A STUDY OF THE SAFETY AND ACCEPTABILITY OF A PLACEBO VAGINAL FILM: FAME 101

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LIST OF ABBREVIATIONS AND ACRONYMS

ACHD	Allegheny County Health Department
AE	Adverse event
AIDS	Acquired Immunodeficiency Syndrome
BV	Bacterial vaginosis
CVL	Cervicovaginal lavage
CT	*Chlamydia trachomatis*
DAIDS	Division of AIDS
EAE	Expedited adverse event
GC	Neisseria gonorrhoeae
HEC	Hydroxyethylcellulose
HIV	Human Immunodeficiency Virus
HSV-1, HSV-2	Herpes Simplex Virus type 1, type 2
IRB	Institutional Review Board
ITT	Intent-to-treat
N-9	Nonoxynol-9
NIAID	National Institute of Allergy and Infectious Disease
NIH	National Institutes of Health
NRTI	Nucleoside reverse transcriptase inhibitor
PCR	Polymerase chain reaction
PSRT	Protocol Safety Review Team
RSC	Regulatory Support Center
RT	Reverse transcriptase
SOP	Standard operating procedure(s)
STI	Sexually transmitted infection

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A study of the safety and acceptability of a placebo vaginal film: FAME 101

PROTOCOL SUMMARY

Short Title:	FAME 101
Protocol Chair:	Katherine Bunge, MD MPH
Protocol Co-chair:	Sharon Hillier, PhD
Sample Size:	Approximately 64
Study Population:	HIV-uninfected women, 18 – 45 years old
Study Sites:	Magee-Womens Hospital of UPMC, Pittsburgh, PA
Study Design:	A phase I, 4 arm, single site, open label placebo trial with randomization to timing of follow-up

Visit 1	Visit 2	Visit 3	Visit 4
-45 days	Day 0	Day 3,7,10 or 14*	Day 21

*Randomized to timing of Visit 3

Study Duration: Accrual of approximately 64 participants is expected to take 6 months. The expected duration of study participation for each participant will be approximately 5 to 11 weeks. This includes the screening period. After enrollment, the study duration is 3 weeks.

Study Products: Vaginal Film
One dose placebo vaginal film (2" x 2" size)

Study Regimen: All participants will receive a single dose of vaginal placebo film that will be self-administered

Primary Objective:

- To assess the safety of a single dose of a placebo film which is intended for extended release of antiretroviral drugs.

Primary Endpoint:

- Grade 2 or higher Adverse Events

Secondary Objectives:

- Acceptability
    - To describe the acceptability of the placebo vaginal film and identify qualities associated with higher or lower degrees of acceptability

Secondary Endpoint:

- Acceptability
    - Self-reported assessment of qualities of the experience with the film drawn from existing survey on microbicidal films, and general acceptability ratings drawn from market research methodology

Exploratory Objectives:

- **Vaginal Microenvironment**
    - To compare the effects of the placebo film on the vaginal microbiota and glycome pre and post study product use

- **Persistence**
    - To describe the persistence of the film at 3,7,10 and 14 days after insertion

Exploratory Endpoints:

- **Vaginal Microenvironment**
    - Cervicovaginal lavage (CVL)
        - Levels of anti-viral activity
        - Lectin microarray
        - Levels of glycoproteins and mucins
        - Levels of cytokine and innate immunity factors
    - Vaginal
        - Quantitative vaginal cultures and quantitative PCR for selected microbiota
        - Nugent score

- **Persistence**
    - Eudragit ®levels in CVL

1 KEY ROLES

1.1 Protocol Identification

Protocol Title: A study of the safety and acceptability of a placebo vaginal film:

Short Title: FAME 101

Date: September 20, 2017

1.2 Funding

Funding Agency: DAIDS/NIAID/NIH
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2 INTRODUCTION

2.1 HIV prevention and microbicides

Since 1981, HIV has directly or indirectly resulted in the deaths of 25 million people[1]. Each year millions of people worldwide are newly infected with HIV, most recently 2.7 million people in 2008[2]. Sexual transmission accounts for the majority of new HIV infections worldwide. While most cases of sexual transmission involve both a male partner and a female partner, women are disproportionately impacted by the sexual transmission of HIV [2]. This disproportionate impact arises from a combination of biologic, social, and economic factors [2]. Women at risk face formidable challenges in protecting themselves from HIV infection, including power differentials, gender norms, and economic dependence.

Topical microbicides are agents designed to prevent or at least substantially reduce the acquisition and transmission of HIV when applied to the genital or gastrointestinal mucosa. In addition to preventing HIV infection, topical microbicides may satisfy two critical needs: affordability and female control. Topical microbicides also have the potential for high acceptability for both male and female partners. Acceptability and utility of topical microbicides can be maximized by the development of an array of dosing formulations. For all dosing formulations, safety, stability, and efficacy must be established.

Vaginal films are an attractive dosing option for several reasons. In terms of manufacturing, films are inexpensive, scalable, physically and chemically stable, uniform with regards to product content, and amenable to combinations of active ingredients. In terms of use, films are discreet, portable, and easy to store. Vaginal films can deliver fixed doses of microbicidal agents with minimal mess and without an applicator. The small volume of vaginal films may also result in less dilution of endogenous antiviral or antibacterial properties of vaginal fluids compared to vaginal gels. In studies of spermicidal film, women have found vaginal films to be acceptable[3-5]. When Vaginal Contraceptive Film (VCF) was compared to foaming tablets containing nonoxynol-9 (N-9), contraceptive film was preferred in three different countries [3]. Ninety-seven percent of 59 Mexican women, 60 percent of 52 Dominican women, and 86 percent of 51 Kenyan women reported liking contraceptive film. In particular, they cited the following favorable characteristics: ease of use, general feel or comfort, additional lubrication, and lack of side effects [3]. Over eighty percent of Cameroonian sex workers, who participated in a large randomized placebo-controlled trial of N-9 film, reported that they would use the film were it found to be effective against HIV[4]. In Zambian focus groups, placebo vaginal film was preferred over placebo vaginal tablets and soft-gel capsules after each product was used once daily for one week [6]. In a study evaluating the acceptability of placebo microbicidal film, soft gel capsule and tablet, African women were instructed to use each of the three products once daily for 7 consecutive days. The film and soft-gel capsule were chosen significantly more often than the tablet as the preferred dosage form (39%, 37% and 25%, respectively). In this particular study, film and soft gel were preferred because of faster dissolving time and easier insertion.[7] In other studies, participants found vaginal films to be neater than foaming tablets and gel[3,8].

2.2 Integrated Preclinical/Clinical Program

The Film Antiretroviral Microbicide Evaluation (FAME) program brings together an inter-disciplinary group of research scientists from four academic institutions: University of Pittsburgh, University of Washington, Carnegie Mellon University, New York University and the University of Colorado The long term goal of the program is to develop and evaluate an extended delivery vaginal film containing an integrase inhibitor which could provide protection from HIV for one week or more following a single application. The first iteration of the FAME IPCP established proof of concept that vaginal films containing the antiretrovirals (ARVs) dapivirine and tenofovir can deliver drug to target tissues as effectively as gel formulations of those same ARVs. The

second iteration, of which this is the first clinical trial, strives to develop an extended release formulation of an integrase inhibitor MK-2048 which will provide coverage for 7 days after each use.

2.3 Rationale

This is a phase I randomized trial assessing the safety of a single vaginal placebo film application. In order to develop a vaginal film which can provide extended release of an ARV, the film polymers and formulation have been altered from the cellulose and polyvinyl alcohol films used to deliver dapivirine and tenofovir in previous trials. Therefore, the proposed study will evaluate the safety and persistence of these film polymers when applied vaginally.

HIV-negative women will be randomized to the timing of the follow up visit prior to administration of the placebo vaginal film and. Women will be randomized to return for a clinical evaluation either three days, seven days, ten days, or fourteen days after study product insertion. In addition to safety, the acceptability of this vaginal film intended for extended release of ARVs will be assessed, as will its impact on the vaginal microenvironment. Cervicovaginal lavage and vaginal fluid (as sampled by absorptive material, such as sponge or wick) will be used to assess the impact of the film on the on the vaginal microbiota and glycome. The film's impact on cellular immunity and inflammation will also be assessed, and the CVL samples will be assessed for changes to the innate anti-HIV activity.

The film is intended to provide HIV protection for a week after a single application. Understanding how long the film remains in the vagina will be an integral part of this study. Dr. Anderson's lab has developed an assay to detect the polymer Eudragit® in cervical vaginal fluid. Genital samples from Visit 1, Visit 3 and Visit 4 will be evaluated for the presence of Eudragit.®. Eudragit was chosen over the other polymers because of technical feasibility for the assay. The other polymers in the film are cellulose based, and it would be very difficult to differentiate between the polymers in a biologic sample. Eudragit, on the other hand has a unique structure. Of note, all of the visible film will be removed with the CVL performed at Visit 3; therefore, no Eudragit® should be detectable at Visit 4.

This study has been designed to add to our knowledge about film application challenges. Product misplacement was noted in the three clinical trials of vaginal film previously conducted at the University of Pittsburgh. While most participants could successfully insert the vaginal films, incorrect film placement was noted for some. In some instances, the vaginal film stuck to the finger after attempted insertion and in other instances the film was visualized on the external genitalia. Because the primary objective of this study is to assess safety and persistence, administration error will be noted immediately after the participant attempts to insert the film. If most the film is noted to be external to the vaginal introitus, the film will be removed by the clinician and a new vaginal film will be placed inside the vagina by the clinician. This will ensure the integrity of the primary objective.

2.4 Safety Data: Placebo Film

The placebo film consists of ingredients with a proven safety record. Table 1 outlines documented usage with film excipients per the FDA database for inactive ingredients in approved drug products.

Table 1: Placebo Film Excipients

Excipient	Use	Concentration*	History of Vaginal Use
Hydroxypropyl methyl cellulose (HPMC) or Hypromellose**, USP	Film Forming Polymer	Vaginal; gel 3.5% Vaginal; tablet 5 mg Rectal; gel 4.35% (listed as hypromelloses**)	ESTRACE® Cream and VAGIFEM® vaginal tablets, tenofovir and dapvirine vaginal films
Propylene glycol (PG), USP	Plasticizer/Dispersant	Vaginal; emulsion, cream 20% Vaginal; gel 3% Vaginal; suppository 252 mg	VCF dissolving vaginal lubricant film (5%)[35]
Hydroxyethyl cellulose, NF	Film Forming Polymer	Buccal ; film 54.92 mg Oral; tablet, extended release 400mg Topical; gel 1.75%w/w	Tenofovir vaginal film, tenofovir and dapvirine vaginal gels
Polyethylene glycol 400, NF	Plasticizer/Dispersant	Rectal; suppository 24.8% Topical; cream 1.5%w/w Topical; cream, emulsion, sustained release 7.5%w/w	Vaginal suppository
Ammonio Methacrylate Copolymer Type B (Eudragit®), NF	Extended Release Polymer	Oral; capsule, extended release 35.7mg Oral;tablet, extended release 11.05mg Tablet, film coated 8mg	*
Hydroxypropyl cellulose, NF	Film Forming Polymer	Buccal; film 100.04mg Topical; patch Transdermal; film controlled release 19mg Oral; tablet (immed./comp. Release), film coated 24mg	*

*Although there is not a history of vaginal use, these excipients have been used extensively in other pharmaceutical products such as buccal films, oral tablets, and in transdermal and topical films and patches.

Safety evaluation of the extended release placebo film was performed in a nonhuman primate model. Six pigtailed macaques were exposed to two films per week for two weeks, which is double the proposed human dosing (intended dosing is one film per week). In total, four films were delivered to each macaque over fourteen days. The vaginal microbiota, vaginal pH, and number of PMNs on gram stained vaginal smears were assessed before the first film placement and after the fourth film placement. The integrity of the cervicovaginal tissues was assessed by colposcopy at each film placement. Of note, there was visible film in the vagina with each film placement. No adverse events were noted by colposcopy; microbiota remained stable with no effect noted on the beneficial H202 producing microorganisms; vaginal pH remained within normal range; and PMN counts remained within the normal range even after more frequent administration than intended for use (Patton, personal communication). The entire study report is presented in Appendix IV.

Though the films used had different excipients, the tenofovir and dapivirine vaginal films used in FAME-02 and FAME-04 were found to be safe with no Grade 3 or higher Adverse Events attributed to study product and no changes in the vaginal microenvironment noted. The macaque exposure model utilized for this protocol provided the initial assurances of safety for those studies as well.

2.5 Summary

It is appropriate to advance this placebo film into a clinical trial for safety for the following reasons:

- The individual ingredients used in the film have a history of safe use in mucosal compartments (gastrointestinal and/or vaginal).
- The same film was found to be safe in macaques without evidence of epithelial disruption despite twice weekly dosing, twice the frequency of intended human use.

3 OBJECTIVES

3.1 Primary Objectives

- To assess the safety of a single dose of a placebo film which is intended for extended release of antiretroviral drugs

3.2 Secondary Objectives

- **Acceptability**

  To describe the acceptability of the placebo vaginal film and how different qualities are associated with higher or lower degrees of acceptability

3.3 Exploratory Objectives

- **Vaginal Microenvironment**

  - To compare the effects of the placebo film on the vaginal microbiota and glycome pre and post study product use.

- **Persistence**

  - To describe the persistence of the placebo vaginal film at 3, 7, 10 and 14 days after insertion

4 STUDY DESIGN

4.1 Identification of Study Design

This is, single site, open label placebo trial with randomized timing of follow-up

4.2 Summary of Major Endpoints

Grade 2 or higher Adverse Events deemed related to study product

4.3 Description of Study Population

The study population will be HIV-uninfected women who meet criteria outlined in Section 5.

4.4 Time to Complete Accrual

Accrual is expected to be completed in approximately 6 months.

4.5 Study Groups

All participants will receive a single dose of the vaginal film. They will be randomized at enrollment to the timing of Visit 3. In total, there will be four groups:

- Visit 3 at Day 3 (range days 2-4)
- Visit 3 at Day 7 (range days 6-8)
- Visit 3 at Day 10 (range days 9-11)
- Visit 3 at Day 14 (range days12-16)

4.6 Expected Duration of Participation

The duration of study participation per woman is expected to be approximately 5-11 weeks. This includes the screening period.

4.7 Site

There is a single study site: Magee-Womens Hospital, Pittsburgh, PA.

5 STUDY POPULATION

5.1 Selection of the Study Population

The inclusion and exclusion criteria in this section will be utilized to ensure the appropriate selection of study participants.

5.2 Recruitment

Participants will be recruited from a variety of sources including the ambulatory clinic of Magee-Womens Hospital and the surrounding population. Participants will also be referred to the study from other local research projects and other health and social service providers serving the target study population. Recruitment materials will be approved by the University of Pittsburgh Institutional Review Board.

5.3 Retention

Once a participant is enrolled, the study site will make every effort to retain her in follow-up to minimize possible bias associated with loss-to-follow-up. The site will implement the following procedures to enhance retention:

- Thorough explanation of the study visit schedule and procedural requirements during the informed consent process and re-emphasis at each study visit.
- Thorough explanation of the importance of all 4 study groups to the overall success of the study.
- Collection of detailed locator information at the study screening visits, and active review and updating of this information at each subsequent visit.
- Use of appropriate and timely visit reminder mechanisms.
- Immediate follow-up on missed visits.

Study sites will use a participant tracking mechanism to facilitate visit scheduling and timely identification and follow-up on missed visits.

5.4 Inclusion Criteria

Women must meet all the following criteria to be eligible for inclusion in the study:

1) Age 18 through 45 years (inclusive) at screening

2) Able and willing to provide written informed consent to be screened for and to take part in the study.

3) Able and willing to provide adequate locator information

4) HIV-uninfected based on testing performed by study staff at screening (per algorithm in Appendix II)

5) In general good health as determined by the site clinician

6) Agree to be sexually abstinent for 48 hours prior to each visit and from Visit 2 to seven days after Visit 3

7) At screening, agrees to abstain from any other intravaginal product or penetration (including sex toys, excluding tampons) for 48 hours prior to each visit and between Visit 2 and 3.

8) Willingness to undergo all study-related assessments and follow all study-related procedures

9) At screening and enrollment, agrees not to participate in other research studies involving drugs, medical devices, or vaginal products while enrolled in this trial

5.5 Exclusion Criteria

Women who meet any of the following criteria by participant report will be excluded from the study. Of note, the study is limited to premenopausal women with an intact uterus because the mucosal immune environment differs substantially between pre- and post- menopausal women. Therefore, inclusion of post-menopausal women would introduce heterogeneity into the population.

1.) Menopause (as defined as amenorrhea for one year or more without an alternative etiology)

2.) Hysterectomy

3.) Participant report of any of the following:
   a) Known adverse reaction to any of the study products (ever)
   b) Known adverse reaction to latex (ever)
   c) Non- therapeutic injection drug use in the 12 months prior to Screening
   d) Surgical procedure involving the pelvis in the 90 days prior to enrollment (includes dilation and curettage or evacuation, and cryosurgery; does not include cervical biopsy for evaluation of an abnormal pap smear)
   e) Participation in a drug, spermicide and/or microbicide study in the 30 days prior to enrollment or anticipated participation in an investigational drug study until completion of the study
   f) Currently pregnant or pregnancy within 42 days prior to enrollment
   g) Currently lactating
   h) Use of a diaphragm, NuvaRing®, or spermicide for contraception
   i) Internal vaginal use of any device or product (except tampons) in the 48 hours prior to enrollment

4.) Urogenital infection or suspected infection within 14 days of enrollment including: symptomatic candidiasis, trichomonas vaginalis, and symptomatic bacterial vaginosis; or cervical infection, including *N. gonorrhoeae* (GC), *C. trachomatis* (CT), or mucopurulent cervicitis; syphilis; HSV lesions, or other sores (Note: seropositive HSV without active lesions will not be excluded); acute pelvic inflammatory disease; urinary tract infection; recent exposure to a partner with GC, CT, Trichomonas, syphilis, or NGU

5.) Antibiotic or antifungal therapy (vaginal or systemic) within 14 days of enrollment

6.) As determined by the PI, has any significant uncontrolled active or chronic cardiovascular, renal, liver, hematologic, neurologic, gastrointestinal, psychiatric, endocrine, respiratory, immunologic disorder or infectious disease

7.) Menses-like bleeding at the time of the Enrollment visit* or expected menses-like bleeding within 14 days of the Enrollment visit
(*Women who have vaginal bleeding at the scheduled Enrollment Visit may return at a different date to be re-examined and possibly enrolled provided they are still within the screening window and meet all criteria.)

8.) Any condition that, in the opinion of the Investigator, would preclude provision of consent, make participation in the study unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the study objectives

6 STUDY PRODUCT

6.1 Regimen
Each participant will receive one vaginal film.

6.2 Administration
After eligibility is confirmed, site staff will randomize the participant to the timing of Visit 3 and retrieve the study product from the Pharmacy. Participants will be counselled thoroughly on product administration and then handed the film in its packaging. The participant will attempt to insert the film while a site staff member stands behind a curtain to maintain privacy. Should the participant have any concerns or questions, the site staff member will be readily available. Immediately after the participant attempts insertion, the site clinician will do a quick inspection of the external genitalia to assess for proper placement. If the film is visible outside of the vaginal introitus, the clinician will return to the pharmacy to retrieve a second dose of the vaginal film. The clinician will then remove visible film, insert a speculum, remove visible film from inside the vagina, and place a second film in the proximal third of the vagina using a forceps instrument.

6.3 Study Product Formulation
A two inch by two inch (2” x 2”) film containing no active pharmaceutical ingredient will be used. This placebo film is a cellulose based film containing hydroxypropyl methyl cellulose (HPMC) (E5 and K4M), hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC), Eudragit® (Ammonio Methacrylate Copolymer Type B), propylene glycol and polyethylene glycol 400. Placebo films will be manufactured by CMO under cGMP using hot melt extrusion and clinical trial material will be subjected to GMP stability program by the contract manufacturer.

6.4 Study Product Supply and Accountability
Packaged study product is being provided to the Magee-Womens Hospital Pharmacy. All study product will be available to the study staff through the Magee Pharmacy.

The Magee research pharmacist will maintain complete records of all study products received for this protocol and dispensed to participants. Additional documentation will be required for study product returns, destruction (if applicable) and other related issues as outlined in instructions for DAIDS clinical trials. All unused study products must be returned to the Pharmacy.

The film is packaged into a foil-lined pouch and stored at room temperature. Product has R&D stability for 6 months.

6.5 Study Product Dispensing

Study products will be dispensed only to enrolled participants upon receipt of a written prescription signed by an authorized prescriber.

6.6 Concomitant Medications

Enrolled study participants may use concomitant medications during study participation. All concomitant medications, over-the-counter preparations, vitamins and nutritional supplements, recreational drugs, and herbal preparations will be recorded on the concomitant medications log form.

All participants will be counseled to avoid the use of spermicide and other non-study vaginal products (other than tampons during menstruation and female condoms) while participating in the study. Participants who report use of these products will be counseled regarding the use of alternative methods, but reported use of these products does not require any change in study product administration or follow-up procedures. Condoms provided by study staff will not be coated with any type of spermicide.

7 STUDY PROCEDURES

This section describes visit-specific study procedures.

7.1 Pre-Screening

As part of participant outreach and recruitment strategies, study staff may pre-screen potential study participants (e.g. via telephone). During these interactions, study staff may explain the study to participants and ascertain elements of presumptive eligibility, to be confirmed at an on-site screening visit. If the participant is eligible based on a screening script her name and appointment time will be placed on the script. If she signs consent, the telephone script will then become part of her research record. If she does not sign consent, the form will be de-identified.

7.2 Visit 1: Screening Visit

Screening may take place up to 45 days prior to Enrollment. Screening procedures may occur over several visits. A combined written informed consent for screening and enrollment will be obtained before any screening procedures are initiated. For participants who do not meet the eligibility criteria, screening will be discontinued once ineligibility is determined.

The table below outlines procedure to take place at the Screening Visit.

Table 2: Visit 1 Procedures

VISIT 1: SCREENING VISIT	
Component	**Procedure/Analysis**
ADMINISTRATIVE	• Written Informed Consent • Assign Study Number (PTID) • Review Eligibility • Collect Contact Information • Visit Questionnaire • HIV Pre-/Post-Test Counseling • Screening Results • Abstinence/Condom Counseling (& distribution prn) • Schedule Next Study Visit, prn • Participant Reimbursement
URINE	• Pregnancy test • Urine dipstick, as clinically indicated
SALIVA	• Rapid HIV
BLOOD	• Confirmatory HIV, as clinically indicated
VAGINAL	• pH • Wet Mount, as clinically indicated • Trichomonas (NAAT) • Future Use
CERVICAL	• GC/CT (NAAT) • Future Use
CVL	• Levels of antiviral activity • Lectin microarray • Levels of glycoproteins and mucins • Levels of cytokines & innate immunity factors • Eudragit® level
PHYSICAL EXAM	• Physical Exam • Vital Signs (BP) • Height & Weight
PELVIC EXAM	• Bimanual Exam, as clinically indicated • External Genital & Speculum Exam

7.3 Visit 2: Enrollment

Subjects who meet the inclusion and exclusion criteria following the Screening Visit may schedule an Enrollment Visit. Care will be taken to schedule the Enrollment visit at a time when the participant is not expecting to be actively bleeding or anticipating her menses within 14 days.

Before performing any enrollment procedures, study staff will confirm eligibility. The participant will undergo a pelvic examination. Pelvic specimens as described below will be collected and the study staff will assess for bleeding. If no bleeding or other exclusionary findings are evident, the participant will then be randomized to the timing of Visit 3. The act of randomization is considered the act of enrollment for this study. Subjects with menses like vaginal bleeding at the Enrollment visit will not be randomized/enrolled at that time. These subjects may be rescheduled if vaginal bleeding resolves before 45 days from Screening have elapsed. If vaginal

bleeding does not resolve during the window period, and the subject expresses interest in enrollment for the study, she may be offered a rescreening attempt.

After eligibility is confirmed, the clinician will go to the Pharmacy to obtain the study product. Participants will be counselled thoroughly on product administration and then handed the film in its packaging. The participant will attempt to insert the film while a site staff member stands behind a curtain to maintain privacy. Should the participant have any concerns or questions, the site staff member will be readily available. Immediately after the participant attempts insertion, the site clinician will do a quick inspection of the external genitalia to assess for proper placement. If the majority of the film is visible outside of the vaginal introitus, the clinician will return to the pharmacy to retrieve a second dose of the vaginal film. The clinician will then remove visible film, insert a speculum, remove visible film from inside the vagina, and place a second film in the proximal third of the vagina using a forceps instrument. After film placement, participants will be scheduled for Visit 3. When Visit 3 occurs will be determined by randomization (3, 7, 10 or 14 days).

Table 3: Visit 2 Procedures

VISIT 2: ENROLLMENT – DAY 0 (Day 1 of Product Use)	
Component	**Procedure/Analysis**
ADMINISTRATIVE	• Review/Confirm Eligibility • Collect/Update Contact Information • Visit Questionnaire • Screening Results • Abstinence/Condom Counseling (& distribution prn) • Assess/Document Adverse Events • Schedule Next Study Visit • Participant Reimbursement
ACCEPTABILITY	• Acceptability Questionnaire
URINE	• Pregnancy test • Urine dipstick, as clinically indicated
VAGINAL	• pH • Wet Mount, as clinically indicated • Gram Stain • Quantitative Vaginal Culture • Quantitative PCR for microbiota • Future Use
CERVICAL	• Future Use
PHYSICAL EXAM	• Physical Exam, as clinically indicated • Vital Signs (BP)
PELVIC EXAM	• Bimanual Exam, as clinically indicated • External Genital & Speculum Exam • Post-insertion External Genital Exam • Repeat Speculum Exam, prn
RANDOMIZATION	• Randomization to timing of V3
STUDY PRODUCT	• Study Product Distribution • Participant Self-Inserted (1st attempt) • Clinician-inserted (2nd attempt), prn

7.4 Visit 3: Follow-Up Visit

The timing of Visit 3 will be determined through the randomization process. All participants will be randomized to return either 3,7,10, or 14 days after film insertion. Visit 3 procedures are outlined in Table 4. The windows around these study visits are as follows:
Day 3 (days 2-4)
Day 7 (days 6-8)
Day 10 (days 9-11)
Day 14 (days 12-16)

Table 4: Visit 3 Procedures

VISIT 3: FOLLOW-UP VISIT – 3, 7, 10, 14 DAYS POST FILM INSERTION	
Component	**Procedure/Analysis**
ADMINISTRATIVE	• Collect/Update Contact Information • Visit Questionnaire • Abstinence/Condom Counseling (& distribution prn) • Assess/Document Adverse Events • Schedule Next Study Visit • Participant Reimbursement
ACCEPTABILITY	• Acceptability Questionnaire
URINE	• Pregnancy test • Urine dipstick, as clinically indicated
VAGINAL	• pH • Wet Mount, prn • Trichomonas (NAAT), as clinically indicated • Gram Stain • Quantitative Vaginal Culture • Quantitative PCR for microbiota • Future Use
CERVICAL	• GC/CT (NAAT), as clinically indicated • Future Use
CVL	• Levels of antiviral activity • Lectin microarray • Levels of glycoproteins and mucins • Levels of cytokines & innate immunity factors • Eudragit® level
PHYSICAL EXAM	• Physical Exam, as clinically indicated • Vital Signs (BP)
PELVIC EXAM	• Bimanual Exam, as clinically indicated • External Genital & Speculum Exam

7.5 Visit 4: Follow-Up Visit 21 Days Post Enrollment

The final scheduled visit will be 21 (18-25) days after the Enrollment Visit.

TABLE 5: Visit 4 Procedures

VISIT 4: FOLLOW-UP VISIT	
Component	**Procedure/Analysis**
ADMINISTRATIVE	• Collect/Update Contact Information • Visit Questionnaire • Abstinence/Condom Counseling (& distribution prn) • Assess/Document Adverse Events • Participant Reimbursement
ACCEPTABILITY	• Acceptability Questionnaire
URINE	• Pregnancy test • Urine dipstick, as clinically indicated
VAGINAL	• pH • Wet Mount, as clinically indicated • Trichomonas (NAAT), as clinically indicated • Gram Stain • Quantitative Vaginal Culture • Quantitative PCR for microbiota • Future Use
CERVICAL	• GC/CT (NAAT), as clinically indicated • Future Use
CVL	• Levels of antiviral activity • Lectin microarray • Levels of glycoproteins and mucins • Levels of cytokines & innate immunity factors • Eudragit® level
PHYSICAL EXAM	• Physical Exam, as clinically indicated • Vital Signs (BP)
PELVIC EXAM	• Bimanual Exam, as clinically indicated • External Genital & Speculum Exam

7.6 Participants Who Become Pregnant

Urine pregnancy tests will be performed at screening, enrollment, Visit 3, the last study visit, and as clinically indicated. Participants who become pregnant will be referred for care. Should the pregnancy be diagnosed during study follow-up, pelvic exams will only be performed to evaluate a participant's reported symptom. No genital specimens will be collected in a pregnant participant.

Of note, the participant will be encouraged to continue in the study so that safety data might be collected. Participants who are pregnant at the final study visit will be contacted after their study participation to ascertain the pregnancy outcome.

7.7 Interim Visits

Interim visits may be performed at any time during the study (and study procedures repeated as clinically indicated), for the following or other reasons:

- For administrative reasons, e.g., a participant may have questions for study staff, or may need to re-schedule a follow-up visit.

- In response to AEs. When interim contacts or visits are completed in response to participant reports of AEs, study staff will assess the reported event clinically and provide or refer the participant to appropriate medical care.
- In the event of laboratory processing issues (i.e. inconclusive STD testing, lost or inadequate sample
- For other reasons at participant request.

Details of the interim visit will be recorded in the chart notes.

7.8 Clinical Evaluations and Procedures

Physical exams will include the following assessments:

- Vital signs:
  - Blood pressure

- Measurements of:
  - Weight (at Screening only)
  - Height (at Screening only)

- Focused physical exam (at Screening only):
  - General appearance
  - Cardiac exam
  - Respiratory exam
  - Abdomen

Additional assessments may be performed at the discretion of the examining clinician in response to symptoms or illnesses present at the time of the exam.

7.9 Laboratory Evaluations

Clinical Local Laboratory
Urine pregnancy test
Rapid saliva HIV test
Wet mount if clinically indicated
Vaginal pH
Dipstick urinalysis
Confirmatory HIV testing if indicated

MWRI Laboratories

Cervical NAAT for chlamydia, gonorrhea
Vaginal NAAT for trichomonas
Vaginal Gram stain
Quantitative vaginal cultures
Quantitative PCR for selected microbiota
Levels of cytokines and innate immunity factors from CVL
Anti-viral activity from CVL

New York University Laboratory (Mahal) and/or MWRI Laboratory
Levels of glycoproteins and mucin in CVL
Lectin microarray in CVL

University of Colorado Lab

7.10 Specimen Collection and Processing

The site will adhere to the standards of good clinical laboratory practice and site standard operating procedures for proper collection, processing, labeling, handling, transport, and storage of specimens. In cases where laboratory results are not available due to administrative or laboratory error, sites are permitted to re-draw and/or re-collect specimens.

7.11 Biohazard Containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study as recommended by the CDC and NIH. All biological specimens sent to the University of Colorado lab will be transported using packaging mandated by CFR 42 Part 72. Biohazardous waste will be contained according to institutional, transportation/carrier, and all other applicable regulations.

8 ASSESSMENT OF SAFETY AND CLINICAL MANAGEMENT

8.1 Safety Monitoring

The study site investigators are responsible for continuous close safety monitoring of all study participants, and for alerting the Protocol Team if unexpected concerns arise. A sub-group of the Protocol Team, including the Protocol Chair or designee, the DAIDS Medical Officer, and the External Safety Monitor will serve as the Protocol Safety Review Team (PSRT). Close cooperation among the PSRT and the study site will be necessary to monitor participant safety and respond to occurrences of toxicity in a timely manner. Appropriate safety monitoring will be contingent upon excellent communication between study participants and study staff, and upon cooperation among study staff, investigators, the External Safety Monitor, and the DAIDS Medical Officer.

8.2 Clinical Data Safety Review

An External Safety Monitor who is familiar with the pertinent scientific literature related to the study product will be responsible for the first review of data and safety monitoring. This physician, independent of the study sponsor, will be available to monitor data from this site. His/her minimum qualifications will include experience as a physician and experience in the conduct of clinical research. This individual will not receive salary or other support from the grant. The External (Independent) Safety Monitor model has been used successfully for other Magee-Womens Hospital Reproductive Infectious Disease studies involving investigational products. The proposed individual will meet the qualifications outlined above and have training in the importance of the objective treatment of clinical safety data.

The Data Management team for this site will generate data summaries for the Protocol Chair, the External Safety Monitor, and the DAIDS Medical Officer on an at least monthly basis. More frequent ad hoc safety reviews can be implemented during the initial enrollment and throughout the study as needed. These data summaries will include adverse event, accrual and retention data. The External Safety Monitor will evaluate adverse event data independently as well to determine whether the study protocol should continue as originally designed, should be changed, or should be terminated.

Approximately once a month the PSRT will convene via email or telephone to review adverse event data. Only masked safety data will be reviewed by the PSRT. If more urgent safety matters arise, telephone calls can occur more frequently.

The IRB will be notified of any serious and unexpected adverse events according to the policies outlined in the University of Pittsburgh IRB Policy and Procedure Manual[9].

The following information will be submitted to the University of Pittsburgh IRB at the time of renewal of a research protocol, as required by the IRB guidelines:

- The frequency of monitoring during the renewal interval, including the dates of data and safety monitoring;
- A summary of any assessment performed to evaluate external factors or other relevant information that may have an impact on the safety of study volunteers or the ethics of the research study;
- A summary of the outcome of procedural reviews conducted to ensure subject privacy and research data confidentiality;
- Any conclusions regarding changes to the anticipated benefit-to-risk ratio of study participation and final recommendations related to continuing, changing, or terminating the study, with accompanying rationales as appropriate.

8.3 Adverse Events Definitions

An AE is defined as any untoward medical occurrence in a clinical research participant administered an investigational product and which does not necessarily have a causal relationship with the investigational product. As such, an AE can be an unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of an investigational product, whether or not considered related to the product. This definition is applied to all groups beginning from the time of randomization.

Study participants will be provided instructions for contacting the study site to report any untoward medical occurrences they may experience, except for possible life-threatening events, for which they will be instructed to seek immediate emergency care. Where feasible and medically appropriate, participants will be encouraged to seek evaluation at Magee-Womens Hospital of UPMC, where the study clinicians are based, and to request that a study clinician be contacted upon their arrival. All participants reporting a clinically significant untoward medical occurrence will be followed either in person or by phone until the occurrence resolves (returns to baseline) or stabilizes over a four week period.

Study site staff will document in source documents all AEs reported by or observed in enrolled study participants regardless of severity and presumed relationship to study product.

For each study participant, AE documentation and reporting will be undertaken throughout the scheduled duration of follow-up, i.e., through Visit 4.

The PI/designee will grade the severity of each AE and the relationship of the AE to study product:

AE severity will be graded per the DAIDS Table for Grading Adult and Pediatric Adverse Events, Version 2.1, July 2017 and the Female Genital Grading Table for Use in Microbicide Studies (Appendix 1, version 1.0, November 2007) except that asymptomatic BV will not be considered an AE. AEs not included in the Female Genital Grading Table will be graded by the DAIDS AE Grading Table Version 2.1, July 2017. In cases where a genital AE is covered in both tables, the Female Genital Grading Table for Use in Microbicide Studies will be the grading scale utilized.

The relationship of all reported AEs will be assessed based on the Manual for Expedited Reporting of Adverse Events to DAIDS (Version 2.0, dated January 2010) and the clinical judgment of the PI/designee.

Per the Manual for Expedited Reporting of Adverse Events to DAIDS, the relationship categories that will be used for this study are:
Related: There is a reasonable possibility that the AE may be related to the study agent(s)

Not related: There is not a reasonable possibility that the AE is related to the study agent(s)
The study products that must be considered when AE relationships are assigned are the placebo film.

The DAIDS Table for Grading Adult and Pediatric Adverse Events, the Female Genital Grading Table for Use in Microbicide Studies, and Version 2.0 of the Manual for Expedited Reporting of Adverse Events to DAIDS are available on the DAIDS Regulatory Support Center (RSC) web site: http://rsc.tech-res.com/

All AEs will be captured on an AE log form. The form should be reviewed at each study visit and updated as needed. For any serious or expedited AEs (SAEs/EAEs) that are continuing at

a participant's study exit visit, the PI/designee must establish a clinically appropriate follow-up plan for the AE and review with the DAIDS Medical Officer. At a minimum, the AE must be re-assessed by study staff at least 2 weeks after the participant's study exit visit (Visit 4); additional evaluations also may take place at the discretion of the PI/designee. The same approach must be taken for any AEs deemed related to study product that are found to have increased in severity at the study exit visit (Visit 4). For those AEs requiring re-assessment, if the AE has not resolved or stabilized at the time of re-assessment, study staff will continue to re-assess the participant at least once per month while the study is ongoing. After the study has ended, all AEs requiring re-assessment will be re-assessed at least once within the 30-60 days after the study end date.

8.4 Expedited Adverse Event Reporting Requirements

8.4.1 Expedited Adverse Event Reporting to DAIDS

Requirements, definitions and methods for expedited reporting of Adverse Events (AEs) are outlined in Version 2.0 of the DAIDS EAE Manual, which is available on the DAIDS RSC website at http://rsc.tech-res.com/clinical-research-sites/safety-reporting/manual.

The DAIDS Adverse Experience Reporting System (DAERS), an internet-based reporting system, must be used for expedited AE reporting to DAIDS. In the event of system outages or technical difficulties, expedited AEs may be submitted via the DAIDS EAE Form. This form is available on the DAIDS RSC website at http://rsc.tech-res.com/clinical-research-sites/safety-reporting/daids/paper-eae-reporting.

For questions about DAERS, please contact NIAID CRMS Support at CRMSSupport@niaid.nih.gov. Please note that site queries may also be sent from within the DAERS application itself.

For questions about expedited reporting, please contact the DAIDS RSC Safety Office at (DAIDSRSCSafetyOffice@tech-res.com).

EAE reporting procedures specific to this protocol are that once the site has submitted EAEs via DAERS (as above), the RSC Safety Office will also prepare the draft safety reports and send them to the DAIDS Medical Officers for review. The study site will be contacted by the DAIDS Medical Officer if any further information or clarification is needed after the report is evaluated.

For all EAEs submitted, sites must file an RSC update with the final or stable outcome unless the initial EAE submitted had a final or stable outcome noted already.

EAE Reporting Level

This study uses SAE category of expedited AE reporting as defined in the DAIDS EAE Manual.

Study Agents for Expedited Reporting to DAIDS

The study agent that must be considered in determining relationships of AEs requiring expedited reporting to DAIDS is the placebo film.

Reporting Period

AEs must be reported on an expedited basis during the entire study duration for an individual subject (from study enrollment until study completion or discontinuation of the subject from study participation for any reason). After the protocol-defined AE reporting period, unless otherwise noted, only suspected unexpected serious adverse reactions (SUSARs as defined in Version 2.0 of the EAE Manual) will be reported to DAIDS if the study staff become aware of the events on a passive basis (from publicly available information).

8.4.2 Reporting of Critical Events to DAIDS

Protocol violations and unanticipated problems must be reported to DAIDS as critical events. The following information about the critical event will be submitted to DAIDS MO and Clinical Operations Officer:

A detailed description of the problem;

Actions the study site is taking or plans to take to address the problem, such as suspending participant enrollment, termination of research, revising study protocol or informed consent documents, increasing monitoring of the study participant, etc. Any revisions to the study protocol and informed consent must be reviewed and approved by the DAIDS MO and study site IRB prior to implementation.

Serious or continuing noncompliance and suspension/termination of IRB approval must be reported as critical events as well.

8.5 Clinical Management of Adverse Events

By definition, an adverse event can be either new finding or symptom or a worsening of a pre-existing condition. In order to accurately capture adverse events in follow-up, a thorough baseline history will be obtained at Visit 1 and Visit 2. For example, for participants who endorse a history of headache, site staff will probe for and record details surrounding the condition such as frequency, location, duration, medication use, triggers, etc. Only by eliciting a full description will study staff be equipped to determine whether a subsequent event in follow-up is a clinically distinct event or not.

Adverse events will be elicited at each follow-up visit. Referral to appropriate care will be offered to participants as needed. Vaginitis and some sexually transmitted infections can be treated in the study clinic. The PI/designee should manage STI/RTI per CDC guidelines, available at http://www.cdc.gov. Observed single dose treatment should be provided whenever possible. Vaginally applied medications should not be used if possible.

8.5.1 Product Hold

In the unlikely event that a participant is intolerant of the study product immediately after placement, the site clinician will perform a pelvic exam to remove all visible product.

8.6 Criteria for Early Termination of Study Participation

Participants may voluntarily withdraw from the study for any reason at any time. The Site PI/designee also may withdraw participants from the study to protect their safety and/or if they are unwilling or unable to comply with required study procedures. Participants also may be withdrawn if the study sponsors, government or regulatory authorities, including the Office of Human Research Protections (OHRP), or site IRBs/ECs terminate the study prior to its planned end date. Every reasonable effort is made to complete a final evaluation of participants who withdraw or are withdrawn from the study prior to completing follow-up. Study staff members will record the reason(s) for all withdrawals in participants' study records.

9 STATISTICAL CONSIDERATIONS

9.1 Review of Study Design

The primary aim of this study is to assess the safety of a single dose of placebo film. Urogenital, systemic and menstrual symptoms will be collected via symptom review with participants. Testing for sexually transmitted infections and vaginitis will occur at the screening visit to rule out other potential causes of reproductive tract inflammation and epithelial disruption. This testing will be repeated during subsequent examinations if clinically indicated. Data on epithelial inflammation and disruption will be collected during visual examination of the reproductive tract. Pharmacokinetic measures will assess persistence following a single exposure.

9.2 Sample Size and Accrual

The primary aim of the study is to assess the local and systemic safety of a single dose of placebo vaginal film. The primary endpoint is the proportion of participants who experience a Grade 2 or higher adverse event. The proposed total sample size is N=64 divided into 4 arms (placebo film with follow up at days 3, 7, 10, or 14 assigned at a 1:1:1:1 ratio). Based on previous studies of vaginal products conducted at the study site, the accrual of 64 eligible subjects with normal reproductive tracts is expected to require the screening of approximately 150 volunteers. Since unevaluable participants will be replaced, it is anticipated that 64 women exposed to study product will complete study visit 3.

The sample size is based on the exact binomial probability of observing at least two Grade 2 or higher Adverse Events. For a given arm, if the true rate of a given toxicity endpoint is 5%, 16 women per arm provide 80% power to exclude toxicity endpoint rates greater than 27% (the probability of observing zero or one event is less than 0.05 when the true rate is 27%).

Table 6: Power Calculations for FAME 101

Event Rate	P(No events | n=16)	P(1 or more events | n=16)	P(2 or more events | n=16)
1%	0.851	0.149	0.011
5%	0.440	0.560	0.189
10%	0.185	0.815	0.485
15%	0.074	0.926	0.716
20%	0.028	0.972	0.859
25%	0.010	0.990	0.937
27%	0.007	0.993	0.955
30%	0.003	0.997	0.974
35%	0.001	0.999	0.990

In previous studies of two different placebo film products the combined frequency of Grade 2 or higher Adverse Events was 19%. Assuming a similar frequency in the current study, a sample size of 64 women will assure that a 95% confidence interval for the Grade 2 or higher Adverse Events rate has an upper limit of no greater than 30%.

9.3 Study Endpoints

9.3.1 Primary Endpoint

Grade 2 Adverse Events

The safety/toxicity endpoint is clinical or laboratory evidence of a Grade 2 or higher Adverse Event as defined by the DAIDS Table for Grading Adult and Pediatric Adverse Events, Version 2.1, July 2017 and the Female Genital Grading Table for Use in Microbicide Studies (Appendix 1 to the DAIDS Table for Grading Adult and Pediatric Adverse Events, (Dated November 2007).

9.3.2 Secondary and Exploratory Endpoints

The secondary and exploratory endpoints of this study are as follows:

Secondary: Acceptability

Reports of overall acceptability of the film and qualities of the experience with the film will be measured using a self-report survey drawing items from two sources. Overall acceptability is measured using standard language in market research methodology, each using a 5-point agreement scale followed by an open-ended explanation. Assessment of film-specific qualities of the experience is assessed by a 9-item scale drawn from previous research with participant-inserted films. Questions assess perceptions of leaking, vaginal dryness, and sensation of the film inside the vagina during and after insertion.

Exploratory: Vaginal Microenvironment

Given that there is no non-product use arm, participants will serve as their own control. Assessment of baseline microenvironment will take place during the enrollment visit with the exception of CVL properties. CVL will be obtained at the screening visit so that the microenvironment can recover prior to study product administration at the enrollment visit.

Levels of lectin binding and mucins measured in CVL samples

Endogenous and exogenous glycolytic enzymes, formulated microbicide products, and microbial flora interact with mucins and may alter their structure and function. These alterations may be detected with lectins, which are sugar specific binding molecules, and can be bound to reporter molecules (such as horseradish peroxidase) in an ELISA type assay.

Changes in the Glycome

Lectin microarray will be used to assess changes in the glycome.

Nugent Score

Clinically significant changes in vaginal flora will be evaluated by a change in the Nugent score baseline (Visit 2, pre-exposure) to Visit 5. The Nugent score is graded 1 to 10 as follows:

Normal, 0 to 3
Intermediate, 4 to 6
BV, 7-10

Any shift from normal at baseline to intermediate or BV at Visit 3 or 4, or shift from intermediate or BV to normal at Visit 3 or 4 will be considered a scientifically meaningful change in vaginal microbiota.

Quantitative vaginal cultures and quantitative PCR

Meaningful changes will be defined by greater than or equal to 1 log changes in dominant members of the microbiota, including *Lactobacillus* species, *Gardnerella vaginalis*, key anaerobic microorganisms, *Enterococcus* species, *Escherichia coli*, *Staphylococcus aureus*, Group B *Streptococcus*, and *Candida* species.

Levels of anti-viral activity in CVL

In-vitro anti-HIV activity will be determined by the TZM-bl assay.

The TZM-bl assay will determine HIV-1 infection by luciferase for single round of replication. To assess the potency of the CVL, serial dilutions will be made and tested to determine the IC_{50}.

Levels of cytokine and innate immunity factors
Changes in cervical cytokine expression and innate immunity factors in cervicovaginal lavage will be assessed. Changes within each group will be reported.

Exploratory: Pharmacokinetic (PK)/ Persistence of Eudragit® in CVL

Persistence of polymer will be determined as detected levels of polymer in vaginal fluid collected through cervicovaginal lavage.

9.4 Blinding

All participants will receive a single dose of the vaginal film. They will be randomized to the timing of Visit 3. This will not be blinded.

9.5 Random Assignment

The randomization scheme will be generated and maintained by a member of the Data Management Division at the study site. Women will be randomized to one of the four arms at a 1:1:1:1 ratio using a permuted block design with random block sizes of 4 and 8. In order to minimize bias in group assignment, participants will be given study identification numbers. A study staff member will allot the identification numbers in sequential order as participants become eligible to enroll in the study. These numbers will be randomly assigned with timing of Visit 3. The randomization scheme will be created for a total of 72 participants; the overage will be created to compensate for participant withdrawals or unusable products.

9.6 Data Monitoring and Analysis

9.6.1 Data Monitoring

This clinical trial will be conducted in compliance with the protocol, GCP guidelines, and applicable regulatory requirements. All research charts are maintained in locked files in a locked room. The research staff, under the direction of the primary investigator, will create and maintain an electronic database on the hospital system on the computers in the Reproductive Infectious Diseases research offices. The database will be backed up every night onto the server's back-up system, and weekly onto an external storage device. Appropriate firewall and virus scanning software are installed and updated routinely by the hospital support staff.

This study will utilize electronic data capture. Once the source documents are ready for computer entry, site staff will enter data into a data entry system. Study data management staff will review the electronic data elements for completeness and accuracy. If there are any responses that are incomplete, unclear, or inconsistent with related data elements, the staff person will speak with the clinician in question as soon as possible to resolve the problem. If necessary, the study staff will make the appropriate change in the electronic database which tracks time and date a change is made.

9.6.2 Primary Endpoint Analysis

The number of adverse events will be summarized by severity, body system, and relationship to study product using frequencies, percent, and 95% confidence intervals. Individual participants will contribute once to the calculation of event rates.

9.6.3 Secondary and Exploratory Endpoint Analysis

Summary statistics of frequencies, percentages, and 95% confidence intervals will be provided for categorical data. Means, standard deviations, medians, ranges and 95% confidence

intervals will be provided for continuous data. Methods for the analyses of the secondary endpoints are described below.

One-way analysis of variance (ANOVA) and Kruskal-Wallis tests will be used, where appropriate, to evaluate differences in the following continuous variables between the study arms: levels of glycoproteins and mucins in CVL, levels of cytokines and innate immunity factors, and antiviral activity in CVL. Between groups, pre- to post-exposure changes in the above endpoints will also be evaluated using ANOVA and Kruskal-Wallis tests, where appropriate, while paired Student's t- and Wilcoxon signed-rank tests will be used to evaluate these changes within each study arm. Mixed effects linear regression will be used to evaluate associations between days of exposure to study product and these outcomes.

Nugent score will be categorized as follows: normal (score 0-3), intermediate (score 4-6), and bacterial vaginosis (score 7-10). Differences in the categorized Nugent score pre and post exposure levels will be evaluated using Fisher's exact, while McNemar's tests will be used to evaluate pre- to post-exposure shifts between normal (score 0-3) and abnormal (score 4-10) vaginal microflora within each study arm.

Prevalence of microorganisms at each visit will be compared between the study arms using Fisher's exact tests, while pre- to post-exposure shifts will be evaluated within each group using McNemar's tests. Differences between the study arms in the quantity of microorganisms at each visit, as well as the change in quantity, will be evaluated using Mann-Whitney U tests. Meaningful changes will be defined by greater than or equal to 1 log changes in dominant members of the microflora, including Lactobacillus (H_2O_2 positive and negative strains), anaerobic gram negative rods, Gardnerella vaginalis, Candida species, facultative gram negative rods, Streptococci and facultative gram positive cocci and rods. Fisher's exact tests will be used to evaluate differences in these changes between study arms. Mixed effects linear regression (for continuous outcomes) and generalized estimating equations (for binary outcomes) will be used to evaluate associations between days of exposure to study product and these outcomes.

Time since last menstrual period will be included as a potential confounder in these analyses.

9.6.4 Analysis Cohort

The cohort will be defined as participants who are randomized. It will be used for analyses of baseline characteristics, protocol deviations and violations, and trial conduct. The intent-to-treat cohort (ITT) will be defined as those who were randomized. The evaluable cohort (Eval) is a subset of ITT participants. Evaluable participants will be defined as participants who complete the Screening and Enrollment Visits and return for their Visit 3 within the window. Non-evaluable participants will be replaced.

Table 7: Statistical Analysis Plan

Endpoint	Variable	Statistics
Primary	Adverse Events	Frequency, Percent, 95% Confidence Interval
Exploratory	Persistence of Eudragit® in CVL	Frequency, Percent, 95% Confidence Interval
	Levels of glycoproteins and mucins, in CVL	Mean, SD, Median, Range, 95% Confidence Interval
	Antiviral activity in CVL	Mean, SD, Median, Range, 95% Confidence Interval

	Change in vaginal flora (Nugent score)	Frequency, Percent, 95% Confidence Interval
	Culture/qPCR results from vaginal organisms	Frequency, Percent, 95% Confidence Interval
		Mean, SD, Median, Range, 95% Confidence Interval
	Change in cytokine and innate immunity factors	Mean, SD, Median, Range, 95% Confidence Interval
	Lectin Microarray to assess changes in gylcome	Mean, SD, Median, Range, 95% Confidence Interval

10 HUMAN SUBJECTS CONSIDERATIONS

The investigators will make efforts to minimize risks of these products to human subjects. Volunteers will take part in a thorough informed consent process throughout their participation in the study. Before beginning the study, the investigators will have obtained IRB approval. The investigators will permit audits by the NIH or any of their appointed agents.

10.1 Special Populations

Study staff will offer screening to eligible women of all ethnic and racial groups. Members of the study staff are not seeking the screening or enrollment of women in special or vulnerable populations. The following section also discusses special considerations for male partners of participants.

10.1.1 Men

Men are not included as subjects in the study because the study is testing a vaginal application of the study product. The male sexual partners of women participating in this study will not be consented or monitored for several reasons. Protocol-specified guidelines for abstinence and condom use are expected to protect male partners from exposure to the study product. In addition, based on preclinical data, no toxicity is anticipated from the study product.

10.1.2 Children

The NIH has mandated that children be included in research trials when appropriate. This study will enroll women aged 18 to 20 who are able to give informed consent. This study meets “Justifications for Exclusion” criteria for younger children as set forth by the NIH. Specifically, "the research topic to be studied is irrelevant to (young) children" and "a separate, age-specific study in (adolescent) children is warranted and preferable" at a later time.

10.1.3 Prisoners

Prisoners will not be included in this study (for screening or enrollment). Any participants incarcerated during the course of participation in the trial will not be followed during their incarceration, and will be discontinued from the study. Participants who have been released from incarceration will be permitted to return for any protocol specified follow-up or safety visits per the guidelines of the local IRB.

10.1.4 Pregnant women

Pregnancy is an exclusion criterion as the vaginal microenvironment in pregnancy differs substantially from the non-pregnant state. Including pregnant participants would make the interpretation of study results difficult. Women who become pregnant during the study period following randomization will not be excluded from analysis. Participants who become pregnant

during the course of the study will be contacted periodically after study termination in order to determine the pregnancy outcome.

10.2 Informed Consent Process

Written informed consent will be obtained from all potential study participants prior to the initiation of any study-related procedures. The informed consent process will give individuals all of the relevant information they need in order to decide whether to participate, or to continue participation, in this study. Potential research participants will be permitted to ask questions and to exchange information freely with the study investigators. Only listed study investigators may obtain informed consent from potential study participants. The investigators will keep research participants fully informed of any new information that could affect their willingness to continue study participation.

10.2.1 Risk/Benefit Statement

Risks

It is not expected that this trial will expose human subjects to unreasonable risk. The intervention used in this study is unlikely to cause uncomfortable side effects and is only given once. However, there may be unknown risks or side effects associated with the use of the vaginal film. Some women who used the film in other studies have reported vaginal discharge, and irritation and discomfort.

Having genital exams may cause some discomfort from placing the speculum. It is unlikely that collection of genital samples will cause discomfort. Cervicovaginal lavage has been associated with mild discomfort secondary to the introduction of sterile fluid into the vagina and its removal. Standard study procedures do not include a blood draw; however, a blood draw is required if a confirmatory HIV test needs to be run. Phlebotomy may lead to discomfort, feelings of dizziness or faintness, and/or bruising, swelling and/or infection.

Disclosure of sexually transmitted infection (STI) may cause sadness or depression in volunteers. Disclosure of HIV-positive status has been associated with depression, suicidal ideation, and denial as well as social isolation. Participation in clinical research includes the risks of loss of confidentiality and discomfort with personal nature of questions.

Benefits

Participation in this trial likely will have no direct benefit to volunteers. Some volunteers may have the opportunity to access expedient treatment and decreased morbidity due to early diagnosis and treatment of a cervical and/or vaginal abnormality with expedient referral if an abnormality is detected. Lastly, the participant may appreciate the opportunity to contribute to the body of knowledge in the field of microbicide research. However, there is no guarantee that volunteers will receive any of these benefits.

10.3 Incentives

Volunteers will not be charged for any of the study visits, study supplies or examinations. There are no costs to participants in this study other than their time. Pending IRB approval of these compensation guidelines, women will be compensated for their time and inconvenience and for their travel needs while participating in the protocol. The approved amounts of compensation will be given out at each visit. The visits will be prorated and partial payment given in the event that the participant only completes a portion of the study visits. The following proposed compensation amounts were created based on common institutional practice for studies investigating vaginal products, as well as studies utilizing observation periods in the Clinical Translational Research Center.

Visit 1	$50

Visit 2	$50
Visit 3	$100 (if evaluable and compliant with date/time)
Visit 4	$50

In addition a parking pass or a bus pass will be provided to participants as needed.

10.4 Participant Confidentiality

Members of the study staff are all trained in patient confidentiality. The log of study subject names and other protected health information is kept in a locked area. All computer information about study volunteers is kept on a computer with log-on passwords. Laboratory specimens are labeled with study numbers and date, and are delivered by study staff. In addition to the research study staff, the NIH, study monitors, University of Pittsburgh Research Conduct and Compliance Office, the Office of Human Research Protections, and/or the University of Pittsburgh IRB may have access to participant's health information. Each member of the staff has log-on identification and password, logs off before leaving a computer screen unattended, and closes their office door when out of the office. All research records will be kept for a minimum of seven years following closure of this study (per University of Pittsburgh policy).

10.5 Communicable Disease Reporting

Study staff members will comply with all local requirements to report communicable diseases including chlamydia, gonorrhea, and HIV identified among study participants to the Allegheny County Health Department. Study investigators will include discussion of mandated reporting during the study informed consent process.

10.6 Study Discontinuation

NIAID or the University of Pittsburgh Institutional Review Board may discontinue this study at any time. Ongoing safety monitoring will track the incidence of AEs and EAEs. In the event of an abnormal number of reported AEs and/or EAEs judged to be related to study product the External Safety Monitor will contact the Principal Investigator to initiate a temporary hold on further enrollment.

11 LABORATORY SPECIMENS AND BIOHAZARD CONTAINMENT

11.1 Laboratory Specimens

Laboratory specimens will be handled in a manner consistent with institutional, OSHA, and GLP guidelines. Study staff members are trained in the appropriate handling of laboratory specimens. Samples such as urine that will be divided for multiple analyses will be divided according to site SOP.

Table 8: Laboratory Test Methods

SAMPLE	METHOD	LABORATORY
URINE	Pregnancy test	Clinical Research Center CLIA #39D1031322
	Urine dipstick	
SALIVA	Rapid HIV	Clinical Research Center CLIA #39D1031322
BLOOD	Confirmatory HIV	UPMC Presbyterian Shadyside CP PUH CLIA# 39D0911193
VAGINAL*	pH	Clinical Research Center CLIA #39D1031322
	Wet Mount	
	Trichomonas (NAAT)	Magee-Womens Research Institute CLIA #39D1004688
	Gram Stain	
	Quantitative Vaginal Culture	
	Quantitative PCR for microbiota	

CERVICAL*	GC/CT (NAAT)	Magee-Womens Research Institute CLIA #39D1004688
CVL	Levels of antiviral activity	Magee-Womens Research Institute/Dezzutti Laboratory (non-diagnostic laboratory)
	Lectin microarray	Magee-Womens Research Institute/Moncla Laboratory (non-diagnostic laboratory) and/or New York University (Mahal Laboratory)
	Levels of glycoproteins and mucins	
	Levels of cytokines & innate immunity factors	Magee-Womens Research Institute/Moncla Laboratory (non-diagnostic laboratory)
	Eudragit® level	University of Colorado (non-diagnostic laboratory)

*Future Use Samples will be stored at Magee-Womens Research Institute; use and processing lab to be determined

11.1.1 Urine Samples

Study staff at the clinical site will utilize dipstick urinalysis to screen for possible urinary tract infection as clinically indicated. Urine will be tested for HCG via the Sure-Vue® test.

11.1.2 Vaginal Samples

The assessment of vaginal flora will be based on the Nugent Scoring System for Gram Stained Vaginal Smears as well as assessment of several groups of organisms by cultivation (culture) based methods. In addition, selected microbiota will be detected using a quantitative polymerase chain reaction (qPCR) test which has been developed and validated within the laboratory. Gram stained vaginal smears will have neutrophils quantified according to site laboratory SOP. Collection will be done as gently and carefully as possible so as to collect an adequate sample that is unlikely to disturb the mucosa or overall vaginal microenvironment. Trichomonas vaginalis will be detected using an amplified DNA assay and handled according to policies outlined in SOPs for this study.

11.1.3 Cervical Swabs

C. trachomatis and *N. gonorrhoeae* will be detected using an amplified DNA assay and handled according to policies outlined in SOPs for this study.

11.1.4 Cervicovaginal Lavage Samples

Eudragit® Content in CVL

Aliquots of lavage samples will be tested for polymer levels (according to SOP).

Anti-viral Activity in CVL

Aliquots of lavage samples will be tested for anti-viral and antibacterial activity as outlined in the site SOPs.

Cervical Cytokines and Innate Factors in CVL

Cytokines (for example, IL-1β and TNFα) along with innate immune factors (for example, antiproteases) that could contribute to a proinflammatory milieu or changes in the glycomic signatures may be evaluated."

Glycome Studies

Aliquots of lavage samples will be tested for glycoproteins, mucins and lectin microarray.

11.2 Quality Control and Quality Assurance Procedures

Dr. Sharon Hillier, who completed a training program in clinical and public health microbiology certified by the American Board of Medical Microbiology (ABMM), directs the Infectious Disease Research Laboratory at Magee-Womens Research Institute. This laboratory is CLIA-inspected and maintains its own CLIA license. Thus, all testing done in this research laboratory is performed with the same level of quality control as required in a licensed clinical laboratory.

11.3 Specimen Storage and Possible Future Research Testing

Part of the consent form will include an explanation of future use of vaginal and cervical specimens and blood samples. Any future use or residual samples will be stored at Magee-Womens Research Institute, Microbiology Laboratory, 5th floor for an indefinite period of time. The principal investigator will assume primary responsibility for control of this area.

Any results from research done on future use or leftover specimens will not be placed in health records and will be kept confidential. The language and format employed in the consent for these purposes are an IRB-approved means commonly employed in studies performed at this and other study sites within our institution to obtain permission for use of stored samples. All primary study endpoints, protocol-specified testing, and QA/QC testing will be ascertained prior to any additional testing of stored specimens.

11.4 Biohazard Containment

Biohazardous waste will be contained according to institutional and all other applicable regulations.

12 ADMINISTRATIVE PROCEDURES

The study proposal for funding, this protocol, the informed consent document, data collection forms, and advertising flyers are all reviewed by the University of Pittsburgh Institutional Review Board prior to enrollment of participants in the study.

12.1 Protocol Registration

Prior to implementation of this protocol, and any subsequent full version amendments, the site will have the protocol and the consent form approved, as appropriate, by their local institutional review board (IRB)/ethics committee (EC) and any other applicable regulatory entity (RE). Upon receiving final approval, the site will submit all required protocol registration documents to the DAIDS Protocol Registration Office (DAIDS PRO) at the Regulatory Support Center (RSC). The DAIDS PRO will review the submitted protocol registration packet to ensure that all of the required documents have been received.

The site will receive an Initial Registration Notification when the DAIDS PRO receives a complete registration packet. Receipt of an Initial Registration Notification indicates successful completion of the protocol registration process. Sites will not receive any additional notifications from the DAIDS PRO for the initial protocol registration. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

Upon receiving final IRB/EC and any other applicable RE approvals for an amendment, the site should implement the amendment immediately. The site is required to submit an amendment registration packet to the DAIDS PRO at the RSC. The DAIDS PRO will review the submitted protocol registration packet to ensure that all the required documents have been received. The site will receive an Amendment Registration Site-specific ICF (s) WILL NOT be reviewed and approved by the DAIDS PRO and sites will receive an Amendment Registration Notification when the DAIDS PRO receives a complete registration packet A copy of the Amendment Registration Notification should be retained in the site's regulatory files.

For additional information on the protocol registration process and specific documents required for initial and amendment registrations, refer to the current version of the DAIDS Protocol Registration Manual.

12.2 Data Coordination

Data management responsibilities will reside with the Data Management staff at Magee-Womens Hospital.

12.3 Study Monitoring

Monitoring will be carried out by Pharmaceutical Product Development Inc., (PPD, Wilmington, NC). Site monitoring visits will be conducted to assess overall study compliance, as required per *Requirements for On-Site Monitoring of DAIDS Funded and/or Sponsored Clinical Trials,* GCP, and FDA regulations 21 CFR Part 312:

http://www.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/Documents/onsitemonitor_reqs.pdf

Study monitors will visit the site to complete the following:

- Assess compliance with the study protocol, Good Clinical Practices (GCP) guidelines, and applicable regulatory requirements, including US CFR Title 45 Part 46 and Title 21 Parts 50, 56, and 312
- Review informed consent forms, procedures, and documentation
- Perform data verification to ensure the accuracy and completeness of study data as applicable
- Verify proper collection and storage of biological specimens
- Verify proper storage, dispensing, and accountability for investigational study products
- Assess implementation and documentation of internal site quality management procedures
- Assess site staff training needs

Site investigators will allow study monitors to inspect study facilities and documentation (e.g., informed consent forms, clinic and laboratory records, other source documents, case report forms), as well as observe the performance of study procedures. Investigators also will allow inspection of all study-related documentation by authorized representatives of the DAIDS, Sponsor and US regulatory authorities. A site visit log will be maintained at the study sites to document all visits. The outcomes of the monitoring visits and the subsequent reports of resolutions of any identified problems will be provided to the Sponsor.

12.4 Protocol Compliance

All protocol amendments will be submitted to and approved by the University of Pittsburgh IRB and DAIDS prior to the implementation of an amendment.

12.5 Investigator's Records

The investigator will maintain, and store securely, complete, accurate and current study records throughout the study. Study records will not be destroyed prior to receiving approval for record destruction from the Principal Investigator and they will be maintained for a minimum of seven years following completion of the study, per the University of Pittsburgh IRB policy. Applicable records include source documents, site registration documents and reports, correspondence, informed consent forms, and notations of all contacts with the participant.

12.6 Use of Information and Publications

Publication of study results will be governed by DAIDS policies. The investigators will submit any presentation, abstract, or manuscript to DAIDS for review prior to submission.

12.7 Training Procedures

Only study staff trained and experienced in HIV pre-test and post-test counseling and who are investigators on this study will provide these study procedures. Approved written materials consistent with the local clinical standard of care will support pre-test and post-test counseling.

REFERENCES

1. UNAIDS. Report on the global HIV/AIDS epidemic 2008.
2. UNAIDS. AIDS epidemic update: November 2009.
3. Buffa V, Stieh D, Mamhood N, Hu Q, Fletcher P, Shattock RJ. Cyanovirin-N potently inhibits human immunodeficiency virus type 1 infection in cellular and cervical explant models. J Gen Virol 2009;90:234-43.
4. Gupta P, Collins KB, Ratner D, et al. Memory CD4(+) T cells are the earliest detectable human immunodeficiency virus type 1 (HIV-1)-infected cells in the female genital mucosal tissue during HIV-1 transmission in an organ culture system. J Virol 2002;76:9868-76.
5. Collins KB, Patterson BK, Naus GJ, Landers DV, Gupta P. Development of an in vitro organ culture model to study transmission of HIV-1 in the female genital tract. Nat Med 2000;6:475-9.
6. Cummins JE, Jr., Guarner J, Flowers L, et al. Preclinical testing of candidate topical microbicides for anti-human immunodeficiency virus type 1 activity and tissue toxicity in a human cervical explant culture. Antimicrob Agents Chemother 2007;51:1770-9.
7. Anton P, Elliott J, Tanner K, et al. Strong Suppression of HIV-1 Infection of Colorectal Explants following in vivo Rectal Application of UC781 Gel: A Novel Endpoint in a Phase I Trial. In: 16th Conference on Retroviruses and Opportunistic Infections. Montreal; 2009.
8. Anton P CR, Carballo-Diequez A, Kashuba A, Khanukhova E, Elliott J, Janocko L, Cumberland W, Mauck C, McGowan I. RMP-02/MTN-006: A Phase 1 Placebo-controlled Trial of Rectally Applied 1% Vaginal TFV Gel with Comparison to Oral TDF. 18th Conference on Retroviruses and Opportunistic Infections, Boston MA 2011.
9. Steiner M, Spruyt A, Joanis C, et al. Acceptability of Spermicidal Film and Foaming Tablets Among Women in Three Countries. International Family Planning Perspectives 1995;21:104-7.
10. Visness CM, Ulin P, Pfannenschmidt S, Zekeng L. Views of Cameroonian sex workers on a woman-controlled method of contraception and disease protection. Int J STD AIDS 1998;9:695-9.
11. Raymond E, Alvarado G, Ledesma L, et al. Acceptability of two spermicides in five countries. Contraception 1999;60:45-50.
12. Schley A. IPM Product Acceptability Study II (PAS II). In: International Partnership for Microbicides; 2009.
13. Nel AM, Mitchnick LB, Risha P, Muungo LT, Norick PM. Acceptability of vaginal film, soft-gel capsule, and tablet as potential microbicide delivery methods among African women. J Womens Health (Larchmt) 2011;20:1207-14.
14. Raymond EG, Chen PL, Condon S, et al. Acceptability of five nonoxynol-9 spermicides. Contraception 2005;71:438-42.
15. Pittsburgh Uo. Institutional Review Board Manual. http://wwwirbpittedu/manual/.

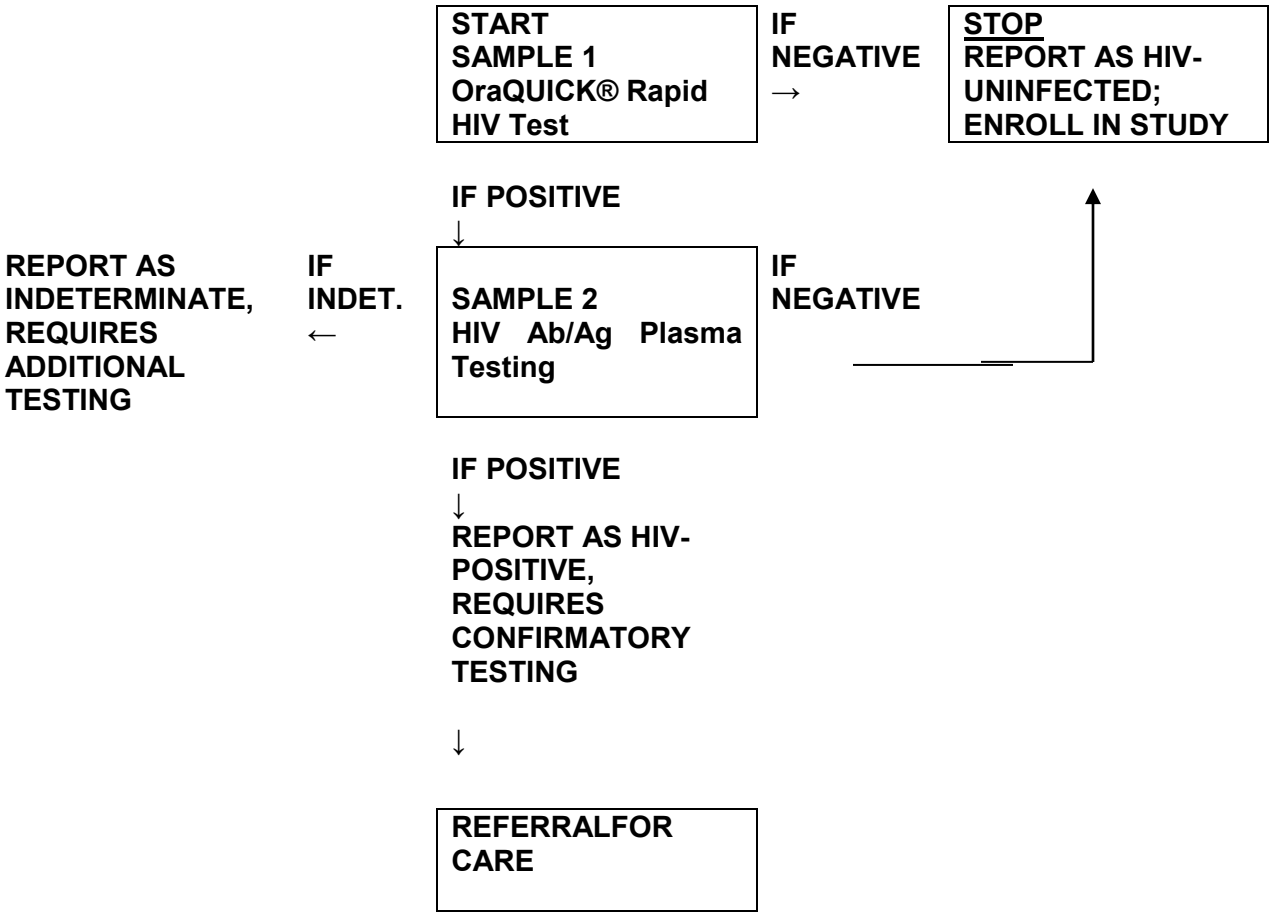
APPENDIX 1: SCHEDULE OF STUDY VISITS AND PROCEDURES*

Component	Procedure/Analysis	V1: Screening	V2: Enrollment	V3: 3, 7, 10 or 14 Day FU	V4: 21 Day FU
ADMINISTRATIVE	Written Informed Consent	X			
	Assign Study Number (PTID)	X			
	Review/Confirm Eligibility	X	X		
	Collect/Update Contact Information	X	X	X	X
	Visit Questionnaire	X	X	X	X
	HIV Pre-/Post-Test Counseling	X			
	Screening Results	X	X		
	Abstinence/Condom Counseling (& distribution prn)	X	X	X	X
	Assess/Document Adverse Events		X	X	X
	Schedule Next Study Visit	X	X	X	
	Participant Reimbursement	X	X	X	X
ACCEPTABILITY	Acceptability Questionnaire		X	X	X
URINE	Pregnancy test	X	X	X	X
	Urine dipstick	^	^	^	^
SALIVA	Rapid HIV	X			
BLOOD	Confirmatory HIV	^			
VAGINAL (SWAB)	pH	X	X	X	X
	Wet Mount	^	^	^	^
	Trichomonas (NAAT)	X		^	^
	Gram Stain		X	X	X
	Quantitative Vaginal Culture		X	X	X
	Quantitative PCR for microbiota		X	X	X
	Future Use	X	X	X	X
CERVICAL	GC/CT (NAAT)	X		^	^
	Future Use	X	X	X	X
CVL	Levels of antiviral activity	X		X	X
	Lectin microarray	X		X	X
	Levels of glycoproteins and mucins	X		X	X
	Levels of cytokines & innate immunity factors	X		X	X
	Eudragit® level	X		X	X
PHYSICAL EXAM	Physical Exam	X	^	^	^
	Vital Signs (BP)	X	X	X	X
	Height & Weight	X			
PELVIC EXAM	Bimanual Exam	^	^	^	^
	External Genital & Speculum Exam	X	X	X	X
	Post-insertion External Genital Exam		X		
	Repeat Speculum Exam		^		
RANDOMIZATION	Randomization to timing of V3		X		
STUDY PRODUCT	Study Product Distribution		X		
	Participant Self-Inserted (1st attempt)		X		
	Clinician-inserted (2nd attempt)		^		

X Required ^ PRN/as clinically indicated

*Safety Follow-up may extend beyond V4 to include AE Collection and any procedure deemed clinically indicated

APPENDIX II: HIV TESTING AGORITHIM



CONSENT TO ACT AS A PARTICIPANT IN A RESEARCH STUDY

Title: A Study of the Safety and Acceptability of a Placebo Vaginal Film
Short Title: FAME 101
Consent Version: 0.7, 13September2017

Principal Investigators: Magee-Womens Hospital of UPMC Department of OB/GYN/RS Pittsburgh, PA 15213 Phone: 412-641-5403	Katherine Bunge MD, MPH, Assistant Professor Sharon Hillier, PhD, Professor
Reproductive ID Research Staff	412-641-4242

Funding Agency: Division of AIDS, US National Institute of Allergy and Infectious Diseases, US National Institutes of Health

You are being asked to take part in this research study because you are a woman between the ages of 18 and 45 years. Approximately 64 women will participate in this study. The product being used in this study is a placebo (i.e. blank, inactive, no drug) vaginal film (similar to Listerine® breath strips). The person in charge of this study is Katherine Bunge, MD. Before you decide if you want to join this study, we want you to know about the study. This consent form gives you information about this study. The study staff will talk with you about it and answer your questions about this study. You may decide to withdraw from the study at any time.

YOUR PARTICIPATION IS VOLUNTARY

Once you read, discuss, and understand the consent form, and if you agree to take part, you will be asked to sign your name on this form. You will be offered a copy of this form to keep.

PURPOSE OF THE STUDY

This research study will involve the use of a placebo vaginal film. Investigators want to evaluate the safety of the film and understand the acceptability and length of time for the film to dissolve once inserted into the vagina. In future studies, this film may have medication added to it to deliver medication over an extended period of time (i.e. extended release).

STUDY PRODUCTS

This placebo film has not been used in humans before, but it is made from ingredients with a proven safety record. The same film was found to be safe in monkey models.

STUDY GROUPS

All of the eligible women will be randomized (distributed by chance, like rolling a dice) at the Enrollment Visit to one of four study groups. The groups will specify what day Visit 3 will be performed.

Group	Study Product	Follow-up Schedule	# of participants
1	Placebo film	Visit 3 at Day 3	16
2	Placebo film	Visit 3 at Day 7	16
3	Placebo film	Visit 3 At Day 10	16
4	Placebo film	Visit 3 at Day 14	16

You have an equal likelihood of being in any one of the four groups. Neither you nor the study staff can choose your group or can change the group you have been placed into. Regardless of which group you are in, you will use the film once during the study. Women in all of the study groups will have the same study visit schedule except for the timing of Visit 3. All of the study groups are important to this study.

FAME 101 STUDY PATICIPATION VISIT REQUIREMENTS

Screening Visit:
Your first visit will happen after you read, discuss, understand and sign this form. The procedures done at this visit will take about an hour.
Study staff will ask you where you live and other questions about you, your medical health (including what medications you are taking), menstrual history, your sexual practices and your understanding of the study requirements.
Study staff will:
Perform a physical exam, including height, weight and blood pressure.
Talk with you about the requirements of the study.
Perform condom counseling and provide condoms if you need them.
Test your urine
A urine pregnancy test will be performed. If you are pregnant you cannot join this study.
If necessary, a urinalysis (urine dipstick) will be performed.
Perform a rapid HIV test using blood or saliva.
You will be told your HIV test result as soon as it is available, approximately 20 minutes. You will talk with the study staff about the meaning of your result, how you feel about it, and ways to prevent HIV and other sexually transmitted infections. Your sample will be tested for the antibody to HIV. An antibody is a substance that blood cells make to fight infection. Exposure (contact) to the HIV virus produces antibodies. Sometimes HIV test results are not clearly positive, but also not clearly negative. In that case, we will do more tests until we know your status for sure. You must receive your HIV test results to be in the study. If the test shows you have HIV, you cannot join the study. We will refer you to available sources of medical care and other services you may need. Your partner(s) may have access to free HIV counseling and testing, if needed.
Perform a pelvic examination:
The study doctor or research clinician will use a speculum, a plastic or metal instrument used to separate the walls of the vagina. The study clinician will check your vagina and cervix for signs of infection, and other problems. They will also take some fluids to test for sexually transmitted infections or diseases (commonly known as STIs or STDs, including gonorrhea, chlamydia, and trichomonas) and other possible problems if they feel it is necessary.
The study clinician will perform a cervicovaginal lavage (CVL). This involves putting saline (salt water) into your vagina and using a pipette (or suction bulb) to draw up the fluid and wash the walls of your vagina and the cervix. The washing lasts for one minute. The purpose of collecting this lavage fluid is to use it to compare future fluid collected during the course of the study after you have been using the study product if you are enrolled into the study.

Provide you with the results of your tests, if available.
If necessary, this visit can occur over more than one visit.
Schedule your next visit to enroll in this study, if you are willing and eligible, within 45 days of this visit. This visit will be scheduled when you are not expecting to be bleeding or anticipating menses within 14 days of the visit.

You must agree to periods of abstinence (no sex) during the study and agree not to use vaginal products throughout the study. The details will be discussed with you.

If you are eligible and decide to enroll, the following may happen at every additional visit:
Your contact information will be reviewed and updated as needed
Your medical history and medication use will be reviewed and updated as needed
You will be asked to complete a study questionnaire
You will have your blood pressure taken and a urine pregnancy test performed
A bimanual exam (to feel uterus and ovaries) will be done as part of the pelvic exam only as clinically indicated
A physical exam may be performed as needed
You will be reminded of abstinence requirements and of the importance of not using other vaginal products throughout the study
Condoms and protocol counseling will be reviewed; condoms will be provided if you need them
The next study visit will be scheduled
You will be provided of results of tests that would impact your care (i.e. STD testing results, results of evaluations as a result of vaginal complaints, pregnancy test results). You will not be given the results of the tests that are done for research purposes (i.e. vaginal fluid and CVL).
You may be offered treatment for infections diagnosed during the study or you will be referred for treatment

Visit 2 (Enrollment; Day 0): Duration of visit = approx. 1 ½ hours
At this visit, you will:
Answer acceptability questions related to the vaginal film.
Have a pelvic examination, including a visual examination of the outside of your vagina, and a speculum examination. The study clinician will check your vagina and cervix for signs of infection or any problems. Vaginal and cervical fluids will be collected to test for bacteria, organisms and baseline tests. If an infection is present you may be offered treatment or referred for treatment as needed.
If you are bleeding, the visit will need to be re-scheduled within 45 days of the screening.
Be randomly assigned by study staff to one of the four study groups.
The film will be obtained from the pharmacy and given to you with verbal/written instructions on how to insert it. A researcher will remain in the room, behind a curtain while you attempt to insert it in case you have questions and to time the insertion.
Following insertion, the clinician will do a quick look at the outside genital area to be sure she cannot see the film.

If the film is seen outside or if you are unable to get the film in, a new film will be dispensed from the pharmacy and will be inserted by the clinician using a speculum.
You will be asked to return for Visit 3 according to your randomized assignment.

Visit 3 (Day 3, 7, 10 or 14) Duration of visit = up to 45 minutes
At this visit, you will:

Answer acceptability questions related to the vaginal film.
Have a pelvic examination performed by a study clinician that will include a check of the external genital area and vagina and cervix (using a speculum) for signs of an infection or problem. Vaginal and cervical fluids will be collected to test for bacteria and organisms, changes that may have occurred during the use of study products and, if necessary, look for any other problems. A CVL wash will be repeated. This fluid will be used for additional testing of the effect of the study film.

Visit 4 (Day 21) Duration of Visit = up to 45 minutes
During this visit, you will:
Answer acceptability questions related to the vaginal film.
Have a pelvic examination including a visual examination of the outside of your vagina and a speculum examination. The study clinician will check your vagina and cervix for signs of infection or any problems. Vaginal and cervical fluids will be collected to test for bacteria and organisms. A CVL wash will be repeated. This fluid will be used for additional testing of the effect of the study film.
Your study participation will end at this visit.

Interim Visits
It may be necessary for additional visit(s) during your participation in this study to have any of the study procedures listed above repeated in the event of unforeseen or unanticipated abnormal results; difficulties in sample delivery, processing, or testing; and/or if you experience any vaginal symptoms. Additional relevant testing may be done as determined by a study clinician (i.e. urine dip to test for infection, herpes culture, etc.).

It may also be necessary for the study staff to call you after your last study visit if you have any side effects that need to be followed. In the rare event you become pregnant while participating in the study, the study staff will refer you for appropriate care. The investigator would encourage you to stay in the study so that safety data could be collected. Additional genital samples will not be collected and a pelvic exam will not be performed unless you are experiencing vaginal symptoms that the investigator feels needs to be evaluated. Study staff would need to follow up with you by telephone until the outcome of your pregnancy is known.

RISKS AND/OR DISCOMFORTS

Blood draw
You may have excessive bleeding, discomfort, dizziness, fainting, a small blood clot, bruising, swelling or infection.

Pelvic Exam and Sample Collection
During pelvic exams and sample/fluid collection, you may feel discomfort or pressure in your vagina and/or pelvis.

Vaginal Film
The vaginal film may cause some side effects. We do not yet know all the side effects of the film. Some, but not all women who used film in other studies have had:
Discharge from the vagina
Irritation and discomfort

Discussing Sexual Practices, Being tested for STDs and Waiting for Test Results

You may become embarrassed and/or worried when discussing your sexual practices, ways to protect against HIV and other infections passed through sex, and your test results. You may be worried while waiting for your test results. If you have HIV or other STDs, learning this could make you worried. Trained study staff will help you deal with any feelings or questions you have. Finding out your HIV status could also cause problems between you and your partner. If you have any problems, study staff will talk with you and/or your partner to try to help resolve them.

Breach of Confidentiality
We will make every effort to protect your privacy and confidentiality during the study visits. Your visits will take place in private. However, it is possible that others may learn of your participation here and, because of this, may treat you unfairly or discriminate against you. For example, you could have problems getting or keeping a job, or being accepted by your family or community.

You could have these side effects or other side effects that we do not know about.

BENEFITS
You may get no benefit from participating in this study. Information learned from this study may help in the development of vaginal products for women.

You will have exams and HIV/STI testing as part of your participation in this study. You may receive treatment for certain STIs as explained above or you will be referred for treatment.

NEW INFORMATION
You will be told any new information learned during this study that might affect your willingness to stay in the study.

STOPPING STUDY DRUG OR BEING WITHDRAWN EARLY
A study doctor may need to remove you from the study early without your permission if:
The study is stopped or cancelled
You are not able to keep appointments
Other reasons that may prevent you from completing the study successfully
Participants who withdraw or who are withdrawn from the study prior to completing follow up may be asked to complete a final study visit.

COSTS TO YOU
There is no cost to you for study related visits, the vaginal film, physical examinations, laboratory tests or other study procedures. Treatments available to you from the study site for infections passed through sex may be given at no cost to you or you may be referred for available treatment while you are in the study.

REIMBURSEMENT
You will receive compensation for your time, effort, and travel at the end of each study visit if all study procedures were completed.

Visit 1 (Screening):	$50
Visit 2 (Enrollment):	$50
Visit 3 (Randomized Visit):	$100 (if compliant with appointment day/time)
Visit 4 (Final Visit) :	$50

You will receive male condoms as needed at no cost to you. Parking passes will be provided as needed.

CONFIDENTIALITY
Every effort will be made to keep your information confidential. However, it is not possible to guarantee confidentiality. Your personal information may be disclosed if required by law. The study staff may use your personal information to verify that you are not in any other research

studies. This includes studies conducted by other researchers about which study staff know. Any publication of this study will not use your name or identify you personally.

Your records may be reviewed by:
NIH, and/or contractors of NIH
Study monitors
University of Pittsburgh Research Conduct and Compliance Office
Study staff

If you test positive for gonorrhea, chlamydia, or HIV, the Commonwealth of Pennsylvania requires that your name be given to the Allegheny County Health Department. You may be contacted and asked questions about your sexual partner(s).

The investigators may use or disclose, for purposes described above, identifiable information (which may include identifiable medical information) related to your being in this study for a minimum of 7 years and for as long (indefinite) as it may take to complete this study.

A description of this clinical trial will be available on http://www.clinicaltrials.gov.. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

FUTURE USE OF STUDY SAMPLES

By participating in this study, you allow the researchers to use any leftover study samples (i.e. vaginal swabs, cervical or vaginal fluid) -- identified only by your unique research number – for future use on the female genital tract. and how the body responds to infections. You would not be told or learn of the results. Your specimens used in this research study may contribute to a new discovery or treatment. In some instances, these discoveries or treatments may be of commercial value and may be sold, patented, or licensed by the investigators and the University of Pittsburgh for use in other research or the development of new products. You will not retain any property rights nor will you share in any money that the investigators, the University of Pittsburgh, or their agents may realize.

RESEARCH-RELATED INJURY

If you believe that the research procedures have resulted in an injury to you, immediately contact the Principal Investigator who is listed on the first page of this form. Emergency medical treatment for injuries solely and directly related to your participation in this research study will be provided to you by the hospitals of UPMC. Your insurance provider may be billed for the costs of this emergency treatment, but none of those costs will be charged directly to you. If your research related injury requires medical care beyond this emergency treatment, you will be responsible for the costs of this follow-up care. At this time, there is no plan for any additional financial compensation. The US National Institutes of Health (NIH) does not have a mechanism to provide compensation for research related injury.

YOUR RIGHTS AS A RESEARCH PARTICIPANT/VOLUNTEER

Taking part in this study is completely voluntary. You may choose not to take part in this study or leave this study at any time. If you choose not to participate or to leave the study, you will not lose the benefit of services to which you would otherwise be entitled at this clinic. If you want the results of the study after the study is over, let the study staff members know.

PROBLEMS OR QUESTIONS

If you ever have any questions about the study, or if you have a research-related injury, you should contact Katherine Bunge, MD or the research staff at (412) 641-4242. If you ever have any questions about your rights as a research participant, you can contact the University of Pittsburgh IRB at 1-866-212-2668.

VOLUNTARY CONSENT

The above information has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions, voice concerns or complaints about any aspect of this research study during the course of this study, and that such future questions, concerns or complaints will be answered by a qualified member of the research team or by the principal investigator listed on the first page. I understand that I may always request that my questions, concerns or complaints be addressed by the Principal Investigator. At any time I may also contact the Human Subjects Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668) to discuss problems, concerns and questions; obtain information; offer input; or discuss situations in the event that the research team is unavailable. By signing this form I agree to participate in this research study for the purposes described above. A copy of this consent form will be offered to me.

_____

Printed Name of Participant

_____	_____	_____
Participant Signature	Date	Time (am/pm)

CERTIFICATION OF INFORMED CONSENT: I certify that I have explained the nature and purpose of this research to the above individual and I have discussed the potential benefits and possible risks of study participation. Any questions the individual has about this study have been answered, and we will always be available to address future questions as they arise. I further certify that no research component of this protocol was begun until after this consent form was signed.

_____	_____
Printed Name of Person Obtaining Consent	Role in Research Study
_____	_____
Signature of Person Obtaining Consent	Date

APPENDIX IV

Study summary for F2-5.1: Placebo (Extended release film): super-dosing SAFETY STUDY

Background: A vaginal film platform has been designed to provide extended release of an API over (at least) seven days. The film is optimized for Appearance, Dissolution, Disintegration and Manufacturability. Placebo films with excipients expected to tolerate MK-2048 drug loading have been provided for pre-clinical testing.

Purpose: This safety study will assess responses in the cervicovaginal environment with placebo film "super-dosing." Rather than once a week dosing, we will expose macaques to two films per week for two weeks.

We will assess the vaginal microflora, pH, PMN influx, and surface tissues after four film placements over two weeks. Additionally, we will detect the level of one of the film platform polymers using PK analysis, as an indicator of residual film presence on day 14 of the study.

Design:

F2-5.1
Placebo extended release film: safety study

	D0 Mon	D3 Thur	D7 Mon	D10 Thur	D14 Mon
Study dates	*9/26*	*9/29*	*10/3*	*10/6*	*10/10*
VF swab (flora)	√				√
VF swab (pH/smear)	√				√
Colposcopy	√	√	√	√	√
CVL					√ x2
Film placement	√	√	√	√	

N=7: A10200 A07101 Z08187 K05007 A10010 T02319 W04064

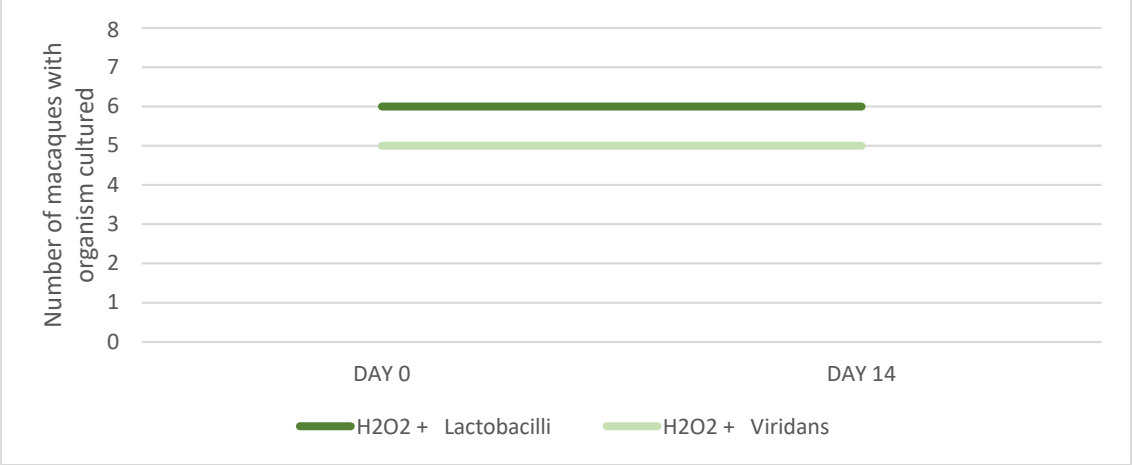
Vaginal Flora: Two Vaginal swabs (dacron) collected from mid-depth vaginal canal and placed in MaxV transporter tubes for overnight delivery to MWRI (Pittsburgh, PA) for semi-quantitative microbiologic assessment.
Vaginal pH/smear: Vaginal pH: Dab head of a Dacron tipped swab onto a pH indicator strip (resolution 0.5 pH units) to document vaginal pH. Vaginal smear-Gram stain: Roll the Dacron tipped swab of vaginal exudate on a glass microscope slide. The smear is air dried and subsequently Gram stained for identification and quantification of polymorphonuclear cells.
Colposcopy: Colposcopy to take place after vaginal secretion collections. If vaginal exudate/product remnant obscures the cervix, save images of the cervicovaginal site prior to clearing out vaginal exudate. Then clear area and continue with colposcopy. Note observations and document with digital photography. Adverse findings include any break in the integrity of the epithelial layer, deeply erythematous or friable tissues, and microulcers.
Note: Limited colposcopic exams and photos were additionally collected just prior to and immediately after each film placement. Remnant film product was noted, but not removed at these visits.

CVL: Collect two 2mL saline CVLs: ("-1") before cleaning the site for colposcopy if necessary, and again ("-2") after conducting colposcopy. Split the recovery fluid from each CVL into two cryovials and freeze (-80C) for future PK analysis [UCD].
Film placement: Administer test film to the vaginal fornix.

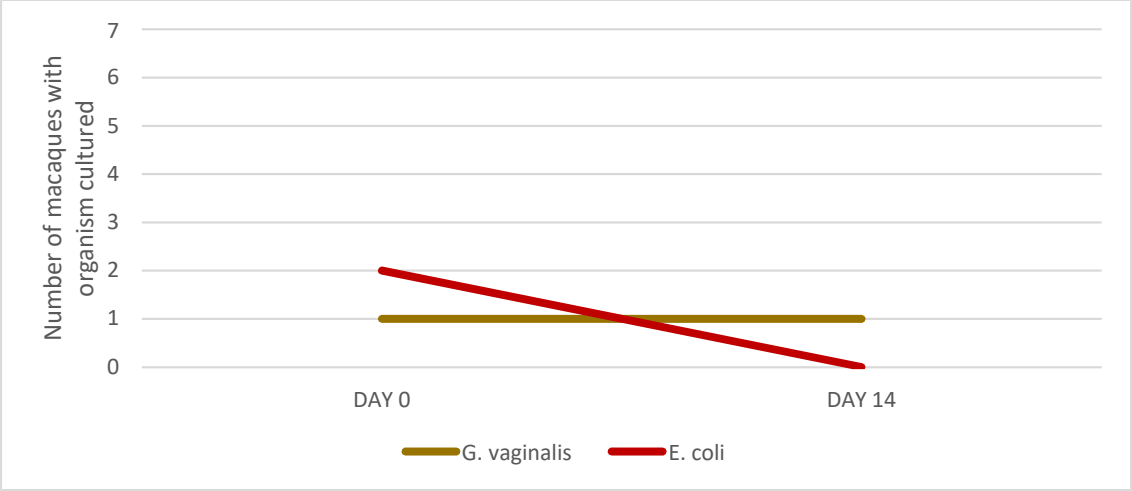
RESULTS

Vaginal Microflora:

Placebo Films had no effect on beneficial (H2O2 producers) microorganisms after repeated exposures:



Shifts in potentially harmful microorganisms after super-dosing:



There was a low prevalence of E. coli and *G. vaginalis* organisms, neither of which increased after film use. Other potentially deleterious organisms *S. aureus* and enterococcus were not cultured from any specimens.

Prevalence of each microorganism detected in vaginal swabs remained quite stable after super-dosing with the placebo film platform for extended release API.

Compiled PREVALENCE: Super-Dosing Placebo for Extended Release API	Day 0 Pre-Exposure	Day 14
H_2O_2 + Lactobacilli	6	6
H_2O_2 - Lactobacilli	1	1
H_2O_2 + Viridans	5	5
H_2O_2 - Viridans	7	5
G. vaginalis	1	1
S. aureus	0	0
coagulase-negative Staph	6	5
group B Strep	0	0
group C Strep	0	0
group F Strep	1	0
group G Strep	0	0
non-groupable Beta-Strep	0	0
diptheroids	7	7
E. coli	2	0
enterococcus	0	0
black anaerobic Gram Negative Rods	7	7
non-pigmented anaerobic GNR	7	7
other anaerobic GNR	0	0
micrococcus	0	0
proteus	0	0
bacillus	0	0
aerobic Gram-positive rods	6	5
aerobic Gram-positive cocci	1	2

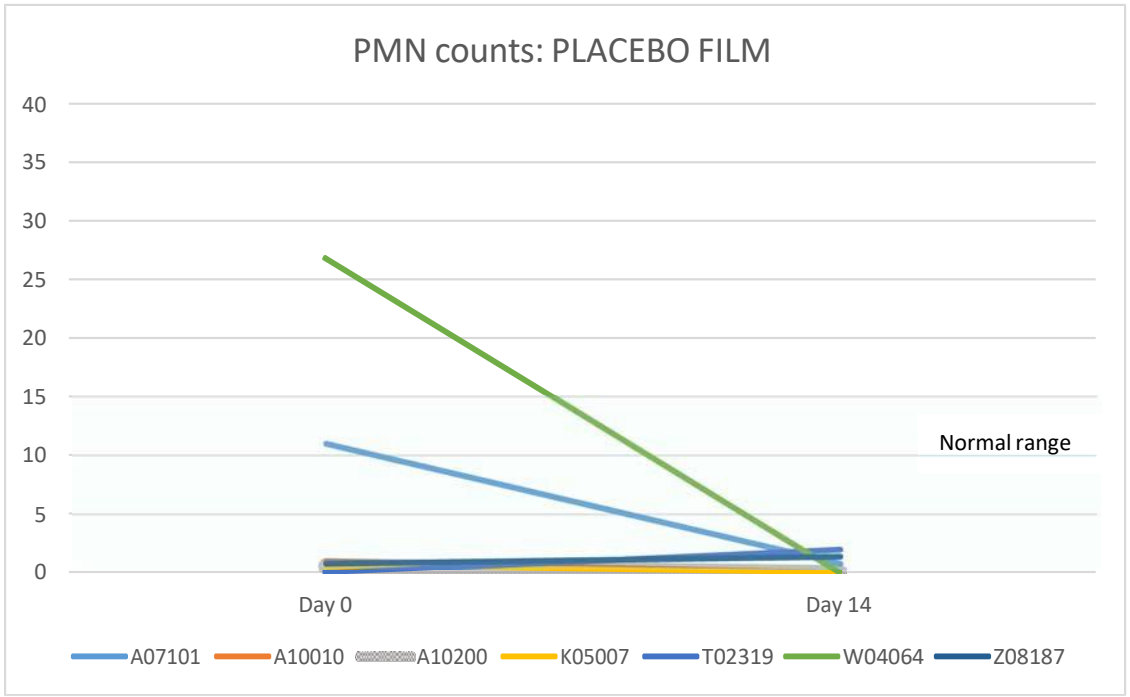
n=7 macaques

A data table displaying semi-quantitative scores for each sample is appended under Raw Data.

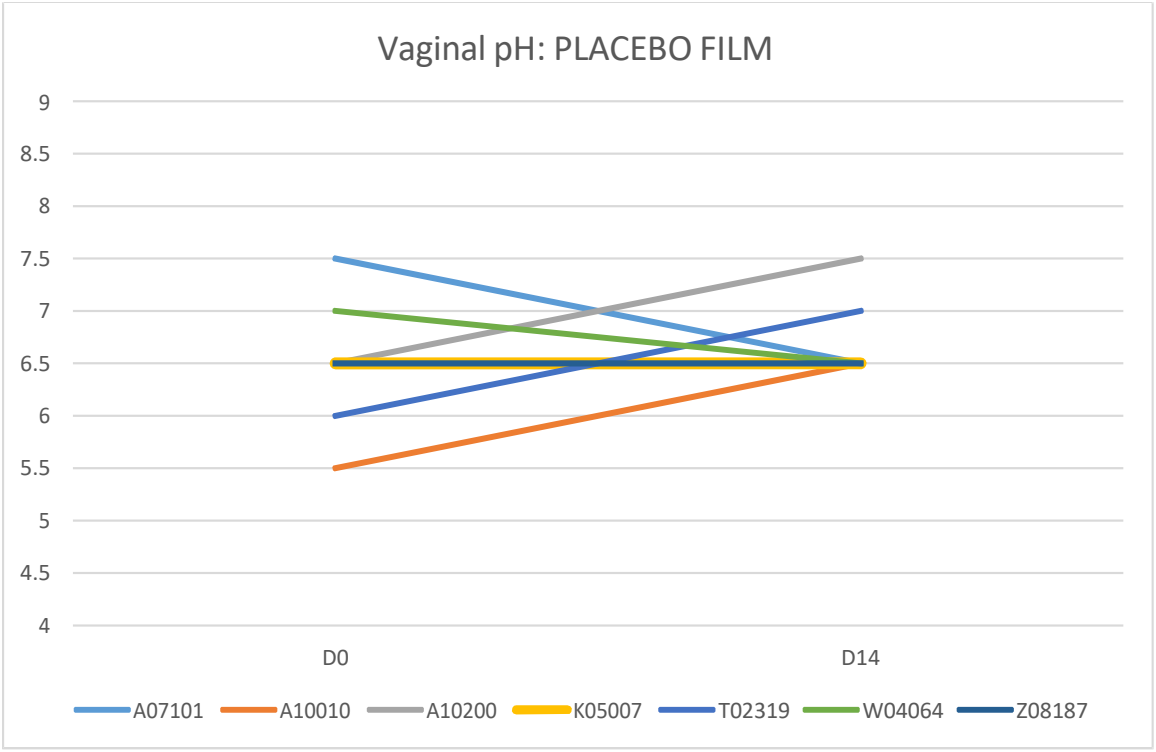
Vaginal PMN response after cumulative exposures to the extended release Film Platform (as noted by Gram stained vaginal smears):

Gram stained vaginal smears were assessed for presence of red blood cells (RBC) and polymorphonuclear cells (PMN) as indicators of a white blood cell infiltrate. RBC are generally associated with menses. In a population of 120 control pigtail macaques to date, we have calculated a normal range of PMNs to fall between 0 and 15 PMNs per high power field (unpublished). This normal range appears lightly shaded in the line graph below.

The graph below plots the mean values (from 5 high power non-adjacent fields) of PMN counts for each macaque at baseline and on day 14. Both of the macaques with elevated PMN counts on Day 0 were menstruating, as was T02319 on Day 14. Clearly this film platform does not elicit a PMN response even when administered more frequently than intended. Raw data is appended.



Vaginal pH Super dosing with extended release vaginal films did not have a deleterious effect on vaginal pH, which normally measures 5.0-8.5 in pigtail macaques. Of four samples that measured pH 7.0 or higher in this study, three were collected during menses.



The weight and temperature of each animal also remained within normal ranges. No indication of adverse health responses to multiple film exposures were noted.

Cervicovaginal Lavage samples were stored at -80°C in the Patton Laboratory, then shipped to UC Denver (Core C). They will be assessed by PK analysis for polymer detection as a marker for film presence in the macaque vagina. Optimizing the conditions for PK detection of the polymer will allow future studies to include drug deposition and film platform presence to be distinguished from each other.

Two CVL samples were collected from each macaque on study day 14. One (CVL-1) was collected prior to clearing residual film for colposcopy; the second (CVL-2) was collected after removing residual film product. In addition to CVL, a sample of residual film from two macaques was frozen and provided to the PK lab.

The data from these samples will be reported separately, and are not expected to impact the safety findings.

	Day 0	Day 3	Day 7	Day 10	Day 14
Colposcopy	√	√	√	√	√

Colposcopy No adverse findings were noted by colposcopy. Residual film was frequently noted. When an animal was menstruating, the residual took on a brown to red tone as seen in photos. Otherwise the residual appeared white. No formulation induced changes to the cervicovaginal tissues were noted.

Colposcopy photo plates follow.

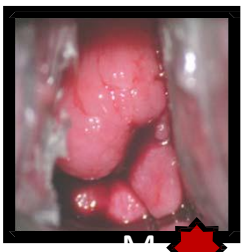
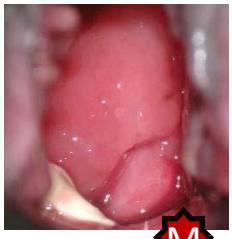
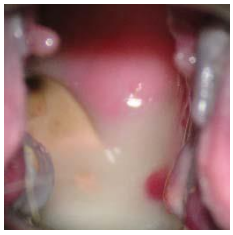
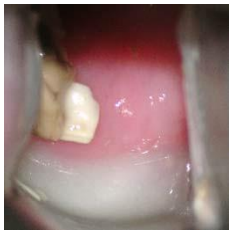
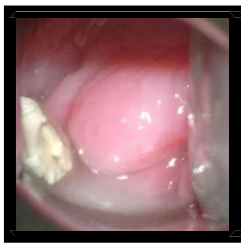
F2-5.1 Extended Release Film Platform: Super Dosing Safety Study

Day 0 → **Day 3** → **Day 7** → **Day 10** → **Day 14**

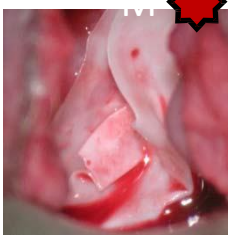
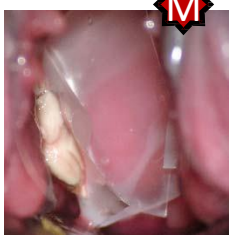
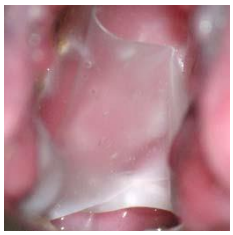
Film 1 Film 2 Film 3 Film 4

A07101

Prior to Film Placement

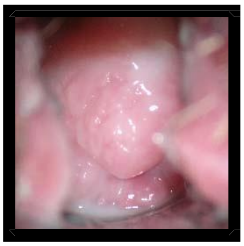
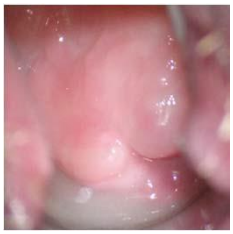
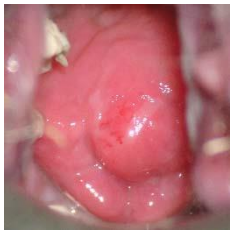
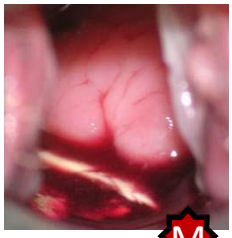
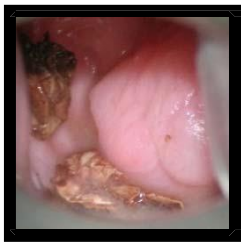
Film placed

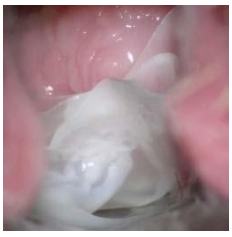
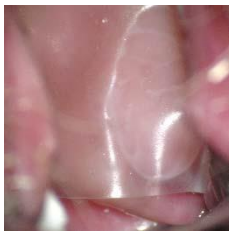
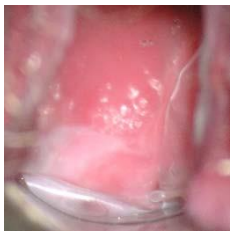

Baseline appearance (menses)	Residual film appears white + menses	Residual film + exudate	Residual film + exudate	Residual film + exudate

A10010

Prior to Film placement

Film placed


Baseline appearance. Edema	Some residual film noted in exudate	Focal erythema (normal near menses), Residual film	Residual film + menses	Residual film + exudate

:Menses

Framed photos indicate time points when specimens

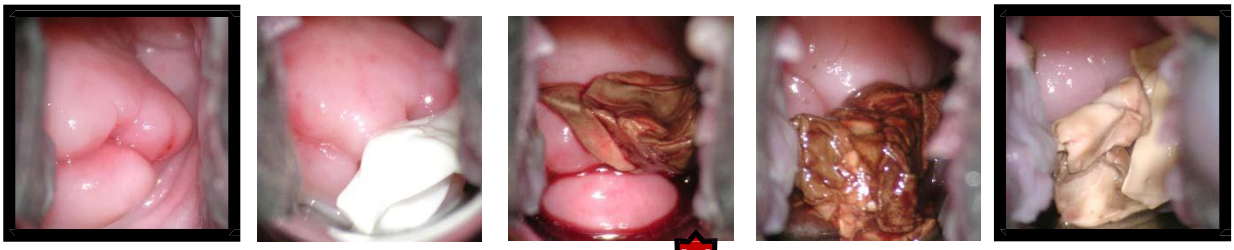
F2-5.1 Extended Release Film Platform: Super Dosing Safety Study

Day 0 → **Day 3** → **Day 7** → **Day 10** → **Day 14**

A10200

Film 1 Film 2 Film 3 Film 4

Prior to Film placement

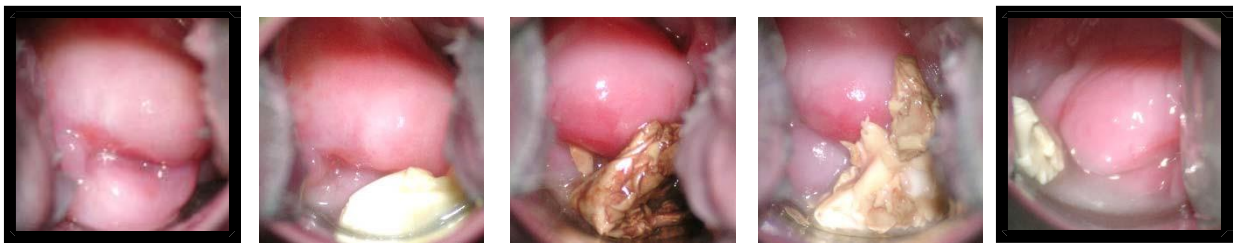


Film placed

Baseline appearance | Residual film appears white | Residual film + menses | Residual film post-menses | Residual film

K05007

Prior to Film Placement



Film placed

Baseline appearance | Residual film appears white/yellow | Residual film (suspected end of menses) | Residual film + exudate | Residual film

Menses

Framed photos indicate time points when specimens

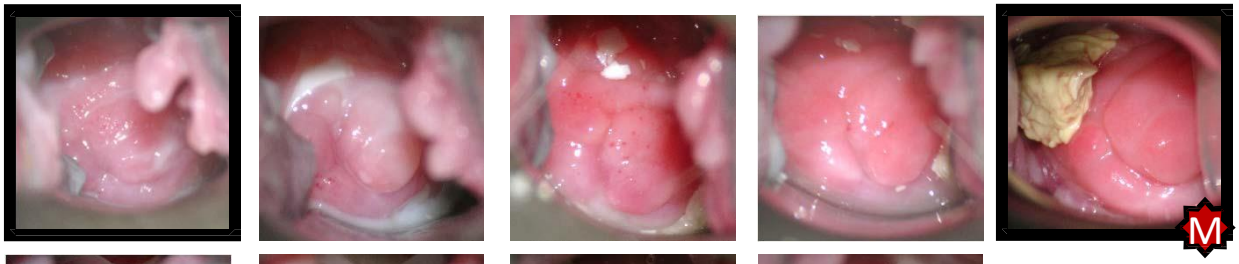
F2-5.1 Extended Release Film Platform: Super Dosing Safety Study

Day 0 — **Day 3** — **Day 7** — **Day 10** — **Day 14**

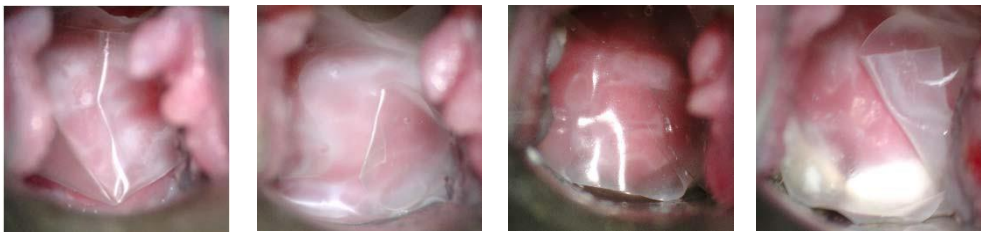
T02319

Film 1 | Film 2 | Film 3 | Film 4

Prior to Film placement



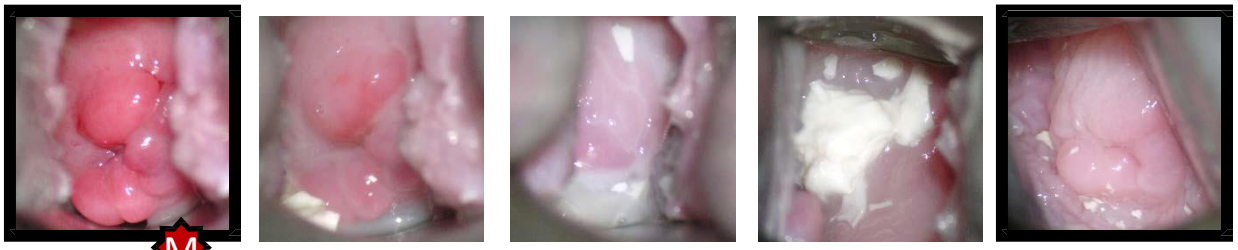
Film placed



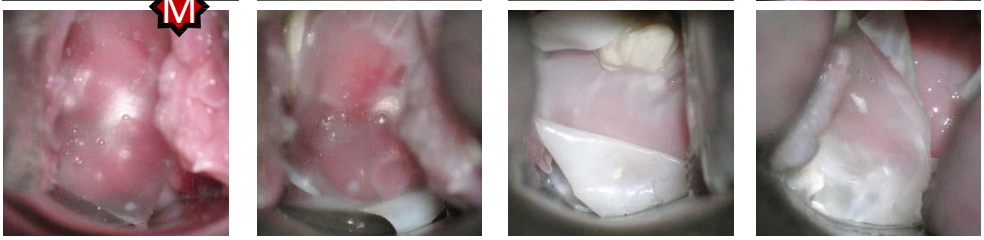
Baseline appearance | Residual film is white & original shape | Residual film is white. Petechiae | Residual film appears white | Residual film + menses

W04064

Prior to Film placement



Film placed



Baseline appearance (menses) | Residual film appears white. Focal erythema. | Residual film | Residual film | Residual film in pieces

M Menses

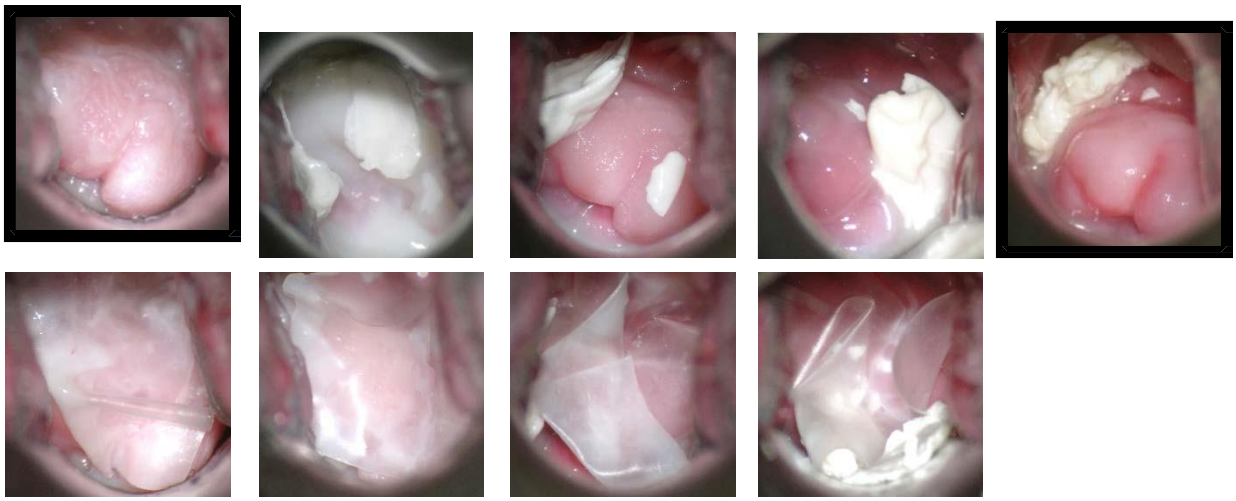
Framed photos indicate time points when specimens

F2-5.1 Extended Release Film Platform: Super Dosing Safety Study

Day 0 — **Day 3** — **Day 7** — **Day 10** — **Day 14**

Film 1 Film 2 Film 3 Film 4

Z08187



Prior to Film Placement

Film placed

Baseline appearance. Edema

Residual film appears white + exudate

Residual film

Residual film

Residual film

Framed photos indicate time points when specimens were collected.

Raw data

Vaginal Flora: Semi-quantitative results (0-4, denoting organism growth on quadrants of a plate) were reported for each organism listed.

N=7 Mn	A07101		A10010		A10200		K05007		T02319		W04064		Z08487	
	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14
H_2O_2 + Lactobacilli	1	1	4	1	1	3	3	4	0	0	3	4	1	1
H_2O_2 - Lactobacilli	0	3	0	0	0	0	2	0	0	0	0	0	0	0
H_2O_2 + Viridans	2	4	2	2	4	4	0	0	0	0	3	4	3	4
H_2O_2 - Viridans	3	2	4	0	2	3	3	0	4	3	3	4	3	4
G. vaginalis	0	0	0	0	0	0	4	4	0	0	0	0	0	0
S. aureus	0	0	0	0	0	0	0	0	0	0	0	0	0	0
coagulase-negative Staph	2	0	2	2	0	2	2	3	4	2	3	0	2	3
group B Strep	0	0	0	0	0	0	0	0	0	0	0	0	0	0
group C Strep	0	0	0	0	0	0	0	0	0	0	0	0	0	0
group F Strep	0	0	0	0	0	0	0	0	0	0	3	0	0	0
group G Strep	0	0	0	0	0	0	0	0	0	0	0	0	0	0
non-groupable Beta-Strep	0	0	0	0	0	0	0	0	0	0	0	0	0	0
diptheroids	3	4	4	4	4	4	3	1	4	4	3	4	3	4
E. coli	0	0	0	0	0	0	3	0	0	0	2	0	0	0
enterococcus	0	0	0	0	0	0	0	0	0	0	0	0	0	0
black anaerobic Gram Negative Rods	3	4	4	4	4	4	3	4	4	4	4	4	3	4
non-pigmented anaerobic GNR	2	4	4	4	3	4	4	4	3	4	4	4	3	4
other anaerobic GNR	0	0	0	0	0	0	0	0	0	0	0	0	0	0
micrococcus	0	0	0	0	0	0	0	0	0	0	0	0	0	0
proteus	0	0	0	0	0	0	0	0	0	0	0	0	0	0
bacillus	0	0	0	0	0	0	0	0	0	0	0	0	0	0
aerobic Gram-positive rods	3	4	3	4	4	2	0	0	4	4	3	0	2	3
aerobic Gram-positive cocci	0	4	0	0	0	0	0	0	0	0	3	0	0	2

Vaginal pH: Vaginal fluid transferred to pH indicator strips (resolution 0.5 pH units) by dabbing VF swab. pH measures are noted for Day 0 and Day 14.

Vaginal pH	D0	D14
A07101	7.5*	6.5
A10010	5.5	6.5
A10200	6.5	7.5
K05007	6.5	6.5
T02319	6	7*
W04064	7*	6.5
Z08187	6.5	6.5

*menses

Gram stain PMN count from vaginal smear: Polymorphonuclear (PMN) cells were counted in 5 representative non-adjacent high power fields. Presence of red blood cells (RBC) was also noted.

		Polymorphonuclear (PMN) Cell Counts								
Animal ID	**Study Day**	**Field 1**	**Field 2**	**Field 3**	**Field 4**	**Field 5**	**Total**	**Avg**	**RBC**	**Comments**
A07101	0	10	14	15	9	7	55	**11**	4	Macaque menstruating
	14	1	3	0	0	0	4	**0.8**	0	
A10010	0	1	1	1	1	1	5	**1**	0	
	14	0	0	0	0	0	0	**0**	0	
A10200	0	0	0	0	0	3	3	**0.6**	0	
	14	0	0	0	0	0	0	**0**	0	
K05007	0	0	1	0	1	1	3	**0.6**	0	
	14	0	0	0	0	0	0	**0**	0	
T02319	0	0	0	0	0	0	0	**0**	0	
	14	2	1	5	1	1	10	**2**	0	Macaque menstruating
W04064	0	22	32	30	20	30	134	**26.8**	0	Macaque menstruating; WBC seen in cervical mucus
	14	0	0	0	0	0	0	**0**	0	
Z08187	0	2	1	1	0	0	4	**0.8**	0	
	14	4	3	0	0	0	7	**1.4**	0	

RBC 0 = none

1+ =less than one per field
2+ =1-4 per field
3+ =5-30 per field
4+ = greater than 30 per field

CVL: Fourteen frozen samples (2/macaque on Day 14) were batch shipped to PK lab at UC Denver. (11/14/2017)

Colposcopy: Colposcopy was conducted prior to each film administration, and on study day 14. Common findings include edema, erythema, petechiae and ecchymoses. These are routinely noted in cycling macaques regardless of vaginal product use. In this study, tissue edema and erythema were noted by quartile of tissue coverage (0, 25%. 50%, 75% or 100%). Petechiae and ecchymoses were noted if present. Adverse findings would include any break in the integrity of the epithelial layer, deeply erythematous or friable tissues, and micro-ulcers. No adverse findings were noted in this study. The raw colposcopic data is compiled in the following table.

			Cervix Appearance					Vagina Appearance					Cervicovaginal Exudate			
Animal ID	Study Visit	Menses	Edema	Erythema	Petechiae/ Ecchymosis	Friable	Epithelial Disruption	Edema	Erythema	Petechiae/ Ecchymosis	Friable	Epithelial Disruption	Amount	Consistency	Appearance	Comments Study Film Lot: MK-2048 Placebo Film SMG-4-24
A07101	D0	Yes	50%	None	None	No	No	75%	None	None	No	No	Profuse	Average	Bloody	Placebo Film 26
A07101	D3	Yes	25%	75%	Both	No	No	25%	75%	Both	No	No	Profuse	Thick	Bloody	Placebo Film 18 Residual film changed to white gummy mass
A07101	D7	No	25%	100%	Petechiae	No	No	None	75%	Both	No	No	Profuse	Average	Opaque white	Placebo Film 49
A07101	D10	No	25%	50%	Both	No	No	75%	25%	Petechiae	No	No	Profuse	Thick	Flecked	Placebo Film 41
A07101	D14	No	None	75%	Both	No	No	None	None	None	No	No	Profuse	Thin	Opaque white	Residual Film appears white in color & broken down into pieces. Film residue removed before CVL-2
A10010	D0	No	75%	50%	None	No	No	None	50%	None	No	No	Profuse	Thick	Opaque white	Placebo Film 17
A10010	D3	No	50%	50%	None	No	No	25%	50%	Ecchymosis	No	No	Profuse	Thick	Opaque white	Placebo Film 47 Less Film residual than in other animals. Some big pieces of film residue noted in exudate.
A10010	D7	No	50%	75%	None	No	No	25%	50%	None	No	No	Profuse	Thick	Flecked	Placebo Film 20 Residual Film is white
A10010	D10	Yes	25%	50%	Both	No	No	None	50%	Ecchymosis	No	No	Profuse	Average	Bloody	Placebo Film 50
A10010	D14	No	75%	25%	None	No	No	100%	25%	None	No	No	Moderate	Average	Translucent white	Residual Film noted & broken down into pieces; removed before CVL-2 taken; some removed and placed in cryotubes for PK.
A10200	D0	No	25%	50%	Ecchymosis	No	No	25%	50%	None	No	No	Profuse	Average	Flecked	Placebo Film 16
A10200	D3	No	None	75%	None	No	No	25%	50%	None	No	No	Moderate	Thick	Opaque white	Placebo Film 46 Residual Film changed to white gummy mass
A10200	D7	Yes	None	75%	None	No	No	None	50%	None	No	No	Profuse	Average	Bloody	Placebo Film 21 Residual Film has black color due to mensing
A10200	D10	No	None	25%	None	No	No	50%	25%	None	No	No	Moderate	Average	Opaque white	Placebo Film 52 Residual Film looks brown/red (prior menses)
A10200	D14	No	50%	25%	None	No	No	100%	50%	None	No	No	Minimal	Average	Clear	Residual Film noted & broken down into pieces; removed before CVL-2
K05007	D0	No	None	50%	Ecchymosis	No	No	25%	50%	Both	No	No	Moderate	Average	Translucent white	Placebo Film 45
K05007	D3	No	None	50%	None	No	No	25%	75%	Both	No	No	Moderate	Average	Translucent white	Placebo Film 37 Residual Film appears as white/yellow gummy mass
K05007	D7	No	25%	50%	Ecchymosis	No	No	25%	50%	None	No	No	Moderate	Thick	Opaque white	Placebo Film 30 Exudate in some areas looks yellowish. Film residual is
K05007	D10	No	25%	50%	None	No	No	25%	25%	None	No	No	Profuse	Thick	Opaque white	Placebo Film 23
K05007	D14	No	25%	25%	None	No	No	None	25%	None	No	No	Profuse	Thick	Opaque white	Film residual noted & broken down into pieces; removed before CVL-2 collected; some removed and placed in cryotube for PK.
T02319	D0	No	25%	75%	Both	No	No	75%	50%	Ecchymosis	No	No	Profuse	Thick	Opaque white	Placebo Film 27
T02319	D3	No	None	50%	Both	No	No	25%	50%	None	No	No	Profuse	Thick	Opaque white	Placebo Film 19 Some big pieces of residual film appear white & original shape
T02319	D7	No	25%	75%	Both	No	No	25%	75%	Ecchymosis	No	No	Profuse	Thick	Opaque white	Placebo Film 48 Film residual color is white
T02319	D10	No	50%	75%	Both	No	No	25%	75%	Both	No	No	Profuse	Average	Flecked	Placebo Film 40
T02319	D14	Yes	75%	75%	None	No	No	None	25%	None	No	No	Minimal	Average	Bloody	Residual Film noted & broken down into pieces, removed before CVL-2 collected, some removed and placed in cryotubes for PK
W04064	D0	Yes	None	75%	Both	No	No	25%	75%	Both	No	No	Moderate	Average	Translucent white	Placebo Film 36 She was at the end of mensing. Blood not seen in exudate,but was on cervix and swabs.
W04064	D3	No	25%	75%	Ecchymosis	No	No	50%	50%	Ecchymosis	No	No	Moderate	Thick	Opaque white	Placebo Film 29 Residual Film appears white and gummy
W04064	D7	No	25%	None	None	No	No	75%	25%	None	No	No	Profuse	Thick	Opaque white	Placebo Film 38 Residual Film is white.
W04064	D10	No	50%	50%	None	No	No	75%	25%	None	No	No	Profuse	Thick	Opaque white	Placebo Film 31
W04064	D14	No	50%	25%	None	No	No	100%	None	None	No	No	Profuse	Thick	Opaque white	Residual Film appears white & broken down into pieces; removed before CVL-2; some removed and placed in cryotubes for PK
Z08187	D0	No	100%	None	None	No	No	75%	50%	None	No	No	Moderate	Thick	Opaque white	Placebo Film 35
Z08187	D3	No	100%	25%	None	No	No	75%	50%	None	No	No	Profuse	Thick	Opaque white	Placebo Film28 Residual Film changed to white gummy mass
Z08187	D7	No	50%	25%	None	No	No	50%	25%	Ecchymosis	No	No	Moderate	Thick	Opaque white	Placebo Film 39 Residual Film is white
Z08187	D10	No	25%	50%	None	No	No	50%	75%	None	No	No	Profuse	Thick	Opaque white	Placebo Film 32
Z08187	D14	No	None	25%	None	No	No	None	75%	None	No	No	Profuse	Average	Opaque white	Residual Film is white & broken down into pieces; removed before CVL-2

1. UNAIDS. Report on the global HIV/AIDS epidemic 2008.
2. UNAIDS. AIDS epidemic update: November 2009.
3. Steiner M, Spruyt A, Joanis C, et al. Acceptability of Spermicidal Film and Foaming Tablets Among Women in Three Countries. International Family Planning Perspectives 1995;21:104-7.
4. Visness CM, Ulin P, Pfannenschmidt S, Zekeng L. Views of Cameroonian sex workers on a woman-controlled method of contraception and disease protection. Int J STD AIDS 1998;9:695-9.
5. Raymond E, Alvarado G, Ledesma L, et al. Acceptability of two spermicides in five countries. Contraception 1999;60:45-50.
6. Schley A. IPM Product Acceptability Study II (PAS II). International Partnership for Microbicides; 2009.
7. Nel AM, Mitchnick LB, Risha P, Muungo LT, Norick PM. Acceptability of vaginal film, soft-gel capsule, and tablet as potential microbicide delivery methods among African women. J Womens Health (Larchmt) 2011;20:1207-14.
8. Raymond EG, Chen PL, Condon S, et al. Acceptability of five nonoxynol-9 spermicides. Contraception 2005;71:438-42.
9. Pittsburgh Uo. Institutional Review Board Manual. http://wwwirbpittedu/manual/.