eligible participants will be enrolled by entry into the Enrollment Log.
- She will be asked if there are any new or worsening medical problems or symptoms or used any medications since the last study visit. In addition, the participant will be asked if her male partner has had any adverse events (AEs) involving the urogenital system or whether he has had any serious AEs.
- She will be provided with an ovulation predictor kit (OPK) to help determine her midcycle point. Instructions for the proper use of the kit will be provided. Participants will be instructed to begin daily urine testing on day 10 of their cycle (adjusted as needed to their normal cycle length) and to contact the study coordinator if an OPK test is positive. Her mucus check visit will then be scheduled for the day of the positive test (preferred) or the next day at the latest. If the participant does not have a positive urine LH test by day 22, she will be considered anovulatory for this cycle.

- The participant will be instructed that from the start of her menses until 72 hours before her expected midcycle, she and her partner should use study-provided condoms for each act of intercourse. There should be no intercourse, use of intravaginal products (including spermicide) and her male partner should not have an ejaculation within 72 hours of expected mid-cycle date (visits 3, 6 and 9).

6.5.3 Visits 3, 6 and 9 (CMC Baseline [no product] Cycle, CMC ZB-06 Cycle, CMC post-product-use safety Cycle)

This visit will be conducted on the day of ovulation or the day before or after, as determined by calculation or as identified by the presence of a positive test result with the OPK, whichever comes first.

- An interval history will be collected. Entry criteria will be reviewed to confirm the participant's eligibility.
- The participant will be asked if she has any new or worsening medical problems or symptoms or used any medications since the last study visit. In addition, she will be asked if her partner has had any AEs involving the urogenital system and whether he has had any serious AEs.
- The participant will be asked whether she refrained from intercourse and the use of intravaginal products and whether her male partner refrained from ejaculation during the previous 72 hours. If instructions were not followed, she will be reinstructed. If she used a spermicide or spermicidally lubricated condom during the previous 72 hours, the visit will be rescheduled in the next cycle. Otherwise, the visit may continue at the investigator's discretion.
- Urine will be obtained for urinalysis and reflex urine culture at visits 3 and 6.
- A urine pregnancy test will be performed at visit 6
- A blood sample (approximately 3 teaspoons) will be obtained at visits 6 and 9. The participant will undergo a pelvic exam during which a sample of cervical and vaginal secretions will be examined for midcycle characteristics and the presence of sperm. At visits 3 and 6, a colposcopy exam of the cervix and vagina will be done to determine if there are signs of irritation of the external genitalia, cervix, and vagina, as observed with the naked eye will be noted.
- Vaginal pH will be determined at visits 3 and 6. A slide for Nugent score will be obtained at visit 6
- A speculum exam will be performed and vaginal swabs, aspirate, endocervical mucus and a cervicovaginal fluid lavage (CVL) will be obtained.
- If sperm are found in the vagina or cervical mucus, the baseline cycle will be rescheduled for the following month and instructions on the use of condoms and avoidance of ejaculation will be reviewed. Longer abstinence may be recommended

for couples that have reportedly complied with the required 72-hour period. Additional supplies will be provided as needed.

- If the cervical mucus score is <10, the participant may be asked to return in 1 or 2 days if it seems likely that the midcycle point has not yet been reached, or in the next cycle if it seems likely that the midcycle point has already passed.
- If no sperm are detected at this visit, and the cervical mucus score is ≥10, the participant will be instructed to return home and have intercourse without a condom 2 to 3 hours prior to Visits 4, 7 or 10, which will be scheduled for the same or following day, within 27 hours of the mucus check visit.
- At visit 6, we will provide the participant with a ZB-06 film to use and instruct her to insert the film deep in to the vagina about 30 minutes prior to intercourse. A back up film may also be provided upon participant request or at the discretion of the investigator.
- A directed physical exam will be performed if indicated.
- The participant will be instructed to ask her partner, prior to her next visit, if he has had any AEs involving the urogenital system or whether he has had any serious AEs.

6.5.4 Visits 4, 7, 10 (PCT Baseline Cycle, PCT ZB-06 Cycle and post-product-use safety cycle

The participant will return to the clinic 2-3 hours after coitus ends and within 27 hours of the mucus check visit (above, visits 3, 6 and 9).

- The participant will be asked if she has any new or worsening medical problems or symptoms or used any medications since the last study visit. In addition, she will be asked if her partner has had any AEs involving the urogenital system or associated with the use of study products (as applicable) and whether he has had any serious AEs.
- A blood sample (approximately 2 teaspoons) will be obtained at visit 7.
- A wet mount or urine dipstick, or other tests, will be performed if indicated. If the test is positive for candidiasis, BV, or UTI the participant will be treated, preferably with oral medication, and continued in the study.
- Vaginal swabs or aspirate, and endocervical mucus will be obtained.
- The participant will again undergo a pelvic exam during which a sample of cervical and vaginal secretions will be evaluated for midcycle characteristics and the presence of sperm. At visits 4 and 7, a colposcopy exam of the cervix and vagina will be performed to observe if there are signs of irritation of the external genitalia, cervix, and vagina will be noted as observed with naked eye. Baseline PCT results will be interpreted and managed as listed in Appendix B.
- A directed physical exam will be performed if indicated.

- If the participant uses the study product prior to intercourse and intravaginal ejaculation cannot be achieved by the male, due to reasons such as, but not limited to: erectile dysfunction, inability to ejaculate, illness, etc., the study team will contact the sponsor and discuss if the participant may be offered a second chance at a product use cycle with a second exposure to the vaginal film, in a subsequent menstrual cycle. The participant will not present to the clinic for visit 7 in the current cycle.
- If the participant is willing and able to participate in a second product use cycle, after a failed first attempt, they will continue in the study with the intent of completing all 10 study visits.

If the participant and their partner do not want to attempt a second product use cycle, or if the sponsor does not allow this, the participant will be asked to return for visit 8 after which they will be exited from the study.

6.5.5 Visits 5 and 8 (24 hours post PCT safety checks)

- The participant will be asked if she has any new or worsening medical problems or symptoms or used any medications since the last study visit. In addition, she will be asked if her partner has had any AEs involving the urogenital system or associated with the use of study products (as applicable) and whether he has had any serious AEs.
- A colposcopy assessment will be performed
- Vaginal swabs or aspirate, endocervical mucus and a cervicovaginal fluid lavage (CVL) will be obtained.
- A blood sample (approximately two teaspoons) will be obtained.
- Urine will be obtained for urinalysis and reflex urine culture.
- Vaginal pH and slide for Nugent score will be obtained at visit 8.
- At the end of Visits 5 and 8, the participant will be given additional condoms if needed and instructed to contact the study coordinator on the first day of her next menses at which time instructions regarding sexual activity and checking urinary LH will be given. The participant will be reminded that from the start of her menses until menstrual cycle day 10, she and her partner should use study provided condoms for each act of intercourse. There should be no intercourse or use of intravaginal products (including spermicide) after menstrual cycle day 10.
- The participant will be provided with a new ovulation prediction kit. When she contacts the clinic on the first day of her next menses, she will be instructed to begin daily urine testing starting on day 10 of her cycle. She will be instructed to contact the study coordinator if a urine test result is positive. Her test cycle mucus check visit will be scheduled for the day of the positive test (preferred) or the next day at the latest. However, if she has not had a positive urine test result by day 22, she will be considered anovulatory and will either be discontinued from the study or allowed to try another cycle, depending on ZabBio, Inc. and investigator discretion.

- A wet mount or urine dipstick, or other tests, will be performed if indicated. If the test is positive for candidiasis, BV, or UTI the participant will be treated, preferably with oral medication, and continued in the study. Note that these vaginal infections may be treated with vaginal medication subsequent to 24 hr check visits.
- At visit 8, the participant will fill out an acceptability questionnaire.
- At visit 8 arrangements will be made to contact the participant's sexual partner within the next 48 hours (within approximately 72 hours from intercourse) for him to answer male acceptability questionnaire questions.

6.6 Visit Windows

- Visit 2 must be within 35 days of Visit 1.
- Visits 3, 6, and 9 (CMC visits) timing is determined according to midcycle timing according to calculations and/or OPK testing results rather than visit windows.
- Visits 4, 7, and 10 (PCT visits) must be between 2 and 3 hours after intercourse, with intercourse timing determined by the findings at the preceding CMC visit.
- Visits 5 and 8 (24 hour assessments after the baseline and product PCT visit) must be within 4 hours either side of that scheduled 24 hour follow up time.

7 DATA ANALYSIS METHODS

7.1 Data Management

Data Management will be overseen by Joseph Politch, PhD of BUSM (Lead Data Manager), via a 21CFR11 compliant electronic database (StudyTRAX®; ScienceTRAX, Macon, GA) with direct data entry via secure web portal. Database construction and validation will be performed in coordination with the Biostatistics and Epidemiology Data Analytics Center (BEDAC) at Boston University School of Public Health. Data analysis will be performed by Howard Cabral, PhD, Professor of Biostatistics at the Boston University School of Public Health.

7.2 Sample Size Determination

To assess the primary endpoint (number of progressively motile sperm in aspirated endocervical mucus 2-3 hours after sexual intercourse), fifteen couples are planned to complete both the baseline and the product PCT visits. We calculate that this sample size will provide a power of 90% to detect a clinically significant (10-fold) reduction of progressively motile sperm per high power field, from a mean of 17 in baseline no-product cycles (Archer *et al.,* 1995; Mauck *et al.,* 1997; Mauck *et al.,* 2004; Burke *et al.,* 2010; Mauck *et al.,* 2017) to <1.7 progressively motile sperm per high power field (PMS/HPF) using study product.

7.3 Subject Populations for Analysis

Data from all women who complete both the baseline and the product PCT visits will be analyzed in the effectiveness analysis, thus including all women exposed to product. Data from all participants who receive the study product will likewise be included in the safety analysis.

7.4 Interim Analysis

No interim analyses are currently planned.

7.5 Statistical Analysis

Participants will be considered evaluable if they complete both the baseline and product PCT visits. If necessary, we will seek to recruit additional participants to achieve a total of 15 evaluable participants.

7.5.1 Primary Endpoint:

The endpoint for the primary objective (surrogate for contraceptive efficacy) will be the average number of PMS (across 9 HPF) in aspirated endocervical mucus 2-3 hours after sexual intercourse without study product versus with study product. A 10-fold or greater reduction in PMS from baseline will be considered indicative of likely contraceptive effect based on prior experience with detergent spermicides and with cervical barrier devices (Mauck *et al.,* 1997b; Mauck *et al.,* 1997c; Mauck *et al.,* 2004; Amaral *et al.,* 2004; Archer *et al.,* 1995; Mauck *et*

al., 1996; Archer *et al.,* 1997; Schwartz *et al.,* 2008; Mauck *et al.,* 2017). Therefore, the main comparison of interest for the primary endpoint will be baseline [2-3 hours after intercourse without product use (Visit 4)] vs. post study product [2-3 hours after intercourse with product use (Visit 7)]. For comparison of the two time points, the Wilcoxon Signed Rank Test will be utilized.

An additional categorical analysis will compare the proportion of women with an average (also across 9 HPF) of fewer than 5 PMS/HPF, calculated separately for each test cycle to the proportion of women with ≥5 PMS/HPF at baseline [2-3 hours after intercourse without product use (Visit 4)] vs. post study product [2-3 hours after intercourse with product use (Visit 7)]. For this analysis, the McNemar's test will be used.

7.5.2 Secondary Endpoint:

The secondary endpoint will be the incidence of Grade 2 or greater adverse events in the participant and her male partner in the interval between the baseline intercourse without product (Visit 4) and Visit 6, in comparison to the incidence of Grade 2 or greater adverse events in the interval between intercourse with product use (Visit 7) and Visit 9. Additional related analyses will include the incidence of *total* adverse events in the participant and her male partner in the interval between the baseline intercourse without product (Visit 4) and Visit 6, in comparison to the incidence *total* adverse events in the interval between intercourse with product use (Visit 7) and Visit 9, as well as comparisons of adverse event severity in the two intervals. For comparison of the two intervals, the Wilcoxon Signed Rank Test will be utilized. An assessment of relatedness to study product will be made for adverse events as supplemental information. Adverse event evaluations will include symptoms, epithelial defects assessed by colposcopy, and laboratory safety blood tests. Emphasis will be placed on adverse events that are urogenital, product-related and/or serious among female and male participants.

7.5.3 Exploratory Endpoints:

Exploratory endpoints will include the following:

1. Antisperm antibody concentrations in *vaginal fluid* measured by ELISA at baseline, at 2-3 hours and at 24 hours after intercourse with and without product use and at 1 month after product (ZB-06 film) use.
2. Sperm agglutination titer in *vaginal fluid* measured by endpoint dilution at baseline, and at 2-3 hours and at 24 hours after intercourse with and without product used, and at 1 month after product use.
3. Concentrations of antisperm antibody in *endocervical mucus* samples measured by ELISA at baseline, and at 2-3 hours and at 24 hours after intercourse with and without product use, and at 1 month after product use.
4. Sperm agglutination titer in *endocervical mucus* measured by endpoint dilution at baseline, and at 2-3 hours and at 24 hours after intercourse with and without product use, and at 1 month after product use.

5. Concentrations of antisperm antibodies in *serum*, and measured by ELISA at baseline, at 24 hours, and at 1 month after exposure to ZB-06 film.
6. Sperm agglutination titer in *serum* by endpoint dilution measured at baseline, and 24 hours and 1 month after exposure to ZB-06 film.
7. Concentrations of immune mediators in *cervicovaginal lavage* measured via multiplex assays at baseline and at 24 hours after intercourse with and without study product.
8. Vaginal pH at baseline and 24 hours after intercourse with vs. without study product.
9. Nugent Score at Visit 6 baseline and 24 hours after intercourse with study product.

The continuous variables described in the Exploratory Endpoint section will be analyzed as follows: For two time point comparisons, the Wilcoxon Signed Rank Test will be utilized; for three time point comparisons, mixed linear model analysis will be performed. If dependent variables are not normally distributed, they will be (natural) log transformed prior to mixed linear model analysis. A significant effect will be followed by Tukey-Kramer multiple comparison tests. The mixed linear model analysis accounts for missing values should some participants fail to return for the 1 month time point. Statistical significance will be assumed when $p<0.05$. All analyses will be performed with SAS (Version 9.4; SAS Institute Inc., Cary, NC, USA).

An equivalence test will be performed between the average number of PMS (across 9 HPF) in aspirated endocervical mucus 2-3 hours after sexual intercourse without study product at Baseline (Visit 4) versus the average number of PMS (across 9 HPF) in aspirated endocervical mucus 2-3 hours after sexual intercourse without study product one month following exposure to study product (Visit 10). The purpose of this test is to determine whether the postcoital PMS concentrations one month following product exposure are equivalent to baseline levels. The equivalence test will be performed utilizing the two one-sided t-tests (TOST) procedure.

8 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

The investigator is responsible for the detection and documentation of events meeting the definition of an AE or serious adverse event (SAE) as provided in this protocol. AE event monitoring for this study will begin at the time of informed consent (Visit 1) through the last visit or follow up phone call. In order to fulfill safety reporting obligations, the investigator should promptly report any SAEs resulting from study participation.

8.1 Definition of an AE

An AE is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product, regardless of the suspected causal relationship with the study treatment. An AE can therefore be any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding), symptom or disease temporally associated with the use of a medicinal product, whether or not considered related to the use of the medicinal product.

The definition of an AE includes:

- An exacerbation of a pre-existing illness
- An increase in the frequency or intensity of a pre-existing episodic event or condition
- Any condition detected or diagnosed after the start of study treatment even though the condition may have been present prior to the start of the study
- A continuous persistent disease or symptom present at baseline that worsens after the start of the study

The definition of an AE does not include:

- A medical or surgical procedure such as surgery, endoscopy, tooth extraction, or transfusion (although the condition that leads to the procedure may be an AE)
- A pre-existing disease or condition present at the start of the study that does not worsen during the study
- Any situation where an untoward medical occurrence has not occurred (for example, hospitalizations for cosmetic elective surgery or social admissions)
- An overdose of either the study drug or a concurrent medication without any resulting signs or symptoms

AEs may also include post-treatment events that occur as a result of protocol-mandated procedures (e.g., invasive procedures or modification of a subject's previous therapeutic regimen).

8.2 Definition of an SAE

A serious adverse event (SAE) is an AE which meets any of the following criteria:

- Results in death
- Is life-threatening
- Requires hospitalization or the prolongation of existing hospitalization
- Results in a persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect

In addition, important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based on appropriate medical judgment, the investigator determines that the event may jeopardize the subject 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 hospitalization, or the development of drug dependency or drug abuse.

Hospitalization for elective treatment of a pre-existing condition that did not worsen during the study is not considered an AE. Any complications that occur during hospitalization are AEs. If a complication prolongs hospitalization, then the complicating event is an SAE.

Life-threatening means that the subject was, in the view of the investigator, at immediate risk of death from the event as it occurred. It does not mean that the event, had it occurred in a more severe form, might have caused death.

8.3 Clinical Laboratory Abnormalities and Other Abnormal Assessments

Abnormal laboratory findings or other abnormal assessments (e.g., ECGs, vital signs) that are judged by the investigator as clinically significant should be recorded as AEs. Clinically significant abnormal laboratory findings or other abnormal assessments that are detected after the start of study treatment, or that are present before the start of study treatment and worsen after study treatment, are considered AEs.

Clinically significant abnormal laboratory findings or other abnormal assessments that are associated with a disease (unless judged by the investigator as more severe than expected for the subject's condition) or that are present before the start of study treatment and do not worsen after study treatment are not included as AEs.

The investigator should exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

8.4 Method, Frequency, and Time Period for Detecting AEs and SAEs

Each subject will be monitored for AEs from the time of informed consent (Visit 1) through their last visit or follow up phone call. All AEs and SAEs, regardless of attribution (i.e., relationship to study drug), will be collected during this time period. The collection period

will end when the subject completes the Follow-Up phone call unless there is an ongoing AE which requires monitoring beyond this time point.

8.5 Documenting AEs and SAEs

All AEs that occur during the study should be documented in the subject's source documents in accordance with the investigator's normal clinical practice and in the AE section of the CRF.

The following information about each AE should be recorded in the source documentation and in the CRF:

- Dose of study drug
- Onset and cessation times
- Severity
- SAE (yes or no)
- Relationship to study drug
- Action taken in response to the event

Assessments of AE severity and of the possible relationship to study drug will be made by the investigator.

The investigator should attempt to establish a diagnosis of the event based on signs, symptoms, and other clinical information. Whenever possible, a diagnosis should be reported as the AE rather than just a sign or symptom. If a clinically significant abnormal laboratory finding meets the definition of an AE, a diagnosis or any clinical signs and symptoms rather than the abnormal laboratory finding should be recorded if possible. If no diagnosis is known and clinical signs and symptoms are not present, then the abnormal finding should be recorded as the AE.

8.6 Follow-Up of AEs and SAEs

All AEs must be followed until resolution, until the condition stabilizes, until the event is otherwise explained or is judged by the investigator to be no longer clinically significant, or until the subject is lost to follow-up. The investigator is responsible for ensuring that follow-up includes any supplemental investigations necessary to elucidate as completely as practical the nature and/or causality of the AE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

ZabBio, Inc. may request that the investigator perform or arrange for the conduct of supplemental measurements and/or evaluations of an AE. If a subject dies during participation in the study or during a recognized follow-up period, then ZabBio, Inc. should be provided with a copy of any post-mortem findings, including histopathology.

8.7 SAE Reporting

Any SAEs or Adverse Event of Interest should be reported within 24 hours of the investigator becoming aware of the occurrence, regardless of expectedness or causality. The investigator must call and then email the completed Serious Adverse Event Form to the contact information listed below.

The SAE form should be completed as thoroughly as possible and signed by the investigator or his/her designee before transmittal to the Sponsor/ZabBio, Inc.'s designee. It is **very important** that the investigator provide his/her assessment of causality to Study Treatment at the time of the initial SAE report.

SAE Reporting Contact Information

Primary Medical Monitor:	**Thomas Moench** **thomas.moench@mappbio.com**
Phone:	**443-900-6696**
Alternate Phone:	**410-828-4358**

The Site Investigator will also forward an electronic copy of the SAE Report and supporting documentation (including discharge summaries progress notes, laboratory results) to the study team contacts via e-mail within 24 hours of receipt.

The site PI will perform a clinical review of the information provided to identify any missing data. The Site Investigator will also contact the study site to clarify any discrepant or missing information, to answer questions and to provide guidance to the site, if needed. The Investigator will be instructed to report the SAE as an acceptable medical diagnosis. If a preliminary diagnosis has not yet been made, then each symptom will be listed separately. A follow-up report will be issued when a diagnosis is made.

An updated SAE Report should be emailed within 48 hours of receipt of new or updated information. The investigator must also promptly notify the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) of the occurrence of an SAE, including any follow-up information.

ZabBio, Inc. has a legal responsibility to notify regulatory authorities about the safety of a drug under clinical investigation. If a given SAE is considered related to the use of study medication and is unexpected, ZabBio, Inc. will forward a report of the event to the relevant regulatory health authority and to all investigators. It is the investigator's responsibility to promptly inform the IRB/IEC in accordance with local regulations.

An SAE that is considered related to study participation (e.g., as the result of any study procedures or invasive tests), even if it occurs during Screening or after the Follow-Up Visit, should be reported promptly to ZabBio, Inc.

8.8 Post-Study AEs and SAEs

Investigators are not obliged to actively seek AE information from former study participants. However, if the investigator learns of an SAE at any time after a subject has been discharged from the study and the event may reasonably be considered related to the use of study drug, the investigator should promptly telephone ZabBio, Inc.

8.9 Pregnancies

Participants are required to be protected from pregnancy by female surgical sterilization. However, should a pregnancy occur, it must be reported as soon as possible to the Sponsor or designee and recorded on the pregnancy CRF. The participant will be discontinued from the study and appropriate exit procedures will be followed. Note that pregnancy in itself is not regarded as an AE unless there is a suspicion that an investigational product may have interfered with the effectiveness of a contraceptive medication. If the participant has been exposed to study product, the course of the pregnancy should be followed until it has an outcome (spontaneous miscarriage, elective termination, normal birth or congenital abnormality). If the participant seeks care outside the site, every effort should be made to obtain her consent for the site to receive a copy of her medical records related to the pregnancy, its outcome and the health of the neonate, if applicable.

Reports of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as SAEs. Elective abortions without complications should not be reported as AEs.

9 STUDY ADMINISTRATION

9.1 Safety monitoring

9.1.1 Protocol Safety Review Team (PSRT)

The study site investigators are responsible for continuous close safety monitoring of all study participants, and for alerting the Protocol Team and IRB if unexpected concerns arise. A sub-group of the Protocol Team, including the Medical Safety Officer, the Sponsor Project Physician, and the Site Investigator will serve as the Protocol Safety Review Team (PSRT). The PSRT and the study site will cooperate closely to monitor participant safety and respond to occurrences of toxicity in a timely manner. The following findings will serve as the minimum criteria to pause or stop enrollment in the study. However, the National Institutes of Health (NIH) and/or the PSRT can stop the study at any time for any concerning findings of lesser severity than those listed here:

1. If any participant manifests a grade 3 or higher AE that is judged to be related to study product.
2. If any participant develops deep epithelial disruption or ulceration of the genital epithelium.
3. If any participant develops erythema or edema of more than 50% of the combined vaginal and cervical surface (grade 2) or more than 50% of the vulvar surface (grade 2).

If enrollment is paused, its reinstitution will require the unanimous agreement of all members of the PSRT.

9.1.2 Clinical Data Safety Review

The Data Management Team will generate data summaries for the PSRT after 8 couples have completed Study Visit 8. These data summaries will include adverse event, accrual and retention data. The Medical Safety Officer (or designee) will evaluate adverse event data independently and present those to the PSRT at this interim review, or at any time that significant safety concerns arise. The PSRT will determine whether or not the study protocol should continue as originally designed, should be changed, or should be terminated.

The IRB will be notified of any serious and unexpected adverse events according to the policies outlined in the EVMS IRB Policy and Manual of Operations.

9.2 Data Handling and Recordkeeping

9.2.1 Study Data

Study data will be collected in an electronic database (Study-TRAX®) on direct web-portal-entry CRFs, via a study laptop. The database will reside on servers at Biostatistics and Epidemiology Data Analytics Center (BEDAC) Boston University School of Public Health.

These eCRFs will serve as source data. Insler score data, and sperm motility and enumeration data from cervical mucus and vaginal pool samples will be initially recorded on a laboratory form, and transferred to a corresponding eCRF, but in this case, the laboratory form will serve as source data.

9.2.2 Source Documentation

Study documentation includes all case report forms, data correction forms, electronic data files, workbooks, source documents, monitoring logs, appointment schedules, ZabBio, Inc.-investigator correspondence and regulatory documents (e.g., signed protocol and amendments, IRB correspondence and approved consent form and signed patient consent forms, Statement of Investigator form, and clinical supplies receipt and distribution records).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study. Source documents include, but are not limited to, laboratory reports, ECG tracings, X-rays, radiologist reports, patient diaries, ultrasound photographs, patient progress notes, hospital charts, pharmacy records and any other similar reports or records of any procedure performed in accordance with the protocol.

Whenever possible, the original recording of an observation should be retained as the source document; however, a photocopy is acceptable provided that it is a clear, legible, and exact duplication of the original document.

9.3 Regulatory and Ethical Considerations

9.3.1 Regulatory Authority Approval and Study Conduct

In accordance with all applicable national regulations, ZabBio, Inc. will obtain regulatory approval prior to initiating the study.

The clinical trial described in this protocol will be conducted in compliance with the current revision of the Declaration of Helsinki, International Conference on Harmonization (ICH) guidelines for Good Clinical Practice (GCP), and applicable local regulatory requirements.

9.3.2 Institutional Review Board/Independent Ethics Committee Approval

It is the investigator's responsibility to ensure that the protocol (and any protocol amendment, if applicable) is reviewed and approved by an appropriate IRB/IEC.

The IRB/IEC must also review and approve the site's ICF and any other written information provided to the subjects, including any advertisements to be used for subject recruitment. The investigator or a designee must forward to ZabBio, Inc. or its representative copies of the IRB/IEC approval and the approved informed consent materials prior to the enrollment of any subjects in the study.

If it is necessary to amend either the protocol or the ICF during the study, the investigator will ensure that the IRB/IEC reviews and approves all amended documents. IRB/IEC approval of the amended ICF must be obtained before any subjects consent to take part in the study using this version of the form. Copies of the IRB/IEC approval of the amended protocol or ICF must be forwarded to ZabBio, Inc. or its representative as soon as available.

9.3.3 Subject Informed Consent

The investigator or designee will inform potential subjects of all study requirements and the risks and benefits pertaining to participation in the study. The process for obtaining subject informed consent will be in accordance with all applicable regulatory requirements.

The investigator or a designee and the subject must both sign and date the ICF before the subject can enroll in the study or any study related procedures may be conducted. The subject will receive a copy of the signed and dated ICF; the original ICF will be retained in the site study records.

The decision to participate in the study is entirely voluntary. The investigator or a designee must emphasize to the subject that consent regarding study participation may be withdrawn at any time without penalty or loss of benefits to which the subject is otherwise entitled.

9.3.4 Investigator Reporting Requirements

In accordance with regulatory requirements, the investigator may be obliged to provide periodic safety updates on the conduct of the study at the site and notification of study closure to the IRB/IEC. Such periodic safety updates and notifications are the responsibility of the investigator.

9.4 Amendment of the Protocol

Changes to the protocol during the study will be documented as amendments. These will form an integral part of the protocol and will be signed by the relevant personnel at ZabBio, Inc., and by the investigator(s).

Depending on the contents of the amendment and local legal requirements, the amendment will be submitted to the IRB/IEC and, where necessary, to the relevant competent authorities.

The investigator should not implement any deviations from, or changes to the protocol, without agreement by ZabBio, Inc. and prior review and documented approval/favorable opinion of the appropriate IRB/IEC and, if legally required, competent authorities, except where necessary to eliminate an immediate hazard to the subjects.

If an amendment substantially alters the study design, increases the potential risk to the subjects or affects the treatment of the subject, then the ICF must be revised and submitted to the IRB/IEC, and where necessary, to the relevant competent authorities, for review and approval. When a subject is currently undergoing study procedures and is affected by the amendment,

then the subject must be asked to consent again using the new ICF. The new ICF must be used to obtain consent from new subjects prior to conducting any study-related activities.

9.5 Subject Data and Data Protection

Permission for direct access to subject's data will be sought in writing by the investigator and from the subject as part of the informed consent procedure. This gives permission to examine, analyze, verify, and reproduce any records and reports that are important to the evaluation of the study. Any party (e.g., domestic and foreign regulatory authorities, clinical research associates and auditors) with direct access must take all reasonable precautions within the constraints of the applicable regulatory requirement(s) to maintain the confidentiality of the subject's identities and ZabBio, Inc.'s proprietary information.

It is the investigator's responsibility to ensure that each subject has consented, in writing, to direct access. The clinical research associate will verify that this has occurred.

The investigator must ensure that the documents that are given to ZabBio, Inc. or its representatives do not contain the name or address of the subject, or other information that would affect the anonymity of the subject (apart from his/her initials).

9.6 Study Personnel and Study Monitoring

The ZabBio, Inc. should ensure that it is specified in the protocol or other written agreement that the investigator(s)/institution(s) will permit trial-related monitoring, audits, IRB/IEC review, and regulatory inspection(s), providing direct access to source data/documents.

ZabBio, Inc. or a ZabBio, Inc. appointed monitor will visit the site in person, or remotely prior to the start of the study to discuss the protocol and data collection procedures with site personnel.

Study monitoring will continue to be conducted on a regular basis to verify that the study data are authentic, accurate and complete, to ensure that the safety and rights of subjects are being protected and to verify that the study is conducted in accordance with the currently approved protocol, GCP, and all applicable regulatory requirements. A monitor will periodically contact the study site during study conduct and will conduct regular on-site or remote visits. The extent, nature, and frequency of on-site visits will be based on such considerations as the study objective and endpoints, the purpose of the study, study design complexity, and enrollment rate. During site visits, the monitor will:

- Assess the progress of the study
- Review all study data collected
- Determine if study data are entered completely and accurately in the CRFs
- Conduct source document verification
- Confirm that ICFs for all subjects are present and accurately completed

- Identify any study conduct issues and address their resolution

The investigator agrees to allow ZabBio, Inc. and the monitor direct access to all relevant documents and to allocate his or her time, and that of the study staff, to discuss any findings and relevant issues.

At study closure, the monitor will also conduct all activities indicated in Section 9.8, Study Site Closure.

9.7 Quality Assurance

At its discretion, ZabBio, Inc. or its representative may conduct a quality assurance audit of this study. Also, regulatory agencies may conduct a regulatory inspection of this study. If such audits or regulatory inspections occur, the investigator agrees to allow direct access to all relevant documents and to allocate his or her time, and that of the study staff, to the auditor or inspector to discuss any findings and relevant issues.

9.8 Study and Site Closure

Upon completion of the study, the following activities, when applicable, must be conducted by ZabBio, Inc. or it's designee in conjunction with the investigator, as appropriate:

- Accounting, reconciliation, and final disposition of used and unused study drug and study drug containers;
- Return of all study data to ZabBio, Inc. or designee;
- Completion of any remaining data clarifications and/or other data resolutions;
- Review of site study records for completeness;
- Ship blood samples to the respective laboratories, if applicable, in accordance with the instructions described in the Study Reference Manual provided for this study.

ZabBio, Inc. reserves the right to temporarily suspend or prematurely discontinue this study at any time and for any reason. The study will be suspended or terminated if continuation of the study represents a serious medical risk to the subjects in the opinion of the investigator or ZabBio, Inc. Other possible reasons for study discontinuation include, but are not limited to: the occurrence of an SAE or other AE unacceptable in nature, severity, or frequency to the investigator or ZabBio, Inc., a failure to observe GCP, or an inadequate level of subject entry.

If the study is suspended or discontinued early, ZabBio, Inc. will inform the investigator of the reason for taking such action. ZabBio, Inc. will also promptly inform the regulatory authorities of the suspension or termination of the study and the reason. If required by applicable regulations, the investigator must inform the IRB/IEC promptly and provide the reason for the suspension or termination.

If the study is prematurely discontinued, all study data must be returned to ZabBio, Inc. In addition, the study site must conduct final disposition of all unused study drug in accordance

with ZabBio, Inc.'s procedures for the study. Financial compensation to investigators and/or institutions in this instance will be in accordance with the agreement established between the investigator and ZabBio, Inc.

9.9 Records Retention

In accordance with applicable regulatory requirements, the investigator will maintain a copy of all site study records in a safe and secure location. After completion of the study, a copy of all study records should be maintained by the investigator for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; if such an application is not approved or not filed, the investigator must maintain a copy of all study records for 2 years after investigation is discontinued and the regulatory authorities are notified. The investigator should contact ZabBio, Inc. prior to destroying any required study documentation.

9.10 Information Disclosure and Inventions

9.10.1 Ownership

All data and records provided by ZabBio, Inc. or generated during the study (other than a subject's medical records) and all inventions discovered in the course of conducting the study are the property of ZabBio, Inc. If a written contract for the conduct of the study which includes ownership provisions inconsistent with this statement is executed, then that contract's ownership provisions shall apply rather than this statement.

9.10.2 Confidentiality

The investigator and other study site personnel will keep confidential any information provided by ZabBio, Inc. related to this study, including this protocol, and all data and records generated in the course of conducting the study, and will not use the information, data, or records for any purpose other than conducting the study. These restrictions do not apply to: (1) information that becomes publicly available through no fault of the investigator or study site personnel; (2) information disclosed in confidence to an IRB/IEC solely for the evaluation of the study; (3) information disclosed in order to provide appropriate medical care to a study subject or (4) study results that may be published as described in the next paragraph. If a written contract for the conduct of the study which includes confidentiality provisions inconsistent with this statement is executed, then that contract's confidentiality provisions shall apply rather than this statement.

9.10.3 Publication

In the event the Institution and/or its employees, agents, consultants or other representatives (including the Principal Investigator) wish to make a publication (including any oral disclosure made without obligation of confidentiality) relating to the Study or the Institution's performance hereunder following completion of the Study, the Institution will deliver to ZabBio, Inc. a copy of the proposed written publication or an outline of such oral disclosure at

least thirty (30) days prior to submission for publication or presentation, as applicable. ZabBio, Inc. will have the right (a) to propose modifications to the publication for protection of Confidential Information or other reasons, and (b) to request a delay in publication in order to protect patentable information. If ZabBio, Inc. requests such a delay, the Institution will delay submission or presentation of the publication for a period of sixty (60) days to enable patent applications protecting ZabBio, Inc.'s rights in such information to be filed. Upon the expiration of thirty (30) days from delivery of the proposed written publication or the outline of any oral disclosure, as applicable, to ZabBio, Inc., the Institution will be free to proceed with the written publication or the oral presentation, respectively, unless ZabBio, Inc. has requested the delay described above. At least one employee of ZabBio, Inc., designated by ZabBio, Inc., will be considered an author on any publication or presentation regarding the Study. In no event shall the Institution publish any Confidential Information in such proposed publication without ZabBio, Inc.'s prior written consent. Notwithstanding anything to the contrary herein, in no event will any Interim Results (as defined below) be published, presented or disclosed without the prior written consent of ZabBio, Inc. For the purposes hereof, "Interim Results" shall mean results from the Study prior to complete compilation of all Study data and review by ZabBio, Inc. and, for multicenter studies, prior to the complete set of final results of the Study from all centers have been published, presented or disclosed by ZabBio, Inc.

If a written contract for the conduct of the study which includes publication provisions inconsistent with this statement is executed, then that contract's publication provisions shall apply rather than this statement.

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APPENDICES

Appendix A: Schedule of events

Appendix B: Table of actions according to PCT results

Appendix A.
Schedule of Events

PROCEDURE	Visit 1 Screening	Visit 2 Enrollment	Visit 3 Baseline (No-product) CMC	Visit 4 Baseline (No-product) PCT	Visit 5 24 h safety check	Visit 6 Product CMC	Visit 7 Product PCT	Visit 8 24 h safety check	Visit 9 No-product CMC	Visit 10 No-product PCT and Study Exit
Informed consent	X									
Confirm eligibility	X	X								
Dispense informed consent for male partner	X									
Enroll		X								
History	X									
Adverse Event Collection	X	X	X	X	X	X	X	X	X	X
Log concomitant medications	X	X	X	X	X	X	X	X	X	X
Condom and abstinence counseling	X	X	X	X	X	X	X	X	X	
Condom distribution	X				X			X		
Ovulation predictor kit distribution		X			X			X		
Film insertion training/review	X					X				
Film dispensed						X				
Urine pregnancy test	X	Δ	Δ	Δ	Δ	X	Δ	Δ	Δ	Δ
Urinalysis with reflex urine culture	X		X		X	X		X		
NAATS for CT, GC, Trichomonas	X									
HIV blood test	X									
CBC, Metabolic Panel	X							X		
Vaginal pH	Δ		X			X		X		
Wet Prep	Δ	Δ	Δ		Δ	Δ		Δ		
Slide for Nugent scoring						X		X		
Pap	Δ									
Serum for HC4-N ELISA					X	X	X	X	X	
Serum for sperm agglutination					X	X	X	X	X	
Vaginal swab or aspirate for sperm count, HC4-N ELISA, and sperm agglutination assay			X	X	X	X	X	X	X	X
Endocervical aspirate for Insler score, sperm count and motility, HC4-N ELISA, sperm agglutination assay			X	X	X	X	X	X	X	X
CVL for immune mediators			X		X	X		X	X	
Vital signs	X	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ
Directed Physical exam	X	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ
Pelvic exam	X		X	X	X	X	X	X	X	X
Colposcopy			X	X	X	X	X	X		
Woman's acceptability questionnaire								X*		
Man's acceptability questionnaire								X**		

X Procedure done
Δ Procedure done if indicated
* Women's questionnaire administered at this visit.
**Men's questionnaire administered by telephone within 72 h of intercourse, and will include questions to elicit AEs.

Appendix B.
Table of actions according to PCT results

Vaginal pool - any sperm present (including fragments) *	Mucus - adequate (thin)	Endocervix			Action at Baseline or Safety Cycle	Action at Test Cycle
		No sperm	Some sperm but <5 progressively motile	≥5 progressively motile		
Yes	Yes	✓			Similar results in test cycle would make it appear that the product was successful. Discontinue participant if baseline cycle. If this is safety cycle, may indicate induction of anti-sperm antibodies	**Barrier successful** Continue to next cycle.
Yes	Yes		✓			
No	Yes		✓			
Insufficient material	Yes		✓			
Yes	Yes			✓	Good PCT. Continue to next cycle if baseline cycle and exit participant from safety cycle	**Barrier failed.** Continue to safety cycle. Discontinue participant if the result was clearly due to participant misuse of the product.
Yes	No			✓		
No	Yes			✓		
No	No			✓		
Insufficient material	Yes			✓		
Insufficient material	No			✓		
Yes	No	✓			Poor mucus may be responsible for scarcity or lack of sperm in cervix. Repeat cycle.	Poor mucus may be responsible for scarcity or lack of sperm in cervix. Repeat cycle.
Yes	No		✓			
No	No		✓			
Insufficient material	No		✓			
No	Yes	✓			Ejaculation may not have taken place. Repeat cycle.	Ejaculation may not have taken place. Repeat cycle.
No	No	✓				
Insufficient material	Yes	✓				
insufficient material	No	✓				

*Absence of sperm in vagina means either that ejaculation did not take place or the vaginal environment is unusually hostile to sperm, possibly due to presence of spermicide in test cycle.