EARLY DEVELOPMENT OF VAGINAL MICROBICIDES TO DECREASE HIV ACQUISITION IN WOMEN

by

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ABSTRACT

Women are disproportionately affected by HIV, with most infections in women occurring as a result of heterosexual intercourse. New HIV prevention strategies are needed that women could employ discretely, potentially without consulting male partners. Vaginal spermicidal gels and films can successfully prevent pregnancy, and similar products can be used to deliver antiretroviral medications. This thesis presents two phase 1 studies of vaginal microbicides containing dapivirine or tenofovir and will demonstrate that these products are safe, effective, and successfully deliver their respective antiretrovirals in the vaginal environment.

The FAME 02b study describes pharmacokinetic and pharmacodynamic evaluation of a single dose of dapivirine vaginal gel and film. By assessing drug concentrations in plasma, cervicovaginal fluid, and cervical tissue, we show that both vaginal film and gel formulations are able to deliver concentrations of dapivirine to these anatomic locations that should be protective against HIV infection. An explant HIV infectivity assay performed on cervical tissue biopsy specimens supports the further development of both dapivirine products.

The FAME 05 study employs a complementary design to FAME 02b to evaluate similar film and gel products containing tenofovir. The same schedule and type of samples were collected following a single dose of tenofovir vaginal film or gel, including the cervical tissue explant assay, with the addition of rectal fluid drug concentration. Tenofovir film was associated with higher drug concentrations in plasma, cervicovaginal fluid, and cervical tissue, while rectal fluid tenofovir was higher after gel dosing. There was no difference between the two products in preventing cervical tissue explant HIV infection.

Early studies of both oral and vaginal pre-exposure prophylaxis against HIV were promising, finding up to 70% protection from HIV acquisition when products were used consistently. Follow-up randomized clinical trials failed to confirm the protective effect of either form of PrEP among women, partly due to poor product adherence. New vaginal microbicides that can be used episodically around the time of intercourse may be more preferable to some women than a daily preventive medication. Clinical trials of dapivirine and tenofovir vaginal gels and films are warranted in order to provide a variety of HIV prevention strategies.

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To my family (Mary, Matthew, and Sierra), who encouraged me to pursue this degree and to never give up – you're a bunch of enablers, and I love you.

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LIST OF ABBREVIATIONS

AE – adverse event

AIDS – Acquired Immune Deficiency Syndrome

ARV – antiretroviral

AST – aspartate amino transferase

AUC_{last} – area under the concentration curve to last sample

BLQ – below the limit of quantification

CAPRISA – Center for the AIDS Program of Research in South Africa

C_{max} – maximum concentration

CT – cervical tissue

CV – coefficient of variation

CVF – cervicovaginal fluid

CVL – cervicovaginal lavage

DAIDS – Division of AIDS

DDU – Drug Development Unit

DMEM – Dulbecco's Modified Eagle Medium

DPV – dapivirine

ELISA – enzyme-linked immunosorbent assay

FACTS – Follow-on African Consortium for Tenofovir Studies

FAME – Film Antiretroviral Microbicide Evaluation

FDA – Food and Drug Administration

fmol – femtomole

FTC – emtricitabine

HAART – highly active antiretroviral therapy

HBSS – Hanks' Balanced Salt Solution

HIV – Human Immunodeficiency Virus

HPTN – HIV Prevention Trials Network

HR – hazard ratio

IC_{50} – 50% inhibitory concentration

IC_{90} – 90% inhibitory concentration

Imax – maximum inhibitory effect

iPrEx – Iniciativa Profilaxis Pre-Exposicion (translation: pre-exposure prophylaxis initiative)

IQR – interquartile range

LC-MS/MS – liquid chromatography-tandem mass spectrometry

LLOQ – lower limit of quantification

mg – milligram

mL – milliliter

MTN – Microbicides Trials Network

ng – nanogram

NNRTI – non-nucleoside/nucleotide reverse transcriptase inhibitor

NRTI – nucleoside/nucleotide reverse transcriptase inhibitor

PBMC – peripheral blood mononuclear cells

PD – pharmacodynamic

pg – picogram

PK – pharmacokinetic

PrEP – pre-exposure prophylaxis

RAI – receptive anal intercourse

RF – rectal fluid

RF – rectal fluid

SAE – serious adverse event

SD – standard deviation

SHIV – simian/human immunodeficiency virus

SIV – simian immunodeficiency virus

$T_{1/2}$ – half-life

TDF – tenofovir disoproxil fumarate

TDF-FTC – combination of tenofovir disoproxil fumarate and emtricitabine

TFV – tenofovir

TFV-DP – tenofovir diphosphate

T_{max} – time to maximum concentration

VOICE - Vaginal and Oral Interventions to Control the Epidemic

CHAPTER 1

1.1 Introduction

Women currently make up more than half of the over 36 million adults and children worldwide who are infected with the human immunodeficiency virus (HIV)[1]. Sub-Saharan Africa is the region of the world most affected by the HIV epidemic, with over a dozen countries reporting HIV prevalence rates greater than 5% among women[2]. Only about half of people living with HIV have access to antiretroviral treatment[1]. Preventing new HIV infections remains an important international health goal[3], and while significant strides in reducing the spread of HIV have been made, there remains a need for new interventions.

Heterosexual intercourse (and specifically penile-vaginal intercourse) remains the most common route of infection for women living with HIV. Young women have been found to have higher incident HIV infection compared to men in the same age group[4]. There are many structural and behavioral factors that enhance women's susceptibility to HIV including poverty, concurrent sexual relationships, transactional sex, and difficulty negotiating condom use[5]. There may also be biological differences that increase women's likelihood of HIV acquisition through penile-vaginal intercourse. Hormonal changes that occur during the phases of the menstrual cycle, or during pregnancy and the postpartum period, appear to influence HIV susceptibility. The presence of other sexually transmitted infections, such as herpes simplex virus or human papilloma virus, have also been linked with increased risk of acquiring HIV.

The projects presented in this thesis are early drug development studies of two nucleoside reverse transcriptase inhibitors administered as vaginal microbicides. The FAME 02b and

FAME 05 studies describe the pharmacokinetics and pharmacodynamics of a single dose of dapivirine vaginal gel or film (FAME 02b) or tenofovir vaginal gel or film (FAME 05). These studies informed subsequent trials of serial doses of tenofovir and dapivirine vaginal gel and film[6,7], demonstrating that these products were safe, well-tolerated, and have the potential to reduce a woman's risk of HIV acquisition through heterosexual intercourse.

Having female-controlled prevention strategies has the potential to overcome some of the weaknesses of relying on male condoms, which while they are effective at preventing transmission of HIV when used, are not always used consistently or correctly. Oral microbicides to prevent HIV transmission have been shown to be effective, but they suffer from several shortcomings including that people do not take them reliably. It may be preferable for some women and couples to have prevention products that are intended to be used only at the time of intercourse, so that they do not have to remember a daily dosing schedule. It is also advantageous for women to be able to use a product without needing the consent of her male partner. These gaps in prevention strategies have the potential to be filled by vaginal microbicides.

1.2 Pre-Exposure Prophylaxis for HIV Prevention

Since the beginning of the HIV epidemic, the primary strategy for prevention of sexual transmission has been to encourage the correct and consistent use of male condoms. Despite extensive efforts to facilitate and encourage condom use, even by known serodiscordant partners, this strategy has been ineffective in slowing the spread of HIV. Only since highly-active

antiretroviral therapy (HAART) for treatment of HIV has been made widely available has there been any measurable decrease in global HIV transmission[3].

The next big leap forward in HIV prevention was made when it was determined that daily oral dosing of tenofovir (TFV, TDF) and emtricitabine (FTC) as pre-exposure prophylaxis (PrEP) could reduce an individual's risk of HIV acquisition. The iPrEx study was the first randomized, placebo-controlled trial evaluating whether daily oral TDF-FTC could reduce HIV transmission among trans-women and men who have sex with men[8]. The overall reduction in HIV risk was 44% in the TDF-FTC group, but the protective effect of oral PrEP was as high as 92% among participants who had detectable study drug levels in their blood [8]. The Partners PrEP study used a similar design among serodiscordant heterosexual couples, although this trial included a third arm in which the HIV-negative partner received daily oral TDF without FTC[9]. The Partners PrEP Study found even more promising results than iPrEx: an overall 75% risk reduction in HIV acquisition with TDF-FTC, with protection as high as 90% among participants with detectable study drug levels (compared to those without detectable drug levels)[9]. There was no difference in the protective effects of TDF-FTC in women compared to men.

Subsequent studies specifically investigating the efficacy of oral PrEP in women found disappointing results. In FEM-PrEP, HIV-negative women were randomized to take either daily oral TDF-FTC or placebo, in a design mirroring the iPrEx trial. Unlike in iPrEx or Partners PrEP, FEM-PrEP found no difference in HIV acquisition with oral PrEP compared to placebo (HR 0.94, 95% CI 0.59-1.52, p=0.81)[10]. Participants in FEM-PrEP reported a high level of adherence to the daily PrEP regimen, and pill counts were consistent with 88% adherence.

However, when plasma tenofovir concentrations were measured in HIV seroconverters and matched controls, adherence ranged from 15% to 37%[10]. The VOICE trial had similar adherence challenges. In VOICE, HIV-negative women were randomized to daily use of one of five products: oral TDF, oral TDF-FTC, oral placebo, TFV vaginal gel, or placebo vaginal gel[11]. Compared to placebo, there was no difference in HIV acquisition with oral TDF (HR 1.49, 95% CI 0.97-2.29, p=0.07), oral TDF-FTC (HR 1.04 (0.73-1.49, p=0.81), nor TFV vaginal gel (HR 0.85, 95% CI 0.61-1.21, p=0.37). Despite high levels of adherence by participant self-report (mean = 90%) and returned product counts (mean = 86%), half of the participants assigned to the active product arms had no detectable tenofovir in plasma at any quarterly visit. When participants who had detectable concentrations of TFV in plasma were compared to those without, TFV in plasma was associated with greater than 60% reduced likelihood of HIV infection (adjusted HR 0.34, 95% CI 0.13-0.87, p=0.02).

Data regarding vaginal microbicides for HIV prevention have been similarly mixed. CAPRISA 004 was the first randomized trial to demonstrate efficacy of a vaginal product at reducing HIV acquisition. In this study, a 1% TFV vaginal gel applied before and after intercourse (pericoital use) led to an overall 39% decrease in risk of HIV infection compared to placebo, with the risk reduction as high as 54% among women with high adherence [12]. In contrast, VOICE found no difference in HIV risk among women randomized to daily use of 1% TFV vaginal gel compared to placebo [11]. The FACTS 001 trial repeated the design of CAPRISA 004 (pericoital use of 1% TFV vaginal gel vs placebo) on a larger scale, but found no difference in HIV acquisition between arms (IRR 0.98, 95% CI 0.7-1.4)[13]. In a subgroup analysis, women with high

concentration of TFV in cervicovaginal lavage samples had a 48% lower risk of HIV acquisition (aHR 0.52, 95% CI 0.27-0.99).

1.3 Microbicides in HIV Prevention

Microbicides are products designed to be applied to the vagina or rectum prior to intercourse with the goal of reducing the risk of HIV acquisition by the receptive partner. An ideal microbicide should maintain the integrity of the mucosal epithelium, distribute into the tissues where HIV infection likely occurs without significant systemic absorption, and persist in the site of application for sufficient time to provide protection[14]. Products must also be acceptable to the individuals for whom they are designed. Early products studied as vaginal microbicides included various vaginal gels (nonoxynol-9, cellulose sulfate, surfactant, BufferGel®, Carraguard®) without antiretroviral drugs. These products were thought to prevent HIV transmission by various mechanisms including altering blocking HIV adherence and entry into target cells, altering the vaginal mucosa, or maintaining the acidic pH of vaginal fluid[14]. Despite promising results from animal studies, human trials failed to demonstrate efficacy at preventing HIV transmission.

Several drugs have been investigated as microbicide candidates, with promising results seen for tenofovir and dapivirine. Tenofovir is a nucleoside reverse transcriptase inhibitor (NRTI)[15] that is a key component of first-line oral HAART regimens used for treatment of HIV[16]. Tenofovir has been shown to be effective for HIV prevention when taken orally by individuals at high risk of HIV infection and is well tolerated with few serious side effects[9]. The oral formulation of tenofovir is a prodrug of the active compound tenofovir diphosphate (TFV-DP) which inhibits

HIV replication in target cells[17]. An oral formulation of tenofovir combined with emtricitabine (another nucleoside reverse transcriptase inhibitor) is the only product currently being marketed as HIV pre-exposure prophylaxis[18]. Dapivirine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) that has poor oral bioavailability, but when dosed via a non-oral route has potent anti-HIV activity[19]. Dapivirine is being investigated for HIV prevention in the forms of vaginal film[6,20], gel[21,22], and ring delivery systems[23-27].

A successful microbicide drug must have several features including the ability to penetrate the mucosa of the application site (vagina or rectum) and achieve sufficient concentrations in the local environment to prevent HIV transmission. Microbicides are generally not trying to achieve systemic drug concentrations; the local presence of drug in the vagina or rectum is most important in reducing HIV transmission. The systemic drug concentrations are important for determining the likelihood of adverse drug effects and could also contribute to antiretroviral resistance if a user seroconverts despite using the microbicide.

1.4 FAME 02b and FAME 05

The FAME 02b and FAME 05 studies were designed to assess the pharmacokinetic properties of tenofovir and dapivirine gels and films in various compartments. Participants received a single dose of dapivirine (FAME 02b) or tenofovir (FAME 05) vaginal gel or film and subsequently underwent serial sampling of serum, rectal fluid (FAME 05 only), cervicovaginal fluid (CVF), and cervical biopsies over the next 7 days. Half of study participants were randomized to be dosed with gel first, followed by film after a washout period. The other half were randomized to be dosed with film, followed by gel. Serum, rectal fluid, CVF, and cervical biopsies were

assessed for relevant drug concentrations. Cervical biopsies collected after study product dosing and collected off all study products were used in explant challenges to assess for HIV infectivity in the presence and absence of drug.

All products assessed in these studies were found to be well tolerated, with no serious adverse events and no AEs deemed related to study product use. Dapivirine concentrations in all compartments assessed were similar after film and gel dosing. In contrast, tenofovir film was associated with higher CVF and cervical tissue drug concentrations than tenofovir gel, while the tenofovir gel was associated with higher rectal fluid concentrations than film. Cervical tissue explant challenge suggested protective effect of dapivirine vaginal products but not tenofovir, although there was significant variability in results for all four products. Taken together, these studies indicate that vaginal films and gels containing dapivirine and tenofovir are promising HIV prevention strategies.

CHAPTER 2

FAME 02b Study

This chapter is published as "Robinson JA, Marzinke MA, Bakshi RP, Fuchs EJ, Radebaugh CL, Aung W, Spiegel HM, Coleman JS, Rohan LC, Hendrix CW. Comparison of dapivirine vaginal gel and film formulation pharmacokinetics and pharmacodynamics (FAME 02B). AIDS Res Hum Retroviruses. 2017 Apr;33(4):339-346. Pubmed PMID: 27809557."

Permission for this paper to be included in this dissertation can be found in Appendix C.

2.1 Abstract

2.1.1 Objective

While pre-exposure prophylaxis with oral tenofovir/emtricitabine has proven effective in reducing HIV acquisition rates, poor adherence to and acceptability of existing vaginal gels and the potential for evolving drug resistance to proven agents has led to development of vaginal film formulations and other antiretroviral drugs, respectively, including the non-nucleoside reverse transcriptase inhibitor dapivirine.

2.1.2 Study Design

In this two-arm, cross-over study of a novel fast-dissolving dapivirine film and a previously studied semisolid dapivirine gel, nine healthy women received a single 1.25 mg vaginal dose of each study product (a total of 10 women enrolled, but one withdrew after the first dosing visit). Clinical, pharmacokinetic, and antiviral pharmacodynamic assessments (*ex vivo* HIV-BaL challenge of tissue explants) were performed over 7 days following dosing.

2.1.3 Results

Six of the 10 research participants experienced mild to moderate adverse effects, similar between products, with no severe adverse events or adverse events attributed to study products. There were no statistically significant differences in plasma, cervicovaginal fluid (CVF), or cervical tissue dapivirine concentrations between the gel and film (all $p>0.05$). CVF dapivirine concentrations were 1.5 $\log_{10}$ and 6 $\log_{10}$ greater than tissue and plasma concentrations, respectively ($p<0.001$). Both film and gel demonstrated antiviral effect in explant challenges of cervical biopsies collected 5 hours after dosing, compared to biopsies collected without drug and 72 hours after dosing ($p<0.05$ for gel, $p=0.06$ for film). There was no difference in *ex vivo* explant HIV challenge between gel and film.

2.1.4 Conclusion

The dapivirine film and gel performed similarly in terms of tolerability, pharmacokinetics, and antiviral effect. The film product may provide an acceptable alternative to pharmacokinetically comparable dapivirine gel formulations. Effectiveness remains to be tested.

2.2 Introduction

Infection with human immunodeficiency virus (HIV) remains a global health problem, with 2 million new HIV infections reported worldwide in 2014 [28]. Sexual transmission remains the most common mode of transmission, particularly for young women. A promising strategy to reduce sexual transmission of HIV is the use of pre-exposure prophylaxis (PrEP), during which a person takes or applies a drug or combination of drugs in order to reduce his or her risk of HIV acquisition. Randomized placebo controlled trials of PrEP using daily oral tenofovir/emtricitabine [29] and peri-coital 1% tenofovir vaginal gel [12] demonstrated that high levels of product adherence result in reduced HIV acquisition [8,9,30-32]. Poor adherence resulted in no protective effect of the same drug regimens [11,33,34].

Alternative behavioral and biomedical strategies intended to improve adherence to PrEP regimens include: sustained release products that require infrequent dosing and provide long-term protection, or topical products that may be suitable for periodic dosing and provide alternatives to oral dosing desired by some [35-37]. Sustained delivery product development includes: antiretroviral (ARV) vaginal rings replaced monthly; intramuscular injectable formulations of ARVs dosed every 2 months; and implantable ARV formulations with potential for yearly dosing as indicated by pre-clinical pharmacokinetic (PK) studies [38]. Alternative topical

approaches in development include vaginal films and tablets, rectal gels, suppositories, and enemas; these efforts include tenofovir and several other candidate ARV compounds, including dapivirine.

Dapivirine (DPV) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) that has potent anti-HIV-1 activity, both *in vitro* and *in vivo* [39]. While it has very poor oral absorption, making it impractical for oral dosing, DPV has shown promise as a topical microbicide. Studies investigating rings, films, and gels that deliver various doses of DPV have shown the products to be safe and well tolerated when dosed vaginally in women [22,25,40-45]. A DPV gel developed for vaginal use proved safe and acceptable to men, when applied externally to the penis [46].

Sustained delivery products are not necessary for persons with only periodic risk of HIV infection, and some individuals may choose to avoid the increased and sustained risk of systemic toxicity associated with injectable formulations. On-demand microbicide (topical formulation) products for use during periods of anticipated sexual exposure to HIV may be a suitable alternative for such persons. Most of the microbicide products evaluated for effectiveness have been aqueous-based gels applied peri-coitally [11,30,34]. While efficacy was low or absent in several randomized clinical trials of vaginal tenofovir gels in intent to treat analyses (CAPRISA 004, VOICE, FACTS 001), efficacy was demonstrated in the subset of individuals with high levels of adherence in all of these studies [47].

One limitation of the vaginal gel identified in acceptability studies included the participant experience with the product, as the gel often leaked from the vagina after application, as well as

the bulkiness of the applicator that complicated product storage and transport [48]. A quick dissolving vaginal film dosage form – far smaller in size, less packaging to dispose of post-application, less volume to leak from the vagina, and less volume to dilute innate endogenous antibacterial and antiviral properties of vaginal fluid – may overcome some limitations of gel products which impact adherence. The Listerine® breath mint strips are a familiar and acceptable fast-dissolving film formulation for oral dosing. Vaginal films, like the nonoxynol 9 contraceptive film, have proven more acceptable than gel formulations [49,50].

For this study (FAME 02B), we used a soft, flexible, translucent DPV vaginal film composed of a polyvinyl alcohol (PVA) base, with an individual unit size of 1" × 2", and 70 μm thick. *In vitro* studies show the films dissolve rapidly upon exposure to an aqueous environment, releasing more than half of the DPV within 10 minutes [20]. Each individual film contains 1.25 mg of DPV and is comparable to phase 1 studies of DPV gels (0.05% with administration of 2.5 g/2.8 mL). Studies of DPV vaginal gel (including companion study, FAME 02) have shown that these products are safe, generally acceptable to women (apart from leakiness noted above), and lead to low systemic DPV concentrations[22,42,43,45].

The current study describes the multi-compartment pharmacokinetics (blood, cervical tissue, and cervicovaginal fluid [CVF]) and *ex vivo* pharmacodynamics (HIV tissue explant challenge) over one week following a single dose of DPV film compared to a DPV gel formulation. The companion study, FAME 02, involved one week of daily dosing of the same DPV film and gel products, but with additional safety assessments [45].

2.3 Materials and Methods

2.3.1 Study Design and Participants

This was a two-arm, single site, randomized cross-over study of two DPV formulations, conducted at the Drug Development Unit (DDU) of the Johns Hopkins Hospital in Baltimore, MD. The protocol was approved by the Johns Hopkins Medicine Institutional Review Board. Ten healthy, HIV-uninfected women between the ages of 18 and 45 years were recruited to participate. After ensuring eligibility, participants were randomized to receive either DPV vaginal gel (0.05%, 2.5 g/2.5 mL volume, total 1.25 mg applicator dose) followed by DPV vaginal film (1.25 mg/film), or film followed by gel. At the first study visit, the DPV gel product was applied by an investigator using a polyethylene vaginal applicator (HTI Plastics, Lincoln, NE). The DPV film product was applied in the mid-vaginal region by a gynecologist during a speculum exam. The participant remained recumbent for approximately 30 minutes after each dose. Over the next 12 hours, serial blood samples for DPV concentration were collected, and samples of rectal fluid (RF), CVF, and cervical biopsies were collected 5 hours after dosing. CVF samples were taken from 3 locations within the lower female genital tract – mid-vagina, posterior vaginal fornix, and external cervical os using a Dacron swab (Cardinal Health, McGraw Park, IL). Participants returned to the DDU to provide blood samples at 24, 48, 72, and 168 hours after dosing. Cervical biopsies were repeated at the 72-hour visit, and CVF samples were collected at 72 and 168 hours. A final safety assessment for the first study product was conducted 14 days after dosing, after which the second formulation was dosed at a similar time during the participant's next menstrual cycle. The second study product was followed by the same sampling schedule. A final set of cervical biopsies was collected from each participant

several weeks after completing the second product dosing, in order to serve as a negative control for *ex vivo* explant assessments.

2.3.2 Clinical Assessment

Participants were assessed for adverse events (AEs) at each study visit, and if detected, each AE was assigned a grade based on the Division of AIDS (DAIDS) Table for Grading Adult and Pediatric Adverse Events, Version 1.0 and the Female Genital Grading Table for Use in Microbicide Studies (Appendix 1 to DAIDS Table for Grading Adult and Pediatric Adverse Events, Version 1.0) [51,52].

2.3.3 Pharmacokinetic Sample Analysis

Plasma DPV concentrations were measured using a validated ultra-performance liquid chromatographic-tandem mass spectrometric (LC-MS/MS) method that has been previously described [53]. The lower limit of quantification (LLOQ) for this assay is 20 pg/mL, with coefficient of variation (%CV) for intra- and inter-assay precision and accuracy ranging from 5.23 to 13.89.

Cervicovaginal fluid samples were analyzed for DPV concentration using validated LC-MS/MS analysis, with LLOQ of 0.250 ng/swab, corresponding to median 0.01 ng/mg based on CVF sample volumes. Cervical tissue DPV concentration was assessed, following homogenization and protein precipitation, using a validated LC-MS/MS method with a LLOQ of 0.05 ng/sample, corresponding to median 0.07 ng/mg based on biopsy weights [44]. Tissue DPV quantification was performed using calibrators prepared in human plasma and matrix-specific tissue quality control

samples. The assay for rectal fluid (RF) DPV quantification is still being validated; consequently, these results are not presented.

2.3.4 HIV Exposure and HIV-1 p24 Measurement

Two cervical biopsies from each study product and time point (5 hour, 72 hour) and the no drug control were collected in L15 media (Mediatech, Manassas, VA), 1x Pen/Strep/Glutamine, 1x Amphotericin B, and 10% FBS (Gemini BioProducts, Woodland, CA). Biopsies were individually transferred to single wells of a 48 well plate containing 1 mL culture medium - DMEM (Mediatech, Manassas, VA) with 10% human AB serum (Gemini BioProducts, Woodland, CA), 1x Pen/Strep/Glutamine (Gemini BioProducts, Woodland, CA), 1x Non-essential amino acids (Mediatech, Manassas, VA) and 100 units IL-2 (Roche). Biopsies were exposed to HIV-1 BaL at final concentration of 5 x 10^4 $TCID_{50}$/mL and incubated for 2 h at 37 °C and 5% CO_2 in 100% humidity. After infection, biopsies were washed 4 times with 1 mL HBSS, weighed, and transferred to separate wells of a 48 well plate (1 biopsy/well) containing 1 mL fresh culture medium (described above). The day of infection was denoted as Day 0. Plates were incubated for 14 days at 37 °C and 5% CO_2 in 100% humidity. 0.7 mL culture medium was harvested on days 4, 7, 10 and 14 and replaced with fresh medium. Harvested medium was stored frozen at -20 °C until analysis. HIV-1 p24 concentration in the harvested medium was measured using the PerkinElmer Alliance HIV-1 p24 ELISA kit according to manufacturer's instructions. Cumulative p24 produced by biopsies over 14 days was used for analysis.

2.3.5 Statistical Analysis

Plasma values were analyzed calculating area under the concentration-time curve from 0 to last sample (AUC_{last}), peak concentration (C_{max}), and time to peak concentration (T_{max}) with noncompartmental methods using Phoenix WinNonlin® software (version 6.4, Pharsight/Certara, Princeton, NJ). Crude maximum half-life estimates for CVF and cervical tissue DPV were also calculated using imputation of LLOQ/2 where concentration fell below the LLOQ. (Note: these are poor estimates due to very sparse sampling [2 tissue and 3 CVF samples] with highly uncertain time to both C_{max} and LLOQ. Guided somewhat by plasma T_{max} data, the calculated estimates are highly conservative and are very likely longer than the true elimination half-life.) Concentration-time data is presented using SigmaPlot®, Version 13 (Systat Software, Inc., San Jose, CA). Descriptive statistics of data and comparisons between the two formulations and between different matrices were performed using the Wilcoxon rank sum test using Stata®, Version 12 (StataCorp LP, College Station, TX). Differences in CVF among the 3 lower female genital tract sites were tested first with the Friedman test, followed by *post hoc* paired comparisons (Wilcoxon test). A *p*-value of less than 0.05 was considered statistically significant in comparison testing and correlation.

The DPV concentration-response (cumulative p24) relationship was explored through (1) testing correlation of DPV tissue concentration and cumulative p24 (Spearman correlation coefficient), (2) linear least-squares regression modeling (using raw and log-log transform of p24 and DPV concentration) with participant and formulation (film v. gel) as covariates, and (3) a series of pharmacodynamic models using the Hill equation to estimate concentration at which antiviral effect is 50% of maximum (IC_{50}), maximum inhibitory effect (I_{max}), baseline effect (no drug), and sigmoidicity (Hill coefficient) using Phoenix WinNonlin v 6.4 (Pharsight/Certara, Princeton,

NJ). Correlation and pharmacodynamic modeling were performed by both excluding all BLQ (Below Limit of Quantitation) DPV concentrations and imputing those concentrations as LLOQ.

2.4 Results

2.4.1 Subjects

Participants were mean (standard deviation [SD]) 28.7 (±8.3) years of age. Half of participants (n=5) were African-American, with non-Hispanic white, Hispanic, Asian, and mixed race among the other 5. One participant withdrew from the study after her first product dosing (film) and did not have any samples collected after the first day. Pharmacokinetic and pharmacodynamic data are presented from the remaining 9 participants who have complete data, while adverse event data are presented from all 10 enrolled participants.

2.4.2 Adverse Events

A total of 24 AEs were recorded throughout the study, all of which occurred in 6 of 10 participants (i.e.: 4 participants experienced no AEs). The AEs were reported within the week following dosing, except for one AE at baseline (known history of iron-deficiency anemia) and two identified at the follow up visit that occurred between the first and second dosing visits. Reported adverse events included: headache, diarrhea, periorbital edema, upper respiratory infection, phlebotomy site bruising, urinary tract infection, mononucleosis, and dehydration; lab abnormalities included hypoglycemia, hyperglycemia, anemia, hypokalemia and increased neutrophil count. No serious AEs (SAEs) were recorded for any participant, and the majority of AEs were Grade 1 (only 6 of 24 were Grade 2, which occurred in two participants). Of the AEs that occurred during dosing intervals (n=21), they were evenly distributed between the two

products (11 occurred with gel, 10 occurred with film). All AEs were determined to be "not related" to study product exposure.

2.4.3 Pharmacokinetics

Following dosing of each product, plasma DPV concentrations rose to a peak between 12 and 24 hours, then fell in log linear fashion; at the last observation, 168 hours, 5 of 9 participants (2 film, 3 gel) had detectable plasma DPV concentrations (Figure 2.1A). Median (IQR) plasma PK parameter estimates for the film product were: C_{max} 91 (67, 179) pg/mL, T_{max} 12 (5, 18) hours, AUC_{last} 7,952 (5,763, 9,021) pg-hrs/mL, and half-life 59 (51, 82) hours. Gel PK parameter estimates were: C_{max} 132 (93, 169) pg/mL, T_{max} 24 (10, 24) hours, AUC_{last} 7,832 (5,799, 11,807) pg-hrs/mL, and half-life 52 (42, 64) hours. There were no statistically significant differences between the film and gel products for any PK parameter (Wilcoxon rank sum, all p-values >0.05).

CVF DPV concentrations declined from 5 through 168 hours in log linear fashion (Figure 2.1B); at 168 hours, all DPV concentration medians (though not all upper quartiles) were BLQ for both products at all sites. Accordingly, because we did not have reliable concentration estimates for the 168 hour time point, half-life estimates were not made. There were no statistically significant differences between study products for DPV CVF concentrations at any sampling location (Table 2.1, all $p>0.05$). Within participants, there were statistically significant differences among the 3 genital tract CVF sampling site concentrations for both film and gel (Table 2.1; Friedman test both $p\leq0.02$); in pairwise comparisons between sites, the general concentration trend was mid vagina > fornix > cervical os.

Cervical tissue homogenate DPV concentrations were also not different comparing film to gel (Table 2.1, Figure 2.1B). At 72 hours after dosing, only 3 of 9 samples after gel and none of 9 samples after film had DPV cervical tissue homogenate concentrations above the LLOQ. Comparing concentrations across biological matrices 5 hours after study product dosing (where all matrices for all products and subjects are in the quantifiable range), CVF DPV concentrations (median across the 3 genital tract) were 1.5 $\log_{10}$ greater than tissue concentrations and 6 $\log_{10}$ greater than plasma concentrations. Plasma half-life was significantly greater than estimated maximum half-life for both CVF and cervical tissue, which were similar to each other. No statistical comparisons are made between products or among matrices since these CVF and cervical tissue half-life estimates are very crudely based on too sparse data (2 to 3 samples, 5 hour concentrations very likely before peak concentration is achieved, uncertain time to LLOQ, 31% overall BLQ values requiring imputation).

2.4.4 Pharmacodynamics (HIV-1 p24 Measurement)

At least 7 of 9 participants' 5-hour cumulative p24 antigen concentrations were lower than baseline and the 72-hour values for the corresponding product (Figure 2.2A). For cervical biopsies collected 5 hours after film dosing, the cumulative p24 antigen production, median 1.0 pg/mg (IQR 0.5, 252), was significantly less than the cumulative p24 antigen 72 hours after film dosing, 136 pg/mg (2, 3258) (Wilcoxon rank sum test p=0.01) and trended toward being less than the no study product baseline, 55 pg/mg (3, 297) (p=0.07). After gel dosing, 5-hour and 72-hour cumulative p24 concentrations were 1.0 pg/mg (1.0, 1.5) and 543 pg/mg (71, 3437), respectively, with 5 hour values trending toward statistical significance (p=0.06) when compared

to 72 hour values, and were significantly lower than no study product baseline values (p=0.04). Baseline values were not different than 72 hour values for either product (p>0.10). Neither was there any statistically significant difference in p24 production at either 5 or 72 hours post dose when gel was compared to film.

Figures 2.2A and 2.2B demonstrate a highly variable 4 $\log_{10}$ spread of cumulative p24 concentrations from baseline cervical biopsy samples (no drug) as well as for cervical samples collected within 72 hours of dosing, nearly all of which have BLQ DPV concentrations. Using all baseline biopsies and biopsies with BLQ DPV concentrations as "no drug" biopsies, within participant variability was median 169% (IQR 103%, 242%) coefficient of variation; overall coefficient of variation among all "no drug" biopsies was 262%.

DPV tissue concentration was inversely correlated with cumulative p24 values, demonstrating a rough concentration-response relationship (Spearman correlation coefficient = -0.483, p=0.003). With log-log transformation of values, an inverse linear relationship between DPV concentration and p24 production was seen (ß = -0.82 [0.26 SE], p=0.004) with participant (ß = -0.12 [0.05], p=0.03), but not the product arm (ß = 0.79 [0.47], p=0.11) as significant covariates. The data failed to fit any non-linear inhibitory pharmacodynamic models (no statistically significant parameter estimates; data not shown).

2.5 Discussion

Our single-dose comparison study indicated that both DPV film and gel were well tolerated by study participants, with no serious adverse events reported after single doses of either product

and there were no differences in adverse events between products. There were also no statistically significant PK differences between the two formulations in plasma, tissue, or CVF, except for a statistical trend toward a 2-fold greater DPV mid-vaginal CVF concentration with film compared to gel. There were, however, differences in DPV concentrations among anatomic sites at similar sampling times, generally highest at the mid vaginal position, especially compared to the cervical os, for both formulations. Five hours after product dosing, when drug was readily detectable in all participants' samples, DPV concentrations were far greater in CVF compared with either cervical tissue (32-fold greater) or plasma (10^6-fold greater). Plasma DPV half-life (greater than 50 hours) was at least several times longer than the cervical tissue DPV half-life (<10 hours, a significant overestimate).

These anatomic differences are very similar to 2 other DPV studies (FAME 02 and MTN-013) where the samples were analyzed in the same laboratory and paired samples from the same women are available for plasma, cervical tissue, and CVF[44,45]. FAME 02 compared daily use of the same DPV film and gel products as this study, though for a total of consecutive 7 days. In their analysis, Bunge, *et al*., found that 2-4 hours after the final dose, DPV concentrations in cervicovaginal lavage (CVL) samples were 4 $\log_{10}$ greater than in plasma, and were the same as in cervical tissue. Median DPV plasma concentrations (220-310 pg/mL) drawn 2 to 4 hours after the final dose were higher than our values by 2-fold, but samples were collected after 7 daily doses which allows for DPV accumulation in plasma due to a long DPV half-life relative to the daily dosing interval. Median DPV tissue concentration following daily dosing for 7 days was the same as what we detected after a single dose (2 to 7 ng/mg) – no accumulation between our single and their 7 daily doses is also consistent with a half-life in tissue that is much shorter as

compared to plasma. The anatomical differences we showed - vaginal greater than cervical os CVF DPV concentration - is consistent with FAME 02 which showed greater DPV concentrations and antiviral effect (*ex vivo* explants) in vaginal tissue compared to cervical tissue. Median CVL concentrations were 2 to 5 ng/mg, which is ~20 to ~60-fold lower than our 5 hour CVF concentrations, but the dilutional effect of the 10 mL lavage volume on drug in 100-300 µL resident CVF volume largely accounts for this difference. No accumulation would be expected in CVF since the products were identical and repeated dosing should have no effect in CVF concentrations. So, accounting for the multiple dose accumulation of DPV in plasma and the dilutional effect of lavage fluid on CVF, the DPV PK findings are very similar to what was observed in this study.

Unlike our single dose study, FAME 02 noted a statistically significant greater tissue DPV concentration after gel dosing when compared to film dosing. The FAME 02 report suggested that the tissue DPV concentrations after gel dosing were increased, when compared to film, likely due to residual gel adherent to the tissue biopsies; sample handling, therefore, might have differed between studies. In addition, in our FAME 02B study, a gynecologist administered all film doses whereas in FAME 02, research participants self-administered film doses, some of whom had difficulty. The concentration differences we noted might also be attributed to speculum-assisted placement in mid-vagina (5 hour samples) or study-related sampling (72 hours). Finally, FAME 02 was a much larger study and had greater statistical power to detect differences than FAME 02B; however, FAME 02B actually trended in the other direction (film greater than gel tissue DPV concentration). Taken together, these differences support the finding that film and gel likely achieved similar tissue DPV concentrations.

In MTN-013, a 28-day DPV ring comparison study, the average daily dose was 0.16 mg based on the 25 mg DPV ring content as manufactured and 82% DPV dose retained in the rings, on average, after 28 days intravaginally [44]. This is roughly 13% of the 1.25 mg film and gel dose in FAME 02 and FAME 02B. The mean (SD) steady-state (or 28 day) concentrations in MTN-013 were: plasma, 175 (45) pg/mL, which exceeds our single dose median peak plasma DPV concentrations and falls below the plasma concentrations reported in FAME 02, indicating both accumulation of plasma DPV with time as well as more efficient drug delivery per administered dose with the sustained DPV release from the ring; cervical tissue, 0.6 (0.9) ng/mg, which falls below our single dose 5 hour (and FAME 02's steady-state 2-to-4 hour sampled) post-dose cervical tissue concentrations, though proportionally higher than expected accounting for the lower average daily ring dose compared to film and gel and doesn't account for trough concentrations in the FAME studies which were not measured; CVF, 5.7 (18.7) ng/mg, which is lower by 8- to 15-fold compared to our values, also consistent with formulation dose differences. Steady-state CVF DPV concentrations are 5 $\log_{10}$ greater than plasma and 1 $\log_{10}$ greater than cervical tissue, which is highly consistent with FAME 02 and FAME 02B given steady-state release of DPV from the ring and fluctuating concentrations with the film and gel.

A cervical tissue DPV concentration-*ex vivo* explant challenge response relationship was demonstrated when controlling for participant as a covariate; in addition, participant specific differences in p24 production were identified. Product arm, however, was not statistically significant in this model, consistent with our simpler non-parametric comparisons. However, these data failed to fit traditional pharmacodynamic models, which we hoped would provide

useful IC_{50} concentrations. IC_{50} concentrations are useful in evaluating the comparability and appropriate scaling of *ex vivo* IC_{50} contrasted to *in vivo* $IC_{50,}$ which is essential to evaluate the clinical predictive value of such *ex vivo* tests. Lack of success with pharmacodynamic model fitting occurred due to too few concentrations above the LLOQ and, especially, a high degree of intra- and inter-participant variability. This was not, apparently, a limitation for the simpler linear model. For reference, the MTN-013 DPV vaginal ring and FAME 02 DPV film and gel also demonstrated a statistically significant linear relationship with a similar log-log transform of the variables. The consistent explant assay concentration-response seen in MTN-013, FAME 02, and FAME 02B indicates a clear dapivirine antiviral effect. There may also be a vehicle barrier effect contributing to this antiviral effect (as noted for some microbicide vehicles), but this cannot be determined for this product without a vehicle only control for comparison [54].

In summary, we found no significant PK or PD differences between the DPV film and gel products. The PK findings were consistent with those found with one week of daily dosing (FAME 02), allowing for evidence of DPV accumulation in plasma, and dilution of CVL compared to CVF. Comparing both FAME studies (film and gel) with a 28-day DPV ring study (MTN-013), plasma and cervical tissue DPV concentrations were similar, accounting for steady-state versus intermittent dosing differences. In addition, we demonstrated a linear concentration-response relationship using *ex vivo* explant challenge when controlling for participant as covariate, despite a large degree of assay variability. Especially in combination with the data from the companion FAME 02 study, which reported a high degree of acceptability and tolerability of the film product over one week of daily dosing, the film product may provide a more portable alternative to a DPV gel for women interested in periodic PrEP or who wish to

avoid an indwelling DPV vaginal ring. Effectiveness of any vaginal formulation of DPV awaits the outcome of randomized controlled clinical trials.

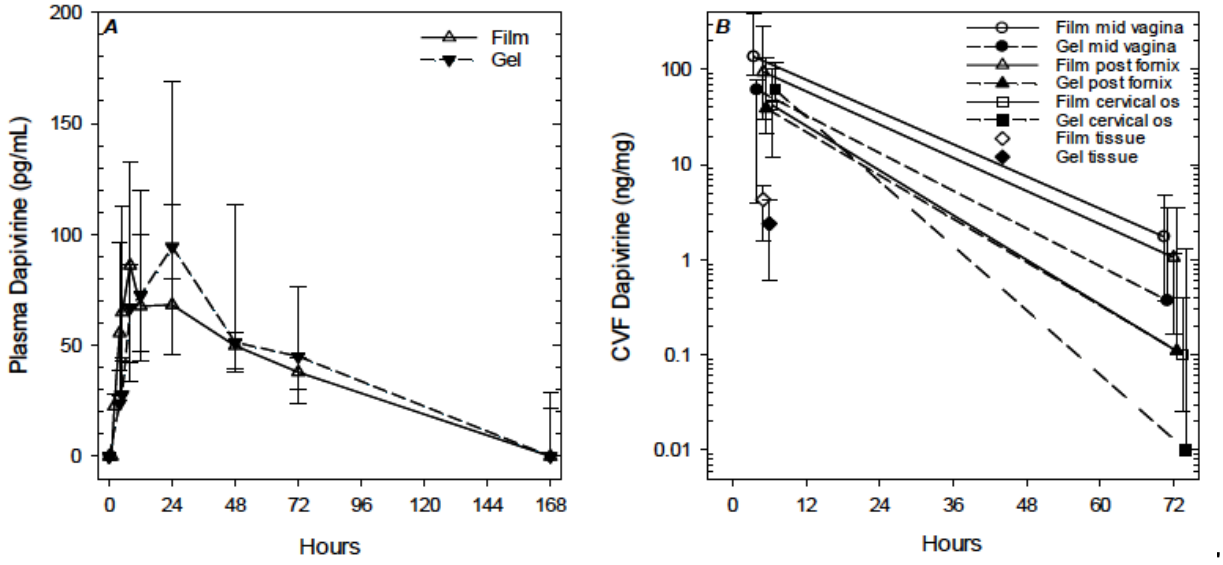
2.6 Tables and Figures

Table 2.1: Pharmacokinetic parameters of DPV in cervicovaginal fluid and cervical tissue homogenates after application of DPV film vs. gel

Matrix – PK Parameter	**Film 5hr Concentration** ng/mg median (IQR)	**Gel 5hr Concentration** ng/mg median (IQR)	**Film 72hr Concentration** ng/mg median (IQR)	**Gel 72hr Concentration** ng/mg median (IQR)	**Film Max Half-life** hours median (IQR)	**Gel Max Half-life** hours median (IQR)
CVF Mid vagina	136 (86, 387)[a]	61 (4, 79)	1.74 (0.37, 4.75)	0.37 (BLQ, 3.52)	11 (9, 15)	13 (4, 18)
CVF Fornix	95 (30, 287)	39 (21, 133)	1.06 (0.17, 1.18)[b]	0.11 (BLQ, 3.47)	10 (7, 12)	12 (8, 25)
CVF Cervical os	42 (12, 103)[c,d]	61 (46, 117)	0.10 (0.03, 0.39) [c,d]	0.01 (BLQ, 1.28) [c,d]	11 (8, 13)	5 (5, 14)
Cervical tissue	4.3 (1.6, 6.0)	2.4 (0.6, 4.2)	BLQ (BLQ, BLQ)	BLQ (BLQ, 0.39)	6 (6, 7)	9 (7, 19)

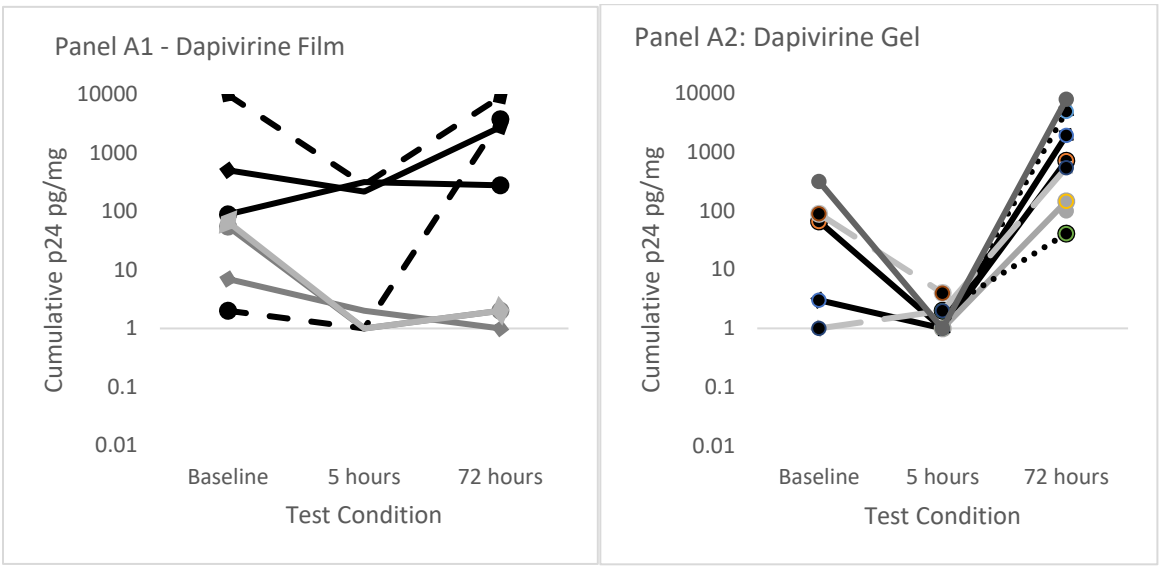
Data for 168 hour not shown as all medians were below limit of assay quantitation (BLQ).
[a]p=0.07 film v. gel (8 of 9 film > gel)
[b]p≤0.05 fornix vs. mid vagina
[c]p≤0.05 cervical os vs. fornix
[d]p≤0.05 mid vagina vs. cervical os
Note: 1 ng/mg is approximately 10^6 pg/mL (units of plasma DPV concentration in text).

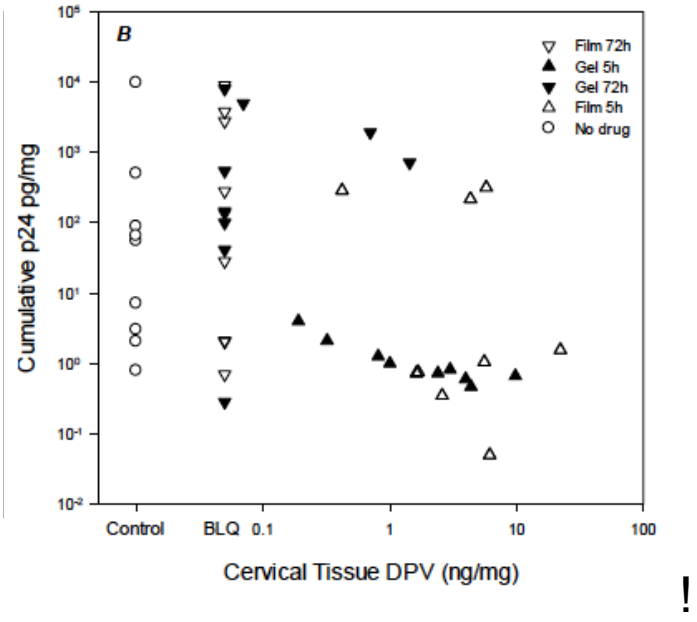
Figure 2.1: Dapivirine plasma and CVF concentration versus time for DPV film and gel



Panel A indicates plasma dapivirine concentration. Open symbols (solid line) are film, closed symbols (dashed lines) are gel. BLQ values are arbitrarily displayed as 0 pg/mL. ***Panel B*** indicates cervicovaginal fluid and cervical tissue homogenate dapivirine concentrations in $\log_{10}$ scale. Open symbols are film and closed are gel. Symbols represent mid vagina (round), posterior fornix (triangle), cervical os (square), and cervical tissue homogenate (diamond). Values are median with upper and lower quartiles. BLQ values are not shown; all medians at 168 hrs are BLQ, therefore that time is not shown. Nominal x-axis values are slightly offset to avoid overlap.

Figure 2.2: Cumulative HIV p24 antigen from *ex vivo* HIV challenge of cervical tissue explants after dosing with DPV film vs. gel





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Panel A indicates cumulative p24 antigen by false sequence categories for comparison. Each symbol-line pair is a unique research participant. Panel A1 presents cumulative p24 antigen at baseline (no drug) and after film dosing; panel A2 presents cumulative p24 antigen after gel dosing. *Panel B* indicates concentration-response relationship of cervical tissue homogenate dapivirine concentration (x-axis). No drug is open circle. Open triangles are film; closed triangles are gel. Upward pointing triangles are samples taken 5 hours after dosing; downward pointing triangles are collected 72 hours post dosing.

CHAPTER 3

FAME 05 Study

This chapter is published as "Robinson JA, Marzinke MA, Fuchs EJ, Bakshi RP, Spiegel HML, Colemen JS, Rohan LC, Hendrix CW. Comparison of the pharmacokinetics and pharmacodynamics of single-dose tenofovir vaginal film and gel formulation (FAME 05). J Acquir Immune Defic Syndr. 2018 Feb 01;77(2):175-182. Pubmed PMID: 29135651."

Permission for this paper to be included in this dissertation can be found in Appendix C.

3.1 Abstract

3.1.1 Objective

While pre-exposure prophylaxis with oral tenofovir (TFV) disoproxil fumarate/emtricitabine reduces HIV acquisition rates, poor adherence to and acceptability of daily vaginal gels has led to development of vaginal film formulations to improve adherence and, potentially, enable episodic use.

3.1.2 Study Design

In this two-arm, cross-over study of a fast-dissolving tenofovir film (40 mg) compared to a previously studied semisolid tenofovir 1% gel (40 mg), 10 healthy women received a single vaginal dose of each study product. Clinical, pharmacokinetic, and antiviral assessments were performed over one week post-dose.

3.1.3 Results

Nine of 10 participants experienced mild to moderate adverse effects, similar between products, with no severe adverse events or events attributed to study products. TFV concentrations after film dosing exceeded concentrations after gel dosing in plasma between 8 and 24 hours ($p \leq 0.02$). TFV concentrations in cervicovaginal fluid and both TFV and TFV diphosphate concentrations in cervical tissue homogenates were higher following film dosing (all p values < 0.04). The differences ranged from median (interquartile range) 2.9-fold (1.1, 9.0; midvaginal cervicovaginal fluid) to 4.4-fold (2.9, 7.7; plasma). Neither film nor gel demonstrated reduced cervical tissue biopsy infectivity after ex vivo HIV challenge.

3.1.4 Conclusion

Single dose tenofovir film demonstrated consistently higher concentrations in plasma and cervicovaginal samples when compared to gel during the first day following dosing. Single dose

cervical tissue TFV-DP concentrations at 5 hours exceeded steady-state concentrations previously reported with daily oral Truvada® dosing. Tenofovir film may provide an alternative to tenofovir gel formulations. Clinical efficacy remains to be tested.

3.2 Introduction

Human immunodeficiency virus (HIV) infection remains a global health problem with sexual intercourse being the most common mode of transmission. Pre-exposure prophylaxis (PrEP) with tenofovir (TFV) containing regimens has proven effective in randomized controlled trials using daily oral tenofovir/emtricitabine. While effectiveness is best when product adherence is high [8,9,30-32], poor adherence may result in no protection, especially in women[11,33,55]. Responding to the negative PrEP impact of poor adherence, alternatives to daily oral dosing have been pursued including sustained release products that provide long-term protection with infrequent dosing and topical products potentially suitable for either episodic or sustained use[35-37]. Topical PrEP efficacy has been demonstrated with vaginal gel and ring formulations of tenofovir and dapivirine, though these have been only modestly effective[11,23,30,47,55,56]. It is hoped that, similar to contraceptive product development where multiple formulation options lead to increased adherence and efficacy across the population, alternative PrEP formulations will boost overall adherence[57].

On-demand microbicide products may be preferred for persons at risk of HIV infection who desire PrEP, but struggle with daily oral dosing, prefer to avoid the potential for long-lasting toxicity from the systemic exposure of injectable formulations, or who only have occasional episodic HIV exposure risks not necessarily requiring long-term formulations. One such option

in development is fast-dissolving vaginal film formulations of dapivirine and TFV, which, in gel and intravaginal ring formulations, have proven PrEP efficacy[11,23,30,55,56,58]. Listerine® breath mint strips are one example of fast-dissolving film formulations on the market. Among topical formulations, film may overcome limitations of the vaginal gel which include messiness; frequent leakage from the vagina after application; and product storage and transport complications due to the bulkiness of the applicator [48]. By contrast, a vaginal film has: less volume to leak from the vagina or dilute innate endogenous antimicrobial factors in vaginal fluid; much smaller size for discreteness of use and portability; and less packaging to dispose of post-application. Vaginal films, like the nonoxynol 9 contraceptive film, have proven more desirable than other dosage formulations, including gels, tablets, and even vaginal rings[49,50,59,60].

Two prior studies have demonstrated the acceptability and pharmacokinetic (PK) equivalence of a fast-dissolving dapivirine vaginal film[45,61]. In this current study (FAME 05) we evaluated the single-dose, multi-compartment pharmacokinetics (blood, cervical tissue [CT], cervicovaginal fluid [CVF], and rectal fluid [RF]) and pharmacodynamics (PD, ex vivo HIV tissue explant challenge) of a fast-dissolving TFV film compared to the TFV 1% gel formulation used in prior clinical trials. A companion study, FAME 04, involved one week of daily dosing of the same TFV film and gel products with additional safety, immunological, and microbiome assessments[62].

3.3 Materials and Methods

3.3.1 Study design and participants

This was a two-arm, single site randomized crossover study of two TFV formulations, conducted at the Drug Development Unit (DDU) of the Johns Hopkins Hospital in Baltimore, MD. The protocol was approved by the Johns Hopkins Medicine Institutional Review Board (IRB00046617) and registered with Clinicaltrials.gov (NCT02280109). Ten healthy, HIV-negative women between the ages of 18 and 45 years were recruited to participate. All participants provided informed consent prior to screening and study procedures. After a baseline evaluation to determine eligibility, each participant was randomized to one of two sequences of a single vaginal dose of one of two study products, either gel then film or film then gel. The study products were TFV 1% gel (a unit dose equivalent to 40 mg in 4 ml of gel) and tenofovir vaginal film (40 mg). The 2-by-2 inch TFV films were composed of hydroxypropyl methyl cellulose, hydroxyethylcellulose, sodium carboxymethylcellulose, and glycerin.

At the first study visit after qualification, the investigational product was applied by a gynecologist during a pelvic exam. The TFV gel was applied using a polyethylene vaginal applicator (HTI Plastics, Lincoln, NE). The TFV film was placed, unfolded, in the midvagina during a speculum exam. The participant remained recumbent for approximately 30 minutes after each dose. Participants returned for additional safety and PK sampling visits for the next 12 hours and 24, 48, 72, and 168 hours after dosing. Serial blood samples were collected post-dose for plasma TFV concentration (0, 0.5, 1, 2, 4, 5, 8, 12, 24, 48, 72, and 168 hours) and peripheral blood mononuclear cell (PBMC) TFV diphosphate (TFV-DP) (0, 2, 4, 8, and 24 hours) concentrations. CVF samples from midvagina, external cervical os, and posterior vaginal fornix were collected at with a Dacron swab (Cardinal Health, McGraw Park, IL) 5, 72, and 168 hours after dosing. RF was collected at 5, 72, and 168 hours after dosing using an anoscope and Dacron

swab. At 5 and 72 hours after dosing, a pair of CT biopsies for PK and PD readouts were collected using Tischler forceps.

Participants were seen on day 14 for a safety evaluation. The second product was dosed during a similar point in a later menstrual cycle (avoiding menses) followed by the same sampling schedule. A final set of cervical biopsies was collected at least two weeks after the second product dosing as negative control for the explant HIV challenge.

3.3.2 Clinical assessment

Participants were assessed for adverse events (AEs) at each study visit. AEs were graded based on the Division of AIDS (DAIDS) Table for Grading Adult and Pediatric Adverse Events, Version 1.0,(December 2004, Clarification dated August 2009) and the Female Genital Grading Table for Use in Microbicide Studies (Appendix 1 to DAIDS Table for Grading Adult and Pediatric Adverse Events, Version 1.0, December 2004, Clarification dated August 2009).

3.3.3 Pharmacokinetic sample analysis

TFV in plasma, CVF, RF , and CT biopsy homogenate as well as TFV-diphosphate (TFV-DP) concentrations in PBMC and CT homogenate were measured using ultra-performance liquid chromatographic-tandem mass spectrometric (LC-MS/MS) methods that have been previously described[63,64]. These methods were validated according to FDA Bioanalytical Method Validation Guidance[65]. The lower limits of quantification (LLOQs) for these assays are: plasma TFV 0.31 ng/mL, PBMC TFV-DP 50 fmol/sample or median 2 fmol/10^6 cells (based on number of cells analyzed), CVF TFV 0.625 ng/swab or median 0.005 ng/mg (based on swab weights), RF TFV

0.625 ng/swab or median 0.2 ng/mg (based on swab weights), CT TFV 0.05 ng/sample or median 0.003 ng/mg (based on biopsy weights), CT TFV-DP 50 fmol/sample or median 3 fmol/mg (based on biopsy weights).

3.3.4 Pharmacodynamic ex vivo HIV explant challenge

As previously described, two CT biopsies were briefly exposed to HIV-1 BaL in the laboratory, and HIV infection was measured over the culture period by HIV-1 p24 ELISA assay (Alliance, Perkin Elmer) of culture supernatant (4, 7, 10, and 14 days after inoculation)[66,67]. The cumulative p24 antigen (sum of p24 antigen concentrations in explant culture supernatant), biopsy weight-normalized and averaged for both biopsies at each time point was the unit of analysis.

3.3.5 Data Analysis

Concentration-time data and PK-PD relationships were visually examined (SigmaPlot, version 13, Systat Software, San Jose, CA). Non-compartmental analysis of concentration data estimated PK parameters including peak concentration (C_{max}), time to peak concentration (T_{max}), area under the concentration-time curve to last sample (AUC_{last}), and time to last concentration (T_{last}) (Phoenix® WinNonlin® version 6.4, Certara USA, Inc., Princeton, NJ). Readouts were summarized using non-parametric descriptive statistics (median, interquartile range), the Friedman test for comparisons among readouts, the Wilcoxon rank sum test for paired comparisons, and the Spearman test for correlations between matrix concentrations (IBM SPSS Statistics v. 24, Armonk, NY). P values less than 0.05 were considered statistically significant. A PK-PD relationship was first assessed with linear regression of log transformed TFV and TFV-DP concentrations in plasma, CT and CVF (each individually as single independent variables)

with log-transformed cumulative p24 concentrations (dependent variable) (IBM SPSS). Iterative model fitting using an E_{max} model (2 to 4 parameter Hill equation) with log-transformed PK and PD was performed to assess PK-PD relationships (Phoenix WinNonlin).

3.4 Results

3.4.1 Subjects

Ten women enrolled in the study, ranging in age from 22 to 45 years (median 33.5). Five women self-identified as African American, four as white (one as Hispanic/Latina), and one as Asian. All participants completed all study visits and evaluations.

3.4.2 Adverse events

Of 22 adverse events captured during the study, none were serious nor related to study product (Table 3.1). All resolved by the end of follow up. Fourteen adverse events occurred after gel dosing, while 7 occurred after film dosing (p=0.20); one participant experienced an adverse event in the washout period between film and gel dosing.

3.4.3 Pharmacokinetics

Following dosing of each product, plasma TFV concentrations reached a peak between 1 and 12 hours, then fell to undetectable concentrations (<0.31 ng/mL) by 48 hours in all but one participant (film 0.39 ng/mL and gel 0.51 ng/mL) (Figure 3.1). The time to peak concentration (T_{max}) was later after film dosing, median (IQR) 8 hours (8, 8), when compared to gel dosing, 4 (2, 8) (p=0.02). Peak concentrations (C_{max}) of TFV were higher in 9 of 10 participants after film dosing, 10.4 ng/mL (3.9, 13.6), when compared to gel dosing, 2.9 ng/mL (1.5, 5.5) which

trended toward statistical significance (Table 3.2, p=0.08). Plasma TFV concentration vs. time curves were similar before 8 hours, but separated (statistically significant) between 8 and 24 hours after dosing, during which time concentrations following film dosing continued to rise and were greater by 3.1- to 4.4-fold when compared to individually-paired gel dosing ($p<0.02$). Overall, plasma TFV non-compartmental C_{max} and AUC_{last} were higher after film than gel dosing by 2.5-fold (1.8, 3.5) and 3.0-fold (2.6, 3.9), respectively (Table 3.2). Finally, the time until the last quantifiable concentration (T_{last}) was later after film dosing, 24 hours (24, 24), when compared to gel dosing, 18 hours (12, 24), trending toward statistical significance (p=0.06). PBMC TFV-DP concentrations were below limits of assay quantification in all samples tested.

CVF TFV concentrations sampled from all 3 intra-vaginal sites declined from 5 hours to 168 hours after dosing, but remained detectable throughout the sampling interval (Figure 3.2). Only 1 of 10 participants had any CVF TFV concentration that was below the limits of assay quantitation (BLQ) 168 hours after dosing (exocervix, gel dosing). Mid-vagina CVF TFV C_{max} and AUC (both p=0.014), as well as concentrations at all 3 sample times, were significantly higher after film compared to gel dosing ($p\leq0.02$) (Table 3.2). The differences ranged from 3.1-fold (2.0, 3.8) higher at 168 hours to 4.4-fold (2.9, 7.7) higher at 24 hours. Forniceal CVF TFV trended toward higher concentrations after film than gel at 5 hours (p=0.08), but none of the exocervical CVF TFV concentrations differed between study products. When comparing among CVF sampling sites at any given time for the same product, forniceal samples were higher than exocervical samples, with a C_{max} and AUC_{last} forniceal:exocervical ratio of 2.0 (1.2, 6.8) and 2.0 (1.2, 6.8), respectively (both $p\leq0.01$). Variability of samples (indicated by the range of quartiles)

was also greater for film compared to gel. CVF concentrations between sites were highly correlated (rho>0.94, p<0.001)

TFV and TFV-DP concentrations in CT homogenates were also higher 5 hours after film dosing, 28 ng/mg (7, 52) and 160 fmol/mg (27, 485), respectively, when compared to gel dosing, 8.7 ng/mg (5.9, 14.0) and 40 fmol/mg (21, 93), respectively (both p<0.05). This resulted in paired film:gel ratios of 3.0 (1.1, 7.3) for TFV and 3.7 (2.0 17.8) for TFV-DP ($p \leq 0.04$). Concentrations were not different 72 hours after dosing.

Using molar concentrations of TFV and TFV-DP, the combined (film and gel) TFV-to-TFV-DP ratio in CT was 532 (332, 999) and not different between products. At 5 hours after dosing when all matrices were available and detectable, combined (film and gel) CVF tenofovir concentrations were 2 $\log_{10}$ and 5 $\log_{10}$ greater than tissue and plasma concentrations, respectively (p<0.001). CT TFV-DP correlated modestly with plasma TFV concentrations (rho=0.522, p<0.001) and correlated highly with both CVF ($rho \geq 0.818$, p<0.001) and CT (rho=0.92, p<0.001) concentrations.

Unlike TFV concentration and time differences (film greater than gel) at all other anatomic sites, RF TFV concentrations 5 hours after gel dosing were 12-fold (3, 39) greater than after film dosing (p=0.004). The RF concentration distribution 5 hours after gel, 2.5 ng/mg (0.5, 26), overlapped the 5 hour CT homogenate TFV concentration for both film, 31 ng/mg (11, 63), and gel, 9 ng/mg (6, 17). Time to peak RF concentrations were more common at 5 hours, but higher concentrations were seen 72 hours post-dose in 4 participants after film and one participant after

gel. RF concentrations correlated least well of all matrices with all other matrices (rho <0.44, p>0.01).

3.4.4 Pharmacodynamics (HIV-1 p24 measurement)

Cumulative p24 antigen in the CT ex vivo HIV challenge assay was not different among values for baseline and both times (5 and 72 hours) for both film and gel formulations (p=0.4) (Figure 3). Pairwise testing showed statistically significant differences only for the film formulation which increased between the 5 and 72 hour sampling times (p<0.01). Linear regression of cumulative P24 against drug concentrations (pooling concentrations for both film and gel dosing within each anatomic matrix) indicated no statistically significant PK-PD relationships (Figure 3.4). Excluding baselines, any imputed values, or both did not result in any statistically significant regression slopes. Similarly, sigmoid E_{max} PK-PD modelling was not successful.

3.5 Discussion

We demonstrated the feasibility of a single dose TFV fast-dissolving film (40 mg) to achieve concentrations of TFV in plasma, CVF, and activated TFV-DP in CT which exceed those after a single dose of TFV 1% gel during the day of dosing; both film and gel had similar concentrations 3 and 7 days later. Supported by the acceptability of the film established in the companion study, FAME 04, and the theoretical adherence advantages of films over other products, we believe this study helps to advance TFV film as a potentially viable product for extended safety and efficacy testing as a topical microbicide.

In contrast to FAME 04, a 7 dose study of the same film and gel, we report greater concentrations in all matrices (at some time points) after film compared to gel dosing. Temporally richer FAME 05 sampling (2, 4, 5, 8, and 12 hours) could detect the later peaking and higher 8-24 hour plasma TFV differences with film, which indirectly indicate differences in CVF and tissue TFV. Complementarily, FAME 04 was more analytically rich with readouts beyond PK and larger sample size. The other study difference was that the film was folded in half prior to dosing in FAME 04, whereas in FAME 05, the film was not folded. Folding can reduce the dissolution rate of the film.

In addition, our FAME 05 concentrations in all matrices were below those in FAME 04. For example, our CT TFV-DP was 40 fmol/mg (20, 93) and 169 fmol/mg (84, 506) 5 hours after gel and film dosing, respectively, whereas, FAME 04 reported 222 fmol/mg (71, 556) and 937 fmol/mg (56, 1456) 2 hours after dosing gel and film, respectively. Accumulation of drug with the 7 daily doses in FAME 04 compared to the single dose in FAME 05 explains this difference. Vaginal tissue concentrations rise even higher with longer daily dosing – 2,000 fmol/mg (4 fmol/0.2 □L) after 2 weeks reported by Schwartz, et al., and 1,807 fmol/mg (591, 5860) after 6 weeks in MTN-001, both of which studies dosed the same TFV 1% gel daily[68,69]. Together, these studies indicate tissue accumulation continues through 2 weeks after which steady-state is achieved. Continuing accumulation of tissue TFV-DP for weeks of dosing is expected based on every 24 hour dosing and a longer cervicovaginal tissue TFV-DP half-life, 53 hours (45, 68); accordingly, steady-state (6 half-lives) would require (IQR) 11 days to 17 days, consistent with the similarity in 2-week Schwartz, et al. and 6 week MTN-001 reports[68-70].

Five hours after a single TFV film dose, CT TFV-DP concentrations easily exceeded vaginal tissue TFV-DP concentrations associated with daily oral TDF dosing (estimated from combined MTN-001 and HPTN 066, 23 fmol/mg [17, 25]) which occurs weeks after commencing daily oral dosing[69,71]. As concentrations with the first dose are even higher with film compared to gel in FAME 05, this evidence recommends advancing the film formulation for further clinical development. Development as a single episodic dose prior to HIV exposure might be considered, but depends on several key unknowns, namely, whether cervicovaginal tissue concentration of active drug is the single critical variable associated with PrEP efficacy and how long the concentration needs to be maintained.

The evidence for the association between cervicovaginal tissue concentration and PrEP efficacy, however, remains indirect. In an analysis of seroconversion rates across the six primary daily dosing PrEP efficacy randomized clinical trials, using oral vs. vaginal tissue TFV-DP concentration-based differences provided a much tighter sigmoid E_{max} PK-PD model fit than systemic drug concentrations alone[8,9,11,31-33,72]. Possibly arguing against this, adherence adjustments in three TFV vaginal gel trials (CAPRISA 004, FACTS 001, VOICE) all demonstrated improved, but modest, efficacy in post hoc analyses[11,30,47,55,56], despite estimates of higher tissue TFV-DP concentrations compared to oral dosing (discussed above). However, these PK-guided adherence adjustments were dichotomous adjustments into adherent and poorly adherent cohorts and lacked quantitative adherence benchmarks (similar to STRAND and HPTN 066 for oral dosing) to fairly judge efficacy in highly adherent women[71,73]. Further, it remains unclear how long protective concentrations must be sustained after HIV exposure. While film achieves tissue TFV-DP concentrations in 5 hours that are 7-fold greater than estimated steady-

state tissue concentrations associated with 90% protection in Partners PrEP (see below), the tissue TFV-DP concentration falls to 40% of the clinical tissue IC_{90} by 72 hours after the single dose and may need additional doses for protection in the episodic dosing setting.

Due to the absence of clinical trial data to clearly indicate the TFV-DP concentration, anatomic site, and duration best predicting efficacy, viral challenge in animal models and ex vivo human tissue explants have been used as surrogates. Macaque models repeatedly demonstrate protection from SHIV and SIV vaginal challenge with prior TFV dosing[74-78]. We used the explant challenge in FAME 05, but failed to demonstrate an antiviral effect. By contrast, FAME 04 demonstrated an antiviral effect with CT TFV-DP IC_{90} of 813 fmol/mg. The difference in explant results may largely be explained by the fact that all, but one, of the single dose FAME 05 CT concentrations fell well below the explant IC_{90} in multiple dose FAME 04. Accordingly, there were too few high concentrations to generate a statistically significant downward slope.

As with selecting tissue concentration targets, interpreting explant challenge model IC_{90} results doesn't map directly to a clinical IC_{90}. Consider, in MTN-001, participants demonstrated high adherence to prescribed daily TDF (indicated by median pre-dose TFV serum concentration of 65 ng/mL), yet the vaginal tissue homogenate TFV-DP concentrations were below 25 fmol/mg[69]. With similar pre-dose serum TFV concentrations in one high adherence Partners PrEP cohort, >40 ng/mL serum TFV, the tissue TFV-DP concentrations are very likely similar[79]. This high adherence Partners PrEP subgroup had relative risk reduction of 89% for TDF only and 91% for TDF/FTC, approximating a clinical IC_{90}[79]. [This assumes no sex differences in efficacy that Partners PrEP wasn't powered to detect.] By comparison, the explant IC_{90} reported in FAME 04

is at least 1.5 $\log_{10}$ greater than the clinical IC_{90} from Partners estimated above. Similarly, the colon tissue explant IC_{90}, 10,233 fmol/mg (RMP-02/MTN-006), is 1.7 to 2.5 $\log_{10}$ greater than the *clinical* IC_{90} for colon tissue TFV-DP estimated in iPrEx[8,73,80,81]. [The iPrEx clinical IC_{90} is based on 90% efficacy associated with 2 to 4 doses per week based on the STRAND study; in HPTN 066, 2 to 4 doses per week achieved colon tissue homogenate TFV-DP concentrations from 27 to 186 fmol/mg[8,71,73].] Accordingly, both the cervicovaginal and colon explant challenge models appear too stringent and need recalibration to estimate the likelihood of clinical protection. In addition, as discussed above, whether the calibration for oral TDF (often complicated by concomitant dosing with emtricitabine) is relevant for topical TFV remains to be demonstrated and will require an efficacy trial.

Concluding, we demonstrated that a single dose of TFV 40 mg film achieved higher and more sustained concentrations in plasma, CVF, and CT compared to TFV 1% (40 mg) gel. Further, CT TFV-DP concentrations exceed tissue concentrations associated with high levels of protection with oral dosing, though the comparability of tissue protective concentrations and timing after oral compared to topical dosing has not been established. With its single dose advantages compared to TFV gel, and several theoretical advantages of films over gels which may favorably impact adherence, the film formulation remains a promising vaginal microbicide candidate. Longer duration safety and efficacy studies are needed to establish the anticipated adherence advantage and, possibly, superior efficacy of the TFV film formulation relative to gel.

3.6 Tables and Figures

Figure 3.1: Plasma TFV vs. time by product (median [IQR]). Nominal sampling times offset for clarity.

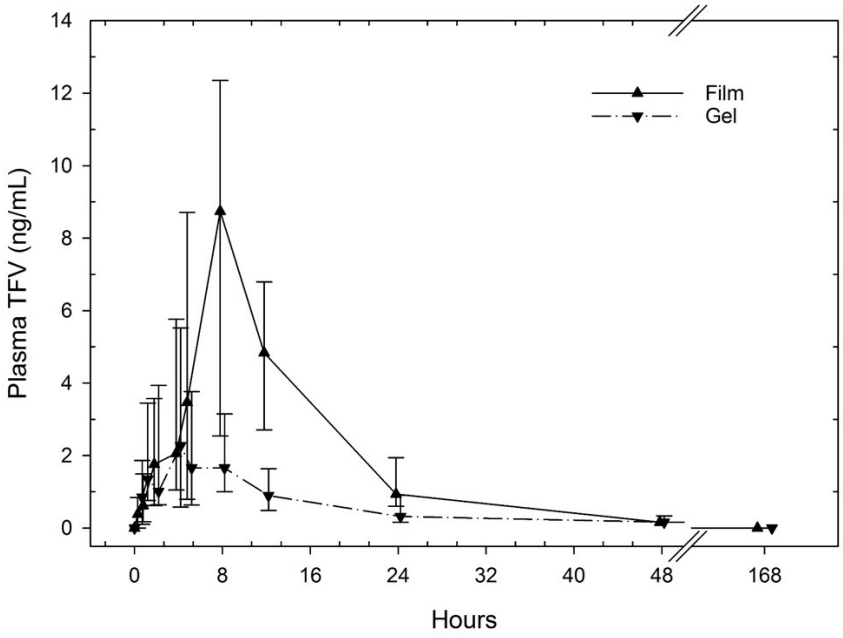


Figure 3.2: Cervicovaginal and rectal fluid, cervical tissue homogenate TFV concentrations vs. time by product (median [IQR]). Nominal sampling time offset for clarity.

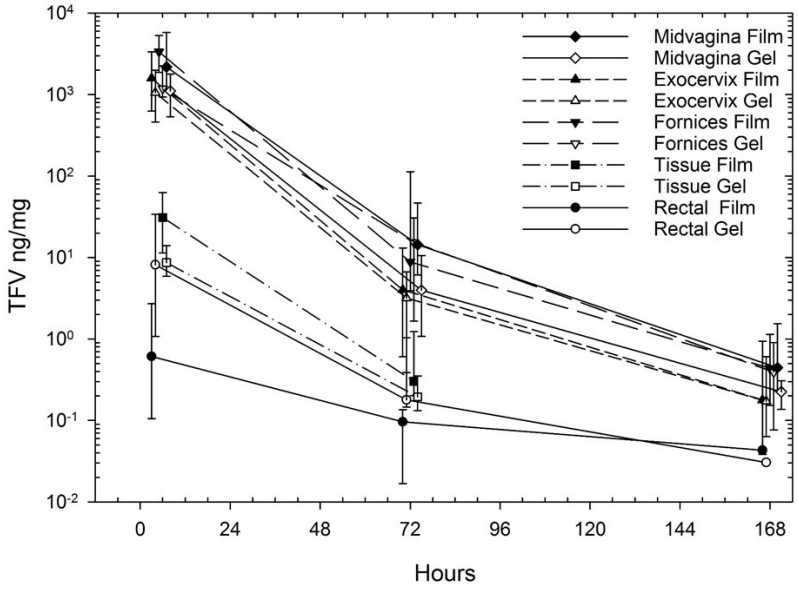
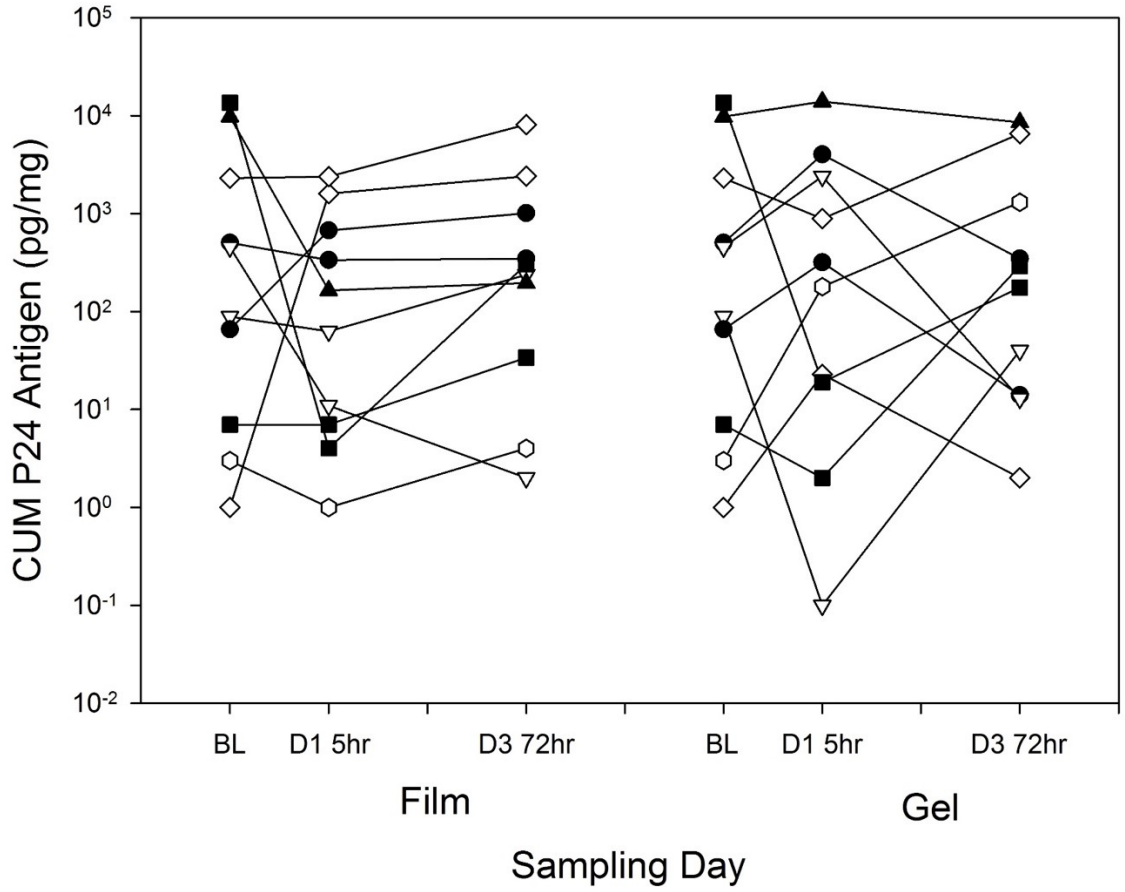


Figure 3.3: Explant *ex vivo* HIV challenge over time by product.



Control (no drug) biopsy is indicated as "BL" (baseline), for comparison, even though the biopsy may have followed the study product dosing by at least one month.

Figure 3.4: Cumulative P24 Antigen v. TFV Concentration across all matrices by product. Regression lines are not shown since no linear regression fitting demonstrated statistically significant slopes (none different from slope=0). Baseline values arbitrarily imputed as 0.01 units in each panel. Concentration values below the lower limit of assay quantitation (LLOQ) are imputed as LLOQ/2.

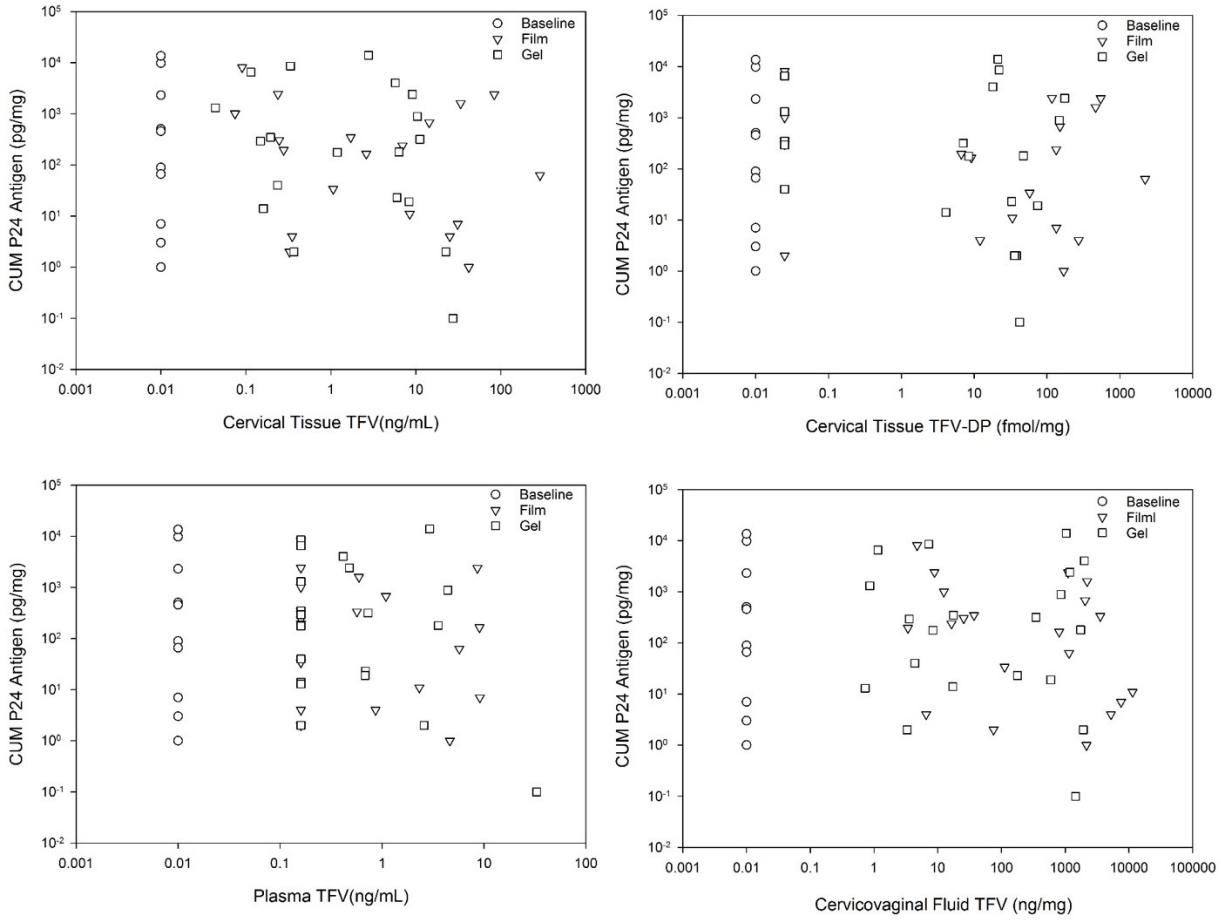


Table 3.1: Adverse events following TFV vaginal film vs gel

DAIDS Category	Film	Gel
Systemic	2, nasal congestion, fatigue	1, upper respiratory infection
Chemistry	0	4, hyperglycemia (non-fasting) (N=3), elevated AST
Hematology	3, anemia (2 Gr 2)	2, anemia
Urinalysis	0	1, hematuria
Gastrointestinal	1, constipation	3, nausea, heartburn, diarrhea (N=2)
Neurologic	1, headache	2, headache 2
Gynecological Pain	0	1, dysmenorrhea

Nine of 10 research participants reported total 22 adverse events, 21 during dosing period which are indicated. None were attributed to study product. No Grade 3 or 4 events were reported. All grade 1, except hematology (3 Grade 2 in parentheses). No differences in adverse event number when comparing Film to Gel (Wilcoxon, P=0.20). Initial number indicates number of participants experiencing the adverse event; if multiple symptoms are included in a category, the number of participants affected by a particular symptom is indicated (unless only one participant was affected).

Table 3.2: Pharmacokinetic parameter estimates by matrix, analytes, and product (median [IQR]).

Matrix-Analyte	C_{max}				AUC_{last}			
	Film	**Gel**	**Film/Gel Ratio**	**p**	**Film**	**Gel**	**Film/Gel Ratio**	**p**
Plasma TFV	10.4 (3.9, 13.6)	2.9 (1.5, 5.5)	2.5 (1.8, 3.5)	0.084	113 (56, 163)	32 (13, 56)	3 (2.6, 3.9)	0.084
CVF Exocervix TFV	1,588 (631, 3,342)	1,056 (461, 1,986)	1.3 (0.8, 2.8)	.	57,683 (23,631, 122,052)	38,181 (16,872, 71,792)	1.3 (0.9, 2.8)	.
CVF Fornix TFV	3,351 (1,872, 5,287)	1,186 (935, 2,250)	3.1 (1.4, 5.6)	0.084	122,474 (67,857, 202,087)	43,488 (36,109, 83,017)	3.1 (1.4, 5.2)	.
CVF Mid-vaginal TFV	2,186 (1,140, 5,813)	1,109 (532, 1,789)	2.9 (1.1, 9)	0.014	79,354 (42,185, 213,176)	40,259 (20,027, 64,548)	2.9 (1.1, 8.9)	0.014
CT TFV	28 (7, 52)	8.7 (5.9, 14.0)	2.2 (0.9, 7)	0.037	1,035 (263, 1,896)	285 (187, 510)	4 (1.2, 8.6)	0.014
CT TFV-DP	160 (27, 485)	40 (21, 93)	3.6 (0.4, 15.1)	0.049	6,637 (1,043, 19,936)	1,509 (596, 2,521)	3.8 (2.2, 10)	0.014
RF TFV	0.50 (0.09, 1.85)	2.65 (1.27, 30.20)	0.08 (0.03, 0.33)	0.004	-	-	-	-

BLQ, below lower limits of assay quantitation
Cmax units: TFV, plasma ng/mL, CVF ng/mg, RF ng/mg, CT ng/mg; TFV-DP fmol/mg
AUC units: TFV, plasma ng-hr/mL, CVF ng-hr/mg, RF ng-hr/mg, CT ng-hr/mg; TFV-DP fmol-hr/mg
P values greater than 0.1 are not shown.

CHAPTER 4: Conclusion

Determining how an investigational product delivers a drug to relevant target tissues, and how the movement of the drug through the body correlates with intended or unintended effects, are crucial initial steps in the process of drug development. The two studies presented in this thesis illustrate the importance of pairing pharmacokinetic and pharmacodynamic investigations in order to best inform the design of larger clinical efficacy trials.

4.1 Vaginal Microbicides for HIV Pre-Exposure Prophylaxis

FAME 02b and FAME 05 provided similar types of data regarding four candidate vaginal microbicides: dapivirine gel and film (FAME 02b) and tenofovir gel and film (FAME 05). FAME 02b demonstrated that dapivirine was safe and well tolerated when applied vaginally in both film and gel formulations. There were no statistically significant differences in dapivirine concentrations across anatomic sampling sites between the two formulations, although the film trended towards a higher dapivirine CVF concentration in the midvagina compared to either the vaginal fornix or cervical os. The half-life of dapivirine was significantly longer in plasma compared to cervical tissue. The cervical tissue explant assay demonstrated reduced HIV p24 production with both dapivirine gel and film at 5 hours after dosing, although there were no differences found between the two study products.

FAME 05 demonstrated a similar safety profile for tenofovir vaginal gel and film. Plasma tenofovir concentrations peaked sooner after product application than dapivirine and fell to undetectable levels by 48 hours after dosing in most participants. In contrast, dapivirine was detectable in half of study participants 168 hours after dosing. While the tenofovir film and gel

were equally well tolerated, they were found to have divergent pharmacokinetics. Tenofovir film led to higher plasma C_{max} and AUC_{last} compared to gel, a difference which was statistically significant between 8 and 24 hours after dosing. The film product also led to higher TFV and TFV-DP concentrations compared to gel in CVF and cervical tissue. Only rectal fluid TFV concentrations were higher following gel dosing compared to film, but correlation of these concentrations with the other tissue matrices was poor. There was no difference in HIV p24 production in the cervical tissue explant challenge between products, nor was there any difference between baseline biopsies and after either film or gel dosing.

Based on these two studies, dapivirine and tenofovir vaginal gel and film are promising products to help women prevent HIV infection. The pericoital dosing of films and gels may be more acceptable to some women than use of a daily PrEP product. Larger randomized clinical trials of both types of products should be pursued to demonstrate effectiveness.

4.2 Challenges

The development of PrEP for prevention of HIV infection has been fraught with setbacks. Early studies of oral PrEP regimens involving TFV alone or in combination with emtricitabine found a 44-75% decrease in HIV acquisition[8,82]. Subsequent studies yielded disappointing results with oral PrEP no more effective at reducing HIV acquisition than placebo[10,11]. A closer look at the data from these more recent studies showed a clear relationship between product adherence and efficacy. Among women who adhered most closely to the recommended dosing regimen in the VOICE trials, there was significant protection against HIV acquisition[11]. Unfortunately, only 25-30% of participants had detectable plasma levels of study drug consistent with product

adherence. Adherence based on detectable drug concentration was similarly low in FEM-PrEP (15-37%)[10], and somewhat better in FACTS 001 (64%)[13].

A similar pattern was seen in studies of early vaginal microbicides. CAPRISA 004 found a 39% decreased risk of HIV acquisition among women who used pericoital vaginal TFV gel[12]. The pericoital regimen involved administering a dose of TFV vaginal gel prior to and after penile-vaginal intercourse, with no more than 2 doses in a 24-hour period. Despite these early promising results, larger trials showed no benefit to vaginal TFV gel[11,13].

Subgroup analyses in large studies of oral PrEP and vaginal microbicides found reduced rates of HIV infection among participants whose plasma drug concentrations were consistent with greater product adherence. While it makes empiric sense that greater use of oral or vaginal PrEP would lead to improved protection against HIV, there remains the possibility of additional confounding factors. There was no analogous way to objectively assess adherence to placebo products – there was no ingredient that could be measured in blood or vaginal fluid to confirm whether women assigned to placebo products used them consistently. It is possible that women with highest levels of microbicide and PrEP adherence experience different risk of HIV infection compared to women with lower adherence. If the behavior of high product adherence is associated with less behavioral risk of HIV, this may partly account for the protection attributed to oral or vaginal PrEP. More consistent use of pericoital TFV gel in the FACTS 001 trial was associated with lower HIV incidence regardless of treatment group (HR 0.48, 95% CI 0.26-0.89), which suggests the behavior of greater adherence may itself be protective[13]. Without a way to objectively measure adherence in placebo users, this confounder cannot be reliably excluded.

In an attempt to understand why self-reported product adherence was so much higher than adherence based on objective measures (i.e. plasma drug concentrations) in the VOICE trial, a qualitative study was performed among a subset of participants[83]. Several reasons for over-reporting product use were elicited, and participants identified receiving real-time feedback and drug concentration monitoring as a way to improve adherence. The logistics of obtaining plasma or CVF drug concentration data in a relevant time for providing this real-time feedback are difficult, but this is one strategy that could strengthen future studies so that the true efficacy of PrEP products can be determined.

4.3 Future Directions

The development of products that a woman can use to reduce her risk of HIV infection without needing the permission of a male partner has been identified as an important part of improving HIV prevention. Daily oral PrEP has been shown to be effective when taken consistently, but poor adherence in several studies suggest that this one intervention will not work for all people. An analogy can be drawn with the different types of female contraception. Daily birth control pills are highly effective at preventing pregnancy when they are taken correctly and consistently, but many women have difficulty maintaining the high level of compliance needed for maximum efficacy[84]. This in part led to the development of multiple non-daily, non-oral contraceptive options including a monthly vaginal ring, 3 month injectable, and longer-acting implants and intrauterine devices that are effective for 3 to 10 years. There are also pericoital contraceptives such as a diaphragm or vaginal spermicides for women who prefer to use these products only when necessary. Meeting the demands of a large variety of women will require the availability of

multiple effective products, as one strategy will not meet the needs of all potential users. It is likely that each individual woman may have varying needs throughout her life, making a daily oral preventive strategy preferable sometimes and an as-needed vaginal preventive product preferable at other times. Having multiple HIV prevention products will allow women to switch between products, or even combine them for greater protection. As vaginal film and gel products are further developed, it will be important to investigate their compatibility with male and female condoms, and to consider whether the protection provided by vaginal microbicides is equivalent to the protection provided by oral PrEP. If the level of protection is similar across products, women could choose to use oral PrEP sometimes and vaginal microbicides at other times, depending on their individual circumstances.

Vaginal microbicides will likely only protect women from exposure that occurs via penile-vaginal intercourse. As demonstrated in this thesis, systemic drug concentrations are achievable following vaginal microbicide dosing, but the systemic drug concentrations are not likely to provide protection from HIV infection in distant sites. For women who have sex with men, the primary route of HIV transmission is through receptive vaginal intercourse, with the vaginal mucosa being the most likely site of HIV infection. Measuring drug concentrations in vaginal mucosa is logistically more complicated than measuring drug concentrations in other, more accessible compartments (i.e. blood, cervicovaginal fluid, cervicovaginal lavage). It is likely that vaginal tissue drug concentrations will be more variable in larger studies when the relationship between product dosing and tissue sample collection is less well defined. Variations in adherence will also increase the variability in tissue drug concentrations. Small studies with more intensive pharmacokinetic sampling strategies, such as FAME 02b and FAME 05, have the potential to

describe relationships among drug concentrations across these varied compartments and to clarify what drug concentration in vaginal tissue is protective.

One question that remains to be answered is whether the use of a vaginally applied microbicide will protect against HIV exposure via heterosexual RAI. FAME 05 demonstrated that tenofovir is detectable in rectal fluid after application of both film and gel products, but it is unclear what level of protection is achieved in the rectum. The prevalence of RAI among heterosexual women is lower than vaginal intercourse, but the risk of HIV exposure is greater[85]. There are currently several studies investigating rectal microbicides to protect against HIV transmission that occurs during RAI. A full description of these products is beyond the scope of this document.

4.3 Conclusion

Stopping the spread of HIV will require multiple types of interventions, including increasing access to antiretroviral treatment, minimizing maternal to child transmission, and preventing new infections from occurring. Vaginal microbicides such as the gels and films described in this thesis offer a non-daily prevention strategy that may be more acceptable to some women than daily oral PrEP. Additional clinical trials of vaginal films and gels are needed to determine product efficacy on a larger scale. Randomized clinical trials of similar vaginal PrEP products to date have been plagued by poor adherence despite rigorous participant education and support during the studies[10,11,13]. Future studies would be enhanced by including objective measures of adherence and real-time feedback to study participants. Having multiple ways to prevent HIV infection is likely to increase use overall and will hopefully achieve population level decrease in HIV transmission. One method will not be perfect for each individual at every moment of their

lives – having options that can meet different needs for the same individual or couple is important to providing comprehensive HIV preventive care.

APPENDIX A: FAME 02b Study Documents

- Study Protocol
- Informed Consent Form

FAME 02b Study Protocol

A.1 Objectives

A.1.1 Primary Objective:

To compare the levels of dapivirine in the cervicovaginal fluid, genital tissue, and blood samples using single dose gel and film formulations of dapivirine microbicide.

A.1.2. Secondary Objectives:

- To compare the effect of single dose dapivirine gel or film formulation on cervical tissue explant challenge with HIV.
- To compare the safety of single dose dapivirine gel and film formulations

A.2 Study Design

A.2.1 Identification of Study design

This is a single site, randomized open-label crossover study. Five subjects will receive the treatment sequence of gel followed by film. Five other subjects will receive the treatment sequence of film followed by gel.

A.2.2 Description of Study Population

The study population will be HIV uninfected women who meet criteria as outlined in section 5.

A.2.3 Time to Complete Accrual

This project will take 3 months to accrue and 6 months to complete.

A.2.4 Study Groups

Subjects will receive dapivirine gel verses dapivirine film in a crossover design in a randomized sequence.

A.2.5 Site

The study will be carried out at the Johns Hopkins Drug Development Unit.

A.3 Study Population

A.3.1 Selection of Study Population

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

A.3.2 Recruitment

Participants will be recruited from a variety of sources. Participants will also be referred to the study from other local research projects. Recruitment materials will be approved by the Johns Hopkins Institutional Review Board

A.3.3 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 completing participation 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.

A.3.4 Inclusion criteria:

1. 18 years of age or older with a history of receptive vaginal intercourse.

2. HIV negative by EIA within 28 days of enrollment (Appendix 1). Note: Only EIA non-reactive subjects will be enrolled. Individuals testing positive by EIA will be excluded, but confirmatory testing and referral for follow-up will be determined by the testing algorithm conducted by the Johns Hopkins Hospital Clinical Laboratory.
3. Understand and agree to local STI reporting requirements.
4. Able and willing to provide written informed consent to take part in the study.
5. Able and willing to provide adequate information for locator purposes.
6. Availability to return for all study visits, barring unforeseen circumstances.
7. Availability to return for the second formulation dosing at the same time in the subject's menstrual cycle as when the first formulation was administered, at least 10 days before menses.
8. Willing to abstain from vaginal intercourse and insertion of anything (e.g., drug, vaginal douche, or sex toy) in vagina for 72 hours before each study product exposure, and 7 days following each vaginal sampling procedure.
9. Willingness to have partner(s) use condoms (must not contain Nonoxynol-9) for the duration of the study.
10. Agree not to participate in other research studies involving drugs and/or medical devices.
11. Negative qualitative urine pregnancy test.
12. Per participant report, using an effective method of contraception at enrollment; hormonal method (except vaginal ring) used continuously for the past 30 days; intrauterine device (IUD [copper or hormonal] inserted at least 30 days prior to enrollment); female sterilization; abstinent from sexual activity with male partner for the past 30 days; or sexual activity with vasectomized partner; and willingness to use

effective method of contraception until the completion of final scheduled study visit if enrolled.

13. Willingness to remain in the research unit for up to 24 hours on each of two dosing days,

A.3.5 Exclusion criteria:

1. Current sexual partner known by participant to be HIV seropositive.
2. Individuals who, by history, engage in condom-less intercourse with HIV-infected partners, or partners that have unknown HIV serostatus, or women who exchange sex for money, shelter, or gifts.
3. Active sexually transmitted infection or documented treatment of sexually transmitted infections including, but not limited to: chlamydia, gonorrhea, syphilis, trichomonas, cervicitis or PID within 6 months prior to enrollment.
4. Known history of genital HSV (diagnosed by either clinical or laboratory test).
5. Symptomatic vaginal candidiasis or bacterial vaginosis.
6. Undiagnosed irregular uterine bleeding
7. Pathology of the female genital tract, which in the judgment of the investigator might increase the risk of the study to the research participant.
8. Individuals who are status post hysterectomy.
9. History of any cervicovaginal procedure (i.e. colposcopy with cervical biopsy) within the past 2 months. Individuals who have a history of cone biopsy or extensive loop electrosurgical excision procedure (LEEP), which in the judgment of the investigator may affect permeability assessment.
10. Any known primary or secondary uro-genital malformations, which in the assessment of the investigator may interfere with the intended urine collection for PK studies.

11. Use of vaginally administered medications within 4 week of enrollment
12. Any active urinary tract infection
13. By history, subjects with irregular menstrual cycles.
14. At screening:
    a. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) greater than 1.5 X the site laboratory ULN (upper limit of normal)
    b. Hemoglobin less than 10.0 g/dL
    c. Platelet count less than 100,000/mm3
    d. Other safety tests outside of the normal range that in the judgment of the investigator may interfere with conduct of the study.
    e. Positive findings on urinalysis that are clinically significant in the opinion of the investigator
15. Estimated creatinine clearance < 60 ml/min based on established nomograms
16. Recent history (past 6 months) of injection drug use or, a level of alcohol use that, in the judgement of the Investigator of Record, may interfere with the conduct of this study.
17. Unwillingness to refrain from aspirin and NSAIDs product use for one week prior to and one week post study procedures.
18. Use of warfarin or heparin.
19. Use of systemic immunomodulatory medications within 4 weeks of enrollment.
20. Use of product containing nonoxynol-9 within 4 weeks of enrollment.
21. Use of any investigational products within 4 weeks of enrollment.
22. Any other medical conditions deemed not safe for participation by the investigator.
23. Any individual that is actively breast feeding.

24. Post-menopausal defined as 12 months of amenorrhea.

A.4 Study Product

A.4.1 Regimen:

Subjects will be randomized to receive one of the two following sequences:

- Single dose of Dapivirine gel 4759 (0.05% w/w dapivirine, in 2.5 grams of gel—total dapivirine content, 1.25 mg per dose), followed by single dose Dapivirine film (1.25 mg/film)
- Single dose of Dapivirine film (1.25mg/film) followed by single dose Dapivirine gel 4759 (0.05% dapivirine, 2.5 g/ 3.4 mL volume)

A.4.2 Administration

A study clinician will administer the product with the aid of a speculum for consistency of placement in the region between the mid vagina and anterior fornix.

A.4.3 Study Product Formulation

Dapivirine Film: Dapivirine film was formulated in a polyvinyl alcohol (PVA) based vaginal film containing hydroxypropyl methyl cellulose (HPMC) 4000 cp, polyethylene glycol 8000 (PEG), propylene glycol, and glycerin. PVA constituted 38.3% (w/w) of the film. The target loading dose for the film is 1.25 mg dapivirine per film.

Dapivirine Gel 4759: Dapivirine gel 4759 (0.05%) is formulated as a hydrophilic semi-solid (gel) for vaginal administration. The excipients in the drug product formula are pharmacopoeia grade components that have a history of use in currently approved vaginal products. Each pre-filled applicator will contain approximately 2.5 g of dapivirine 0.05% gel. Dapivirine gel 4759 should be stored at 15°C to 30°C (59°F to 86°F).

A.4.4 Study Product Supply and Accountability

IPM is the IND holder for dapivirine and will ensure that packaged Dapivirine gel 4759 study product is provided to the Johns Hopkins Investigational Drug Service (IDS) pharmacy. The University of Pittsburgh Magee Women's Research Institute (MWRI) will provide the dapivirine film product to the IDS. All study products will be available to the study staff through the IDS Pharmacy.

The IDS staff will maintain complete records of all study products received for this protocol and dispensed to participants. These records will not be available to other members of the research staff. Additional documentation will be required for study gel 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 after the study is completed or terminated.

A.4.5 Study Product Dispensing

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

A.4.6 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.

Table A.1. Schedule of Events

Event	Screen	Formulation Dose #1					F/U #1	Formulation Dose #2					F/U #2
Visit	1	2	3	4	5	6	7	8	9	10	11	12	13
Post-Dose hours		0-12	24	48	72	168		0-12	24	48	72	168	
Informed Consent	X												
History & Physical	X												
Safety labs	X			X		X	X			X		X	X
STI screening1	X												
Qualitative HCG	X	X4					X	X4					X
Urinalysis	X			▲		▲	X			▲		▲	X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X
AE Assessment	X	X	X	X	X	X	X	X	X	X	X	X	X
Symptom Directed Exam		X	X	X	X	X	X	X	X	X	X	X	X
Dose Product #1		X											
Dose Product #2								X					
PK Plasma 2		X	X	X	X	X		X	X	X	X	X	
Rectal Fluid3		X			X	X		X			X	X	
Cervicovaginal fluid3		X			X	X		X			X	X	
3													
Cervical Biopsy3		X			X			X			X		
Subject Instructions		X	X	X	X	X		X	X	X	X	X	
HIV-1/2 ELISA	X						X						X

▲ = If clinically indicated

(1) STI screening will consist of NAAT testing for gonorrhea, chlamydia, trichomonas, as well as serologic screening for syphilis using syphilis treponemal testing.

(2) Blood PK plasma collection will be obtained at pre-dose, 0.5, 1, 2, 4, 5, 8 and 12 hours (Day 0), 24 hours (Day 1); 48 hours (Day 2); 72 hours (Day 3); and 168 hours (Day 7) following dapivirine formulation dosing. The time points for specimen collections are approximate. Windows will be +/- 5 minutes for 0-12h hours, +/- 1 hour for the period 24-72 hours, +/- one day for 168 hours.

(3) Rectal Fluid collection, cervicovaginal fluid sampling, and cervicovaginal biopsy will be performed 5 hours (+/- 1 hour) and 72 hours following dapivirine formulation dosing. Rectal and Cervicovaginal fluid will also be collected at 168 hours post-dosing.

(4) Qualitative urine pregnancy tests will be performed within 24 hours prior to dosing

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. Vaginal douching will be prohibited for the ten days following dosing with a dapivirine formulation. Condoms provided by study staff will not be coated with any type of spermicide.

A.5 Study Procedures

This section describes visit-specific study procedures.

A.5.1 Visit 1: Screening Visit (within 30 days prior to Formulation #1 Dosing)

All subjects will undergo screening procedures prior to enrollment in the protocol. Screening procedures may occur over several visits. Informed consent will be obtained at screening, prior to the procedures listed in Table 7 above. This will be done by an interactive question and answer process where the potential subject will be asked specific questions to determine her understanding of the purpose, procedures, risks and benefits in participating in the trial. If in the judgment of the investigator the subject cannot demonstrate sufficient understanding, then that subject will be excluded from participation in the protocol.

Screening procedures will include:

- Complete history and general physical examination to determine eligibility for the study.
- Complete GYN examination including collection of swabs
- One cervical swab: to test for gonorrhea, chlamydia, and trichomoniasis.
- Phlebotomy to obtain blood for pregnancy test, complete metabolic panel and complete blood count, HIV antibody testing.
- Urinalysis to rule out urinary tract infection.
- Vital signs, weight, and height will be obtained.

- Assessment for adverse events that occur over the screening period.
- Subjects will be advised to abstain from vaginal intercourse, vaginal products, vaginal douche or vaginal insertion of sex toys at least 72 hours prior to dosing of formulation # 1 and formulation #2, and 7 days following each biopsy (with the exception of study dosings and procedures)
- Pregnancy testing will be repeated prior to administration of formulation #1.

Subjects who meet the inclusion and exclusion criteria following the Screening Visit will be enrolled and randomized to a formulation sequence (gel-film or film-gel). As part of scheduling, care will be taken to attempt scheduling the visit at a time when the participant is not expecting to be actively bleeding or anticipating her menses within 10 days.

Subjects with active vaginal bleeding at the dosing visit will not be randomized/enrolled at that time. Subjects found to have vaginal bleeding on pelvic exam may be rescheduled if vaginal bleeding resolves before 30 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.

A.5.2 Visits 2 & 8: Formulation Dosing Visits

The dose formulation will be vaginally administered by a study clinician.

Study procedures and follow-up will then proceed as outlined in the Schedule of Events.:

Dosing Visit Hour-by-Hour (same for gel and film)

Day 0 (within 30 days of all screening evaluations)

- Subject arrives at the research unit
- Urine pregnancy test performed, required negative to continue with dose.
- Vital signs will be obtained.

- Symptom-directed exam will be performed.
- Assessment for adverse events as noted from the history, and physical examination and as defined in section 9.3.

-1 hour

- Insert saline lock for PK's
- Pre-dose blood collection for drug concentration assessment.

0 hour

- Dose dapivirine formulation.
- Begin blood collections for drug concentration
    - Plasma at following times: 0, 0.5, 1, 2, 4, 5, 8, 12 hours after dosing. The saline lock will be removed following the 12 hour pharmacokinetic timepoint.

5 hours (+/- 1 hour)

- Cervicovaginal samples (fluid sampling with an absorptive device from mid-vagina, fornices, exocervix,) and cervical biopsies (three biopsies for homogenate drug concentration and explant challenge) are being collected.
- Rectal fluid collection

During each study visit, participants will be queried for adverse events and 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.

<u>24 hours (Visits 3 and 9)</u>

- Blood collection for dapivirine plasma.
- Vital signs will be obtained.
- Adverse event assessment

- Symptom-directed physical exam, if needed.
- Subject instructions including counseling to avoid the use of spermicide and other non-study vaginal products (other than tampons during menstruation and female condoms) while participating on the study. Subjects will be counseled to abstain from sexual intercourse and all other insertive vaginal practices for 10 days following each administered dose (or 7 days after the last cervicovaginal sampling at 72 hours).

48 hours (Visits 4 and 10)

- Blood collection for Complete Metabolic Panel, Complete Blood Count, urinalysis (if clinically indicated) and dapivirine plasma concentrations.
- Vital signs will be obtained.
- Assessment for adverse events.
- Symptom-directed physical exam, if needed.
- Subject instructions including counseling to avoid the use of spermicide and other non-study vaginal products (other than tampons during menstruation and female condoms) while participating on the study. Subjects will be counseled to abstain from sexual intercourse and all other insertive vaginal practices for 10 days following each administered dose (or 7 days after the last cervicovaginal sampling at 72 hours).

72 hours (Visits 5 and 11)

- Blood collection for dapivirine plasma concentrations.
- Vital signs will be obtained.
- Assessment for adverse events.
- Symptom-directed physical exam, if needed.

- Cervicovaginal samples (vaginal fluid sampling from mid-vagina, fornices, exocervix,) and cervical biopsy (three biopsies for tissue drug concentrations and explant challenge) are being collected.
- Rectal fluid collection
- Subject instructions including counseling to avoid the use of spermicide and other non-study vaginal products (other than tampons during menstruation and female condoms) while participating on the study. Subjects will be counseled to abstain from sexual intercourse and all other insertive vaginal practices for 10 days following each administered dose (or 7 days after the last cervicovaginal sampling at 72 hours).

<u>168 hours (Visits 6 and 12)</u>

- Blood collection for dapivirine plasma.
- Vital signs will be obtained.
- Assessment for adverse events.
- Symptom-directed physical exam, if needed.
- Cervicovaginal fluid (vaginal fluid sampling from mid-vagina, fornices, exocervix,), 10.
- Rectal fluid collection.
- Safety labs, including a complete metabolic panel and complete blood count and a urinalysis (if clinically indicated).
- Subject instructions including counseling to avoid the use of spermicide and other non-study vaginal products (other than tampons during menstruation and female condoms) while participating on the study. Subjects will be counseled to abstain from sexual intercourse and all other insertive vaginal practices for 10 days following each administered dose (or 7 days after the last cervicovaginal sampling at 72 hours).

A.5.3 Visits 7 & 13. Two Week Post-Dose Follow-up Visit

Subjects will return to the research clinic at Day 14 (Visit 7) to assess their state of health post-study. This evaluation will include a symptom-directed examination and vital signs will be obtained. Safety labs, including a complete metabolic panel, complete blood count, urinalysis, HIV antibody testing, and a qualitative urine pregnancy test will be performed.

Subjects are released to home with a scheduled Dosing Visit (Visit #8) appointment planned to coincide within one week of the time in the menstrual cycle associated with the first dosing visit. The first Follow-up Visit (visit 7) should follow the first Dosing Visit at 14 days (+ 2 days). The final Follow-up Visit (Visit 13) will occur 14 days (+2 days) after Dosing Visit #2 (Visit 8). The events for the second Follow-up visit (Visit #13) are the same as for the first Follow-up Visit (Visit 7).

A.5.4 Clinical Evaluations and Procedures

Physical exams will include the following assessments:

Vital signs:

- Blood pressure
- Pulse
- Temperature

Measurements of:

- Weight (at Screening only)
- Height (at Screening only)

Clinical assessments of:

- Complete history and physical examination a screening
- Adverse events

- Symptom-directed examination including the following systems:
- Genitourinary
- Gastrointestinal
- Systemic
- Menstrual

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.

A.5.5 Laboratory evaluation

- Urine pregnancy test
- Urinalysis, if clinically indicated
- GC/CT/Trichomonas Nucleic Acid Amplification Test
- Syphilis Chemiluminescence Assay
- HIV antibody testing (ELISA)
- Complete metabolic panel
    - Sodium
    - Potassium
    - Chloride
    - Carbon Dioxide
    - Glucose
    - Urea Nitrogen (BUN)
    - Creatinine
    - Calcium
    - Total Protein

    - Albumin
    - Total Bilirubin
    - Alkaline Phosphatase
    - AST
    - ALT
- Complete Blood Count
    - WBC
    - RBC
    - Hemoglobin
    - Hematocrit
    - Indices
    - RDW
    - Platelets
    - MPV
- Dapivirine drug levels in cervical tissue, cervicovaginal fluid, rectal fluid, and plasma

A.5.6 Specimen Collection and Processing

Since samples from up to 6 compartments will be collected, standardized protocols will be established for sample collection, isolation of mucosal mononuclear cells from tissue (above), cell separation and counting.

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 safety laboratory results are not available due to administrative or laboratory error, sites are permitted to re-draw specimens.

A.5.7 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 to the Hopkins University 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

A.6 Assessment of Safety and Clinical Management

A.6.1 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, the IPM 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, IPM Medical Officer, and the DAIDS Medical Officers

A.6.2 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 Johns Hopkins HIV Microbicide 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 External Safety Monitor, the DAIDS Medical Officers and IPM Medical Officer on a monthly basis. These data summaries will include adverse event, type, grade and association with the study products, accrual and retention data. The External Safety Monitor will evaluate adverse event data independently as well to determine whether or not the study protocol should continue as originally designed, should be changed, or should be terminated.

Approximately once a month the PSRT will convene via telephone to review adverse event data. If more urgent safety matters arise, these calls can occur more frequently.

The IRB will be notified of any serious and unexpected adverse events according to the policies of the Johns Hopkins Medicine IRB.

The following information will be submitted to the 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.

A.6.3 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. The term "investigational product" for this study refers to both the gel and the film, as well as the study gel applicator.

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 are instructed to seek immediate emergency care. Where feasible and medically appropriate, participants will be encouraged to seek evaluation at Johns Hopkins Hospital, where the study clinicians are based, and to request that a study clinician be contacted upon their arrival. With appropriate permission of the participant, whenever possible, records from all non-

study medical providers related to untoward medical occurrences will be obtained for review. All participants reporting an untoward medical occurrence will be followed clinically 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.

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 1.0, December 2004 and the Female Genital Grading Table for Use in Microbicide Studies (Appendix 1 to the DAIDS Table for Grading Adult and Pediatric Adverse Events, Version 1.0, December 2004), 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 1.0, December 2004. 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 AEs reported on CRFs will be assessed based on the Manual for Expedited Reporting of Adverse Events to DAIDS, the Investigators Brochures, and the clinical judgment of the PI/designee. The study products that must be considered when AE relationships are assigned are dapivirine gel, dapivirine applicator, and dapivirine 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 Compliance Center (RCC) 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 Officers. At a minimum, the AE must be re-assessed by study staff at least 2 weeks after the participant's study exit visit; 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. 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.

A.6.4 Expedited Adverse Event Reporting Requirements

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 RSC website at http://rsc.tech-res.com/safetyandpharmacovigilance/. 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.

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 IPM and 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 by IPM and DAIDS Medical Officers. The RSC Safety Office will then prepare the final report which will go to IPM for signature and submission to the FDA. Copies of this final report will be filed with IPM and RSC. Additionally, the RSC Safety Office will distribute safety reports to all DAIDS sites that use products under investigation in this study.

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 agents that must be considered in determining relationships of AEs requiring expedited reporting to DAIDS are: dapivirine gel, dapivirine film, and the study gel applicator.

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).

Participants Who Become Pregnant

Urine pregnancy tests will be performed during the investigational protocol. Participants who become pregnant in follow-up will be referred for obstetric care. The participant will be permanently discontinued and the following procedures will be performed at discontinuation:

complete metabolic panel and complete blood count. Pelvic exams will only be performed to evaluate a participant's reported symptom. No genital specimens will be collected in a pregnant participant. Staff will continue follow-up contact with the participant to obtain pregnancy outcome data

Of note, the participant will be encouraged to continue in the study so that safety data might be collected.

A.6.5 Clinical Management of Adverse Events

By definition, an adverse event can be either a 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 during the safety contact telephone call and in-clinic follow-up visits. Referral to appropriate care will be offered to participants as needed. Vaginally applied medications should not be used if possible.

A.6.5.1 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. Product will be permanently discontinued for this participant. If a participant experiences a grade 1 or 2 adverse event following product use, site investigators have the discretion to hold any subsequent product dosing until evaluation can take place in the clinic. The study product will continued to

be held at investigator discretion until the condition resolves and/or the investigator deems it safe to resume product use. If a participant experiences a grade 3 or 4 event following product dosing, the site will temporarily hold any subsequent study product dosings, notify the PSRT, and await its recommendation regarding further product dosing. Participants who discontinue study product will be encouraged to remain in the study for follow up evaluations for safety. With the exception of pregnancy and HIV seroconversion, as outlined in Section 8.4 above, since these are single dose administrations of study product formulations, the study team will attempt to complete all evaluations associated with that dosing formulation according to the study protocol, so long as there is no increased risk for the study participant.

A.6.6 Criteria for Early Termination of Study Participation

Participants may voluntarily withdraw from the study for any reason at any time. Participants will be withdrawn from the study if they test positive for HIV antibody, or become pregnant during the study. Any participant who tests positive for HIV while enrolled in the study will be immediately removed from the study and referred to the appropriate care provider for additional counseling and treatment as necessary. No additional doses of study drug will be given. Patients who become pregnant will not receive additional doses of study drug, and will be discontinued from the study, as described in Section 8.4. Pregnancy outcomes will be collected for all study participants by contacting these women soon after anticipated parturition. After the participant's final study contact, any pregnancy outcomes that meet criteria for SAE reporting as described above (e.g., congenital anomalies) occurring among participants will continue to be expeditiously reported.

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.

A.7 Statistical Considerations

A.7.1 Review of Study Design

The primary aim of this study is to directly compare data among women receiving active microbicide in the form of a water-based gel, the delivery method most widely used in microbicide research currently, and novel thin film formulations of the microbicide. Urogenital, systemic and menstrual symptoms will be collected via symptom review with participants. Testing for sexually transmitted infections will occur at the screening visit to rule out other potential causes of reproductive tract inflammation and epithelial disruption. Pharmacokinetic measures will assess local and systemic absorption of dapivirine following single exposure.

A.7.2 Sample Size and Accrual

Sample size of 10 will allow us to exclude differences as large as 1.2 standard deviation units relative to the mean when comparing film to gel results with 80% power and 5% 2-sided Type I error. In our experience with these intensive PK designs, this sample size has been more than adequate to the exploratory nature of these studies. Research participants without complete key data, for whatever reason, may be replaced. Key data includes plasma, cervical biopsies, and cervicovaginal fluid from 80% of planned collection times, each.

Research participants will be recruited from among prior research participants in similar types of studies. Accrual is expected to take 3 months to accrue and 6 months to complete.

A.7.3 Study Endpoints

Primary Endpoint

Pharmacokinetic (PK)

- Systemic absorption of dapivirine
    - Systemic absorption of dapivirine will be determined following exposure as detected in plasma sample as outlined the study SOP's.
- Local absorption of dapivirine
    - Local absorption of dapivirine will be determined following exposure as detected in genital tissue biopsy samples as outlined the study SOP's.
- Persistence of dapivirine in cervicovaginal and rectal fluid
    - Persistence of dapivirine will be determined as detected levels of dapivirine in cervicovaginal and rectal fluid collected as outlined in the study SOPs.

Secondary Endpoints

Pharmacodynamic (PD)

- Ex vivo explant challenge with HIV
    - Cervical tissue will be challenged ex vivo with HIV and supernatant aliquots will be sampled over 14 days to determine p24 antigen concentration.

Safety

- Adverse clinical and laboratory events will be determined.

A.7.4 Blinding

Complete concealment of allocation will not be feasible in this study. Participants and study staff will not be blinded to drug formulation.

A.7.5 Random Assignments

Johns Hopkins IDS will prepare a randomization sequence (either gel, then film or film, then gel) for subjects and dispense product according to that schedule. -

A.7.6 Data Monitoring and Analysis

A.7.6.1Data Monitoring

This clinical trial will be conducted in compliance with the protocol, GCP guidelines, and applicable regulatory requirements. All research charts are maintained in a double locked room. The research staff, under the direction of the primary investigator, will create and maintain an electronic database on the computers in the Drug Development Unit research offices. The database will be backed up every night onto the appropriate server's back-up system. Appropriate firewall and virus scanning software are installed and updated routinely by the information technology staff.

Case report forms will be used as the first point of data entry for this protocol. Study data management staff will manually review the forms for completeness and accuracy. If queries or discrepancies are noted, the staff person will speak with the clinician in question as soon as possible to resolve the problem. The investigator will review the case report forms for completeness and accuracy at the end of the study.

A.7.6.2 Safety Assessment

Safety endpoints will be determined using all history, physical, and laboratory data prior to and following each of the product doses. The number of adverse events in each treatment 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. Differences in the proportion of participants experiencing adverse events among the treatment arms will be compared using Fisher's exact tests. However, this Phase I study may not have an adequate sample size to detect significant differences between treatment arms.

A.7.6.3 Analysis of PK Data

Blood levels of dapivirine will be evaluated after vaginal administration. PK parameters for plasma and PBMC DPV will be estimated (e.g., Cmax, Tmax, AUC, elimination rate constant) using WinNonlin (Pharsight, Inc., Cary, NC). All PK parameters for plasma and PBMC as well as paired assessments of other matrices will be compared between formulations using a multi-level analysis (STATA/IC 11.2 for Windows software, StataCorp LP, College Station, TX) to assess formulation, sequence, and individual effects.

A.7.6.4 Analysis of PD data

HIV explant challenge will be assessed using cumulative HIV p24 measured in culture media supernatant. These values will be compared between formulations using a multi-level analysis to assess product, sequence, and subject effects. Concentration-response relationships will be evaluated for PK-PD data using sigmoid Emax modeling among other pharmacometric methods (WinNonlin, Pharsight, Inc. Cary, NC).

A.8 Human Subject 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 and

the protocol will have been submitted to the FDA. The investigators will permit audits by the NIH or the FDA or any of their appointed agents.

A.8.1 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.

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 because protocol-specified guidelines for abstinence and condom use are expected to protect male partners from exposure to the study product. In addition, based on both preclinical and clinical data, no toxicity is anticipated from the study product.

Children

The NIH has mandated that children be included in research trials when appropriate. This study will enroll women aged 18 and above who are able to give informed consent. This study meets "Justifications for Exclusion" criteria for 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[86] .

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.

Pregnant women

Pregnancy is an exclusion criterion because there are no current recommendations for the use of dapivirine during pregnancy. Prior to administration of study product, a urine pregnancy test will be performed on all women. During the informed consent process, women will be informed that dapivirine is not known to prevent pregnancy and that the effect of dapivirine on a developing human fetus is unknown. All potential participants will be required to use a reliable method of contraception as outlined in the Inclusion Criteria. Women who become pregnant during the study period following randomization and exposure to study product will not be excluded from analysis. Subjects will be followed during and after pregnancy to determine outcomes.

A.8.2 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 encouraged to ask questions and to exchange information freely with the study team. Only listed research staff 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.

A.8.3 Risk/Benefit Statement

Risks

It is not expected that this trial will expose human subjects to unreasonable risk.

In previous studies involving dapivirine gel and placebo, the following side effects were noted in women exposed to dapivirine but not to placebo: genital itch, vaginal pain, dysuria, vaginal erythema, loose stools, bloated abdomen, neutropenia, vaginal candidiasis, elevated alanine aminotransferase, elevated aspartate aminotransferase, elevated bilirubin, uterine spasm, breast discomfort, cervical erythema, genital herpes, malaise, somnolence, stomach discomfort, and vomiting. Common side effects for both active study group and placebo group participants included headache and metrorrhagia. Serious adverse events that have occurred in clinical trials with dapivirine gel (but considered to be unrelated to gel use) include hospitalization for a diabetic foot, severe headache, gastritis, thrombotic thrombocytopenic purpura, abdominal abscess, and uterine leiomyoma [40,42].

Cervicovaginal lavage has been associated with mild discomfort secondary to the introduction of sterile fluid into the vagina and its removal. Collection of genital tissue by biopsy may cause discomfort and spotting. On rare occasions, chemical cauterization and or suturing may be required if hemorrhaging occurs. Phlebotomy or the intravenous catheter may lead to discomfort which may persist for the duration of the indwelling lock, 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. Confinement to the clinical research unit for a 12 hour period may cause boredom or discomfort.

A.8.4 Minimization of risks

To minimize the risk of a product being dosed to the wrong subject, study personnel will confirm the IDS dispensed drug with the study subject identity, per hospital protocol. The clinical investigation sheet checklist will be signed off in real time to assure there is no redundant sampling outside the requirements of sampling called for by randomization.

A.8.5 Benefits

This is a no benefit study. The participant may appreciate the opportunity to contribute to the body of knowledge in the field of microbicide research.

A.8.6 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, including overnight housing and transport provided by the study team. Using the Johns Hopkins Drug Development unit remuneration schedule, women will be compensated for their time and inconvenience while participating in the protocol. This remuneration schedule is based on standardized payments related to the study visits and study-specific procedures.

A.8.7 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 double locked area. All computer information about study volunteers is kept on a computer with log-on passwords. The data management and clinical staff are the only personnel with access to the protected health information of study volunteers. 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. The computers used by the data groups are connected to the Johns

Hopkins School of Medicine network, which is redundantly firewalled against penetration from the outside. Study-specific files are accessible only to staff of the Division of Clinical Pharmacology via a password-protected server. All research records will be kept for a minimum of five years following closure of this study.

A.8.8 Communicable Disease Reporting

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

A.8.9 Access to HIV-Related Care

The investigators do not expect a screening population at high risk for HIV infection. However, trained clinical staff will refer subjects who test positive or indeterminate via the HIV antibody screen test to a physician for follow-up testing and care. Participants who have positive or indeterminate results will have standard post-test counseling as well as limited follow-up confirmatory testing provided by the study. 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. Various local resources are available for medical care for HIV positive patients, including the Moore Clinic at Johns Hopkins University. Subjects will be offered referral information from among a list of resources (as outlined in SOP #DDU-C-044-07, Attachment 4). Subjects will also be offered Maryland Community Services Locator referral information. In addition to referral information, subjects will be provided with information for local resources for support groups and other services. They will also be offered the Johns

Hopkins Hospital Patient Information handout, "HIV Human Immunodeficiency Virus," per Johns Hopkins medicine policy.

A.8.10 Study Discontinuation

NIAID, the International Partnership for Microbicides, the US FDA, other government or regulatory authorities, or the Johns Hopkins Medicine 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 gel or applicator, or any other condition deemed as an emergency event by the study staff, the External Safety Monitor will contact the Principal Investigator to initiate a temporary hold on further enrollment.

A.9 Laboratory Specimens and Biohazard Containment

A.9.1 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 A2: Designated Labs for Testing

Test	Method	Laboratory
Urine	HCG, urinalysis	JHH Clinical Laboratory
Cervicovaginal fluid	Dapivirine level	CPAL* (non-diagnostic laboratory) Baltimore, MD
Cervical swab	Nucleic Acid Amplification test	JHH Clinical Laboratory
Plasma	Dapivirine level	CPAL (non-diagnostic laboratory)

		Baltimore, MD
Rectal fluid	Dapivirine	CPAL (non-diagnostic laboratory) Baltimore, MD
Blood	Complete Metabolic Panel Complete Blood Count HIV antibody screen (Appendix I) Syphilis Chemiluminesence assay (with confirmatory RPR; FTAABS performed on RPR negative samples)	JHH Clinical Laboratory
Cervical tissue homogenate	Dapivirine concentration	CPAL (non-diagnostic laboratory) Baltimore, MD
Cervical Biopsy	HIV explant challenge	CPAL (non-diagnostic laboratory) Baltimore, MD

*CPAL, Clinical Pharmacology Analytical Laboratory, Division of Clinical Pharmacology, Johns Hopkins University

A.9.2 Urine Samples

A urinalysis will be used to screen for possible urinary tract infection when clinically indicated. Urine will be tested for qualitative HCG.

A.9.3 Cervical Samples

Biopsies to measure dapivirine drug concentrations and tissue explant susceptibility to HIV infection.

A.9.4 Cervical Swabs

C. trachomatis, N. gonorrhoeae, and trichomonas will be detected using an amplified DNA assay.

A.9.5 Cervicovaginal Fluid sampling

Obtained to measure dapivirine concentration.

A.9.6 Rectal Fluid sampling

Obtained to measure dapivirine concentration.

A.9.7 Plasma Samples

Plasma samples will be sent to the Hopkins CPAL for assay of dapivirine concentration.

A.9.8 Quality Control and Quality Assurance Procedures

The Johns Hopkins Drug Development Unit and CPAL have a wealth of experience from taking part in several clinical studies of microbicides. Thus, all testing done in this research laboratory is performed with the same level of quality control as required in a licensed clinical laboratory. Because all of the proposed studies to be conducted in this project will be using an investigational product, the studies will be conducted under IND and additional measures will be undertaken to ensure that all protocols will be conducted under good laboratory practice.

A.9.9 Specimen Storage and Possible Future Research Testing

Participants will be consented for future use of vaginal and cervical specimens and blood samples. Any leftover samples will be stored at CPAL for an indefinite period of time. The principal investigator will assume primary responsibility for control of this area.

Any results from research done on leftover specimens will not be placed in health records and will be kept confidential. Informed consent will give participants the option to withdraw their consent for use of their specimens for future research. The language and format employed in the screening and enrollment consents 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. When all laboratory assays have been completed and the study has been closed, the PI for the study will notify the clinical site Laboratory Manager to discard all samples from volunteers in the study

who chose to have their samples destroyed at the end of the study. These samples will be discarded in the appropriate manner and the disposition will be documented.

A.9.10 Biohazard Containment

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

A.10 Administrative Procedures

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

A.10.1 Study Coordination

Study implementation will follow this protocol, which may not be amended without prior written approval from the Sponsor and DAIDS Medical Officer. Close coordination between protocol team members is necessary to track study progress, respond to queries about proper study implementation, and address other issues in a timely manner. Rates of accrual, retention, follow-up, and AE incidence will be monitored closely by the team.

A.10.2 Study Monitoring

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 source document verification to ensure the accuracy and completeness of study data
- 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 of the IND application.

A.10.3 Protocol Compliance

Amendments to the protocol will require prior written approval from the principal investigators. All protocol amendments will be submitted for DAIDS review and approval facilitated by the DAIDS Regulatory Support Center (RSC). Once approval has been provided by the RSC, such amendments will be submitted to the Johns Hopkins Medicine Institutional Review Board (IRB)

for final review and approval. Once the Johns Hopkins Medicine IRB has given final approval, the amendment can be implemented.

A.10.4 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 DAIDS. Applicable records include source documents, site registration documents and reports, correspondence, informed consent forms, and notations of all contacts with the participant.

A.10.5 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.

Patient I.D. Plate

RESEARCH PARTICIPANT INFORMED CONSENT AND PRIVACY AUTHORIZATION FORM

Protocol Title: **Comparison of the Pharmacokinetics and Pharmacodynamics of Single Dose Dapivirine Vaginal Gel and Film Formulation (FAME-02B) Version 1.0**

Application No.: **NA_00088629**

Sponsor: **Division of AIDS/ NIAID/ National Institutes of Health (NIH)**

Principal Investigator: **Craig W. Hendrix, MD**
600 N. Wolfe Street, Blalock 569
Baltimore, MD 21287
Phone: 410-955-9707
Fax: 410-955-9708

1. What you should know about this study:

- You are being asked to join a research study.
- This consent form explains the research study and your part in the study.
- Please read it carefully and take as much time as you need.
- Please ask questions at any time about anything you do not understand.
- You are a volunteer. If you join the study, you can change your mind later. You can decide not to take part or you can quit at any time. There will be no penalty or loss of benefits if you decide to quit the study.
- During the study, we will tell you if we learn any new information that might affect whether you wish to continue to be in the study.
- Ask your study doctor or the study team to explain any words or information in this informed consent that you do not understand.
- For clinical trials: A description of this clinical trial will be available at www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search the Web site at any time.
- A statement will be added to your medical record that you are in this research study. Results from any clinical tests you have will be included in your medical record. Doctors outside of Johns Hopkins may not have access to this information. You can ask the research team to send this information to any of your doctors.

2. Why is this research being done?

This research is being done to help develop a microbicide, which is a drug placed in the vagina to help prevent HIV transmission. This study will look to see how two vaginally-applied forms of an investigational drug called dapivirine gel and dapivirine film spreads into blood, cervical tissue (the tissue between the vagina and uterus or womb), and cervicovaginal fluid (a mixture of fluids from the vagina and cervix).

The use of dapivirine gel and dapivirine film in this research study is investigational. The word "investigational" means that dapivirine gel and dapivirine film are not approved for marketing by the Food and Drug Administration (FDA). The FDA is allowing the use of dapivirine gel and dapivirine film in this study.

We are asking women to join this study who:

- Are at least 18 years of age
- Have had vaginal sex at least once
- Do not have HIV
- Are using a form of birth control
- Have a womb (uterus)
- Are in general good health

The study will be done at the Drug Development Unit (DDU) of The Johns Hopkins Hospital.

How many people will be in this study?

Up to 10 women will enroll in and complete the study.

3. What will happen if you join this study?

If you agree to be in this study, we will ask you to do the following things:

Screening Visit (Visit 1):

This visit will last about 2 hours. It is performed to make sure that you do not have any health problems that might make some parts of the study more uncomfortable or dangerous for you. The following procedures will be performed during this visit:

- Complete medical history and general physical examination, including a review of any medications you may be taking and a review of your sexual practices.
- Complete GYN exam (pelvic exam) using a speculum (a device used to open the vagina). This will include a swabbing from the opening of your womb (cervix) to be tested for the sexually transmitted infections called gonorrhea, chlamydia, and trichomoniasis. If you have one of these infections you can get treatment from your primary care doctor or another clinic and then you can be re-screened for the study. We will not provide treatment nor pay for treatment of these infections. The law requires us to report positive chlamydia and gonorrhea tests to the health department.
- Vital signs (including blood pressure, heart beat, and temperature) and weight measurement.
- Collect urine for a urinalysis for routine safety testing.

- Collect blood (a little more than 1 tablespoon) for routine safety tests. The blood will be used to make sure that your blood count is good and that your blood clots normally. It will also be tested for liver and kidney function tests. As part of being in this study, you will have a test for syphilis and for HIV virus (the virus that causes AIDS). You will be given the State of Maryland HIV consent form as part of that process. If this test is positive, you will be referred for proper medical care and counseling. The law requires us to report positive tests to the health department.

If the screening tests show that it is safe for you to take part in the study, we will ask you to come back to start the study.

If you join the study, you must not have vaginal sex, nor insert any vaginal products or objects, including sex toys into your vagina for at least 3 days prior to the study visits.

During the study you will receive calls or emails from the study staff reminding you of your visits. We ask that you keep track of all medications you use during the study and bring these to every visit.

There will be two full day visits for the study. Depending on bed availability at the hospital, you may be required to stay overnight for the dosing visits. If beds are not available, each dosing visit will last about 13 hours. Each full day visit will be followed by five follow up visits.

Full Day Visits 2 and 8 (Day 0):
We will ask you to either come to the hospital the night before your dosing visit and stay until the following evening or come to the research unit early in the morning and stay for about 13 hours.

The following procedures will be performed during this visit:

- You may have a physical exam
- Tell us about any changes in your health or medications.
- Pregnancy test
- Vital signs, including blood pressure, heart beat, and temperature
- Intravenous (IV) catheter will be inserted into a vein and kept in place until you are discharged. This is done to help in the collection of blood during the visit. If necessary, the IV catheter may be removed prior to discharge and you may have your blood drawn with a needle instead.
- You will have your blood collected periodically throughout the day to measure the amount of study drug in your blood. Each blood sample will be about 1 teaspoon. There will be a total of 8 samples collected for a total of about 3 tablespoons of blood.
- You will be randomized to receive either the dapivirine gel or film. The order in which you receive the gel or the film will be random, by chance, like flipping a coin. Each participant will receive both the gel and the film during the study.
- A study doctor will then insert a speculum and put the study drug into your vagina.
- A study doctor will insert a speculum and you will have cervicovaginal samples taken (a sample taken from the vagina and cervix using an absorptive device like a swab or sponge), and cervical biopsies (samples of tissue from your cervix) about 5 hours after the dose of study medication.
- You will also have a clear, plastic, lubricated tube gently inserted into the rectum (no more than 4 inches) in order to collect the rectal fluid with a sponge.
- You will be advised to avoid the use of spermicide and other non-study vaginal products (with the exception of tampons and female condoms) during the study.
- You will be asked to not insert anything into the vagina for up to 10 days after you receive the study drug.

24 Hours (Day 1, Visits 3 and 9):
The following procedures will be performed during this visit:

- Vital signs, including blood pressure, heart beat, and temperature
- Blood collections (about 1 teaspoon) to measure the amount of study drug in your blood.
- Tell us about any changes in your health or medications.
- You will be advised to avoid the use of spermicide and other non-study vaginal products (with the exception of tampons and female condoms) during the study.
- You will be asked to not insert anything in the vagina for up to 10 days after you receive the study drug.

48 Hours (Day 2, Visits 4 and 10):
The following procedures will be performed during this visit:

- Vital signs, including blood pressure, heart beat, and temperature
- Blood collections (approximately 3 teaspoons) to measure the amount of study medication in your blood and to assess the health of your blood, liver and kidneys. You may have more blood taken if you are experiencing any problems.
- A urine sample may be collected to look for bladder or urinary tract infections.
- Tell us about any changes in your health or medications.
- You will be advised to avoid the use of spermicide and other non-study vaginal products (with the exception of tampons and female condoms) during the study.
- You will be asked to not insert anything in the vagina for up to 10 days after you receive the study medication.

72 Hours (Day 3, Visits 5 and 11):
The following procedures will be performed during this visit:

- Vital signs, including blood pressure, heart beat, and temperature
- Tell us about any changes in your health or medications.
- Blood collections (about 1 teaspoon) to measure the amount of study drug in your blood.
- A study doctor will insert a speculum into your vagina and you will have cervicovaginal fluid samples and cervical biopsies taken as on Day 0.
- You will also have a clear, plastic, lubricated tube gently inserted into the rectum (no more than 4 inches) in order to collect the rectal fluid with a sponge.
- You will be advised to avoid the use of spermicide and other non-study vaginal products (with the exception of tampons and female condoms) during the study.
- You will be asked to not insert anything into the vagina for up to 10 days after you receive the study drug.

168 Hours (Day 7, Visits 6 and 12):
The following procedures will be performed during this visit:

- Vital signs, including blood pressure, heart beat, and temperature
- Blood collections (about 3 teaspoons) to measure the amount of study drug in your blood and to assess the health of your blood, liver, and kidneys. You may have more blood taken if you are experiencing any problems.
- A study doctor will insert a speculum into your vagina and you will have cervicovaginal fluid samples taken as on Day 0 and Day 3. No biopsies are planned for this visit.
- You will also have a clear, plastic, lubricated tube gently inserted into the rectum (no more than 4 inches) in order to collect the rectal fluid with a sponge.
- A urine sample may be collected to look for bladder or urinary tract infections.

- Tell us about any changes in your health or medications.
- You will be advised to avoid the use of spermicide and other non-study vaginal products (with the exception of tampons and female condoms) during the study.
- You will be asked to not insert anything in the vagina for up to 10 days after you receive the study drug.

Follow-Up Visits (Visits 7 and 13):
About one week following Day 7, you will return to the clinic and the following procedures will be performed:

- You will tell us about any changes in your health and medications.
- You may have a physical exam.
- Vital signs, including blood pressure, heart beat, and temperature.
- Blood collections (about 3 teaspoons) to measure the amount of study drug in your blood and to assess the health of your blood, liver, and kidneys. You may have more blood taken if you are experiencing any problems.
- A urine sample will be collected for a pregnancy test and safety testing to identify bladder or urinary tract infections.
- You will be advised to avoid the use of spermicide and other non-study vaginal products (with the exception of tampons and female condoms) during the study.
- You will be asked to not insert anything in the vagina for up to 10 days after you receive the study product.

How long will you be in the study?
You will be in this study for about 3 months.

Storage of Samples for Future Research
While you are in this research study, there may be some samples of blood, tissue, and/or fluid collected from you that might be useful for future research on sexual transmission of HIV infection. You are being asked to agree to the storage of these samples. Your samples will be labeled with a unique identifier (such as specimen and test type, date, your subject identification number, and study visit number). Your name will not be on the samples.

If, in the future, we decide to analyze the samples, we will ask the IRB (a research review board that protects the rights and welfare of study participants) for permission first. There is no time limit on how long your samples will be stored.

You can still be in this study even if you do not want your samples to be stored for future studies.

On the checklist below, we ask for you to indicate by initialing if you would permit your samples to be shared with other researchers.

_____I agree to have my blood, tissue and/or fluid sample stored for future research.

_____I do not agree to have my blood, tissue, and/or fluid sample stored for future research

4. What are the risks or discomforts of the study?

Risks of blood draws: Blood drawing may cause discomfort. You may feel dizzy or faint, or develop a bruise, swelling or infections where the needle is inserted. The total amount of blood collected during the entire study is less than 1 cup (about 13 tablespoons)

Risks of pelvic (GYN) exam and cervicovaginal fluid sampling: During pelvic exams and the cervicovaginal fluid sampling, you may feel discomfort or pressure in your vagina and/or pelvis. From the pelvic exam you may also have vaginal bleeding or spotting.

Risk of rectal sponge sampling: When rectal fluid is collected via insertion of the plastic tube into the anus, on rare occasion you may experience mild discomfort or have pain (should you have another condition that is already causing pain in the area)

Risks from STD Testing: You may become embarrassed, worried, or anxious when receiving STD counseling. You also may become worried or anxious while waiting for your test results or after receiving positive test results. It may also cause sadness or depression. HIV positive results have been associated with depression, suicidal thoughts, denial, and social isolation. Trained counselors will be available to help you deal with these feelings. Although the study site will make every effort to protect your privacy and confidentiality, it is possible that your involvement in the study could become known to others, and that social harms may result (i.e., because you could become known as "high risk" for HIV infection). For example, you could be treated unfairly or discriminated against, or could have problems being accepted by your family and/or community. In addition to referring you to treatment services, we can refer you to health or mental health services if you wish. However, Johns Hopkins and the NIH do not have funds to pay for treatment once you are referred.

By law, positive results from some STD tests have to be reported to local health authorities. This reporting may result in a potential loss of confidentiality.

Risks from intravenous (IV) catheters: Risks include pain from the needle stick, bruising, and bleeding, but these are usually mild. Sometimes a person can feel dizzy or faint when the IV catheter is being inserted. Other risks include infection, damage to the vein, blood clot, or stroke (if air enters the vein), but these are rare.

Risks from cervical biopsies: You may feel slight to moderate pain at the time of the biopsy (like being pinched) which usually resolves quickly, but could last a few hours. You may have spotting (small amounts of vaginal bleeding) for 1 – 2 days. There is a small risk of the biopsy area becoming infected or having bleeding that is heavier than spotting. It is important for you to know your body is healing for 24-48 hours after the biopsy is collected. However, if you have bleeding heavier than your usual menstrual period, notice a foul odor or a heavier vaginal discharge (more than usual), you should contact the study clinic right away. To ensure that the biopsy sites heal, we will ask you to avoid vaginal intercourse and putting anything into your vagina (such as tampon, sex toy) for 7 days after the biopsy is collected (10 days after receiving the study product).

Risks from study gel and film: The study products can cause some side effects. We do not yet know all the side effects of the film. Some, but not all women who used gel in other studies have had discharge from the vagina and irritation and discomfort.

Risks from the study drug:
The study drug has been compared with placebo in other research studies. A placebo is a substance that looks like the study drug but that contains no active ingredients. Common side effects reported by participants receiving placebo or dapivirine were headache and spotting.

Based on side effects reported among women in previous studies, dapivirine vaginal product may be associated with genital itching, vaginal pain, painful urination, vaginal redness, loose stools, bloated abdomen, low white blood cell count, yeast infection, elevated liver enzymes, uterine spasm, breast discomfort, cervical redness, genital herpes, malaise (uneasiness or generalized discomfort), tiredness, stomach discomfort and vomiting.

Serious side effects reported during dapivirine trials that are thought to be unrelated to dapivirine gel include hospitalization for diabetic foot, severe headache, gastritis, thrombotic thrombocytopenic purpura (a condition where blood clots form in the blood vessels), abdominal abscess (infection in the belly), and uterine fibroids.

There may be side effects and discomforts that are not yet known.

5. Are there risks related to pregnancy?

Pregnant women cannot take part in this study. There are no current recommendations for the use of dapivirine during pregnancy. Dapivirine is not known to prevent pregnancy and the effect of dapivirine on the developing human fetus is unknown. In order to reduce these risks, you will be tested to determine if you are pregnant at the beginning of the study and this test will be repeated the day you receive the dapivirine gel or film. If any of these tests show that you are pregnant, you will not be allowed to continue in the study. It is important for you to let the study doctor know if you become pregnant or suspect that you are pregnant while taking part in this study.

This research may hurt an embryo or fetus in ways we do not currently know.

6. Are there benefits to being in the study?

There is no direct benefit to you from being in this study. If you take part in this study, you may help others in the future.

7. What are your options if you do not want to be in the study?

You do not have to join this study. If you do not join, your care at Johns Hopkins will not be affected.

8. Will it cost you anything to be in this study?

No.

9. Will you be paid if you join this study?

You will receive money for the time and inconvenience of being in the study. The amount you are paid will be based on the number of study visits, study doses, blood collections, cervicovaginal samples, and cervical biopsies.

You will receive $1,400 total for your participation in the study if you keep all appointments and follow the instructions of the study team. $30 of the total is a bonus which may be deducted in part or whole if you fail to keep your appointments on time or fail to follow instruction. All payments are made by check at the end of the study.

If you withdraw before the study is completed, you will be paid only a portion of the $1,400 based on how much of the study you complete. For example, if you only receive one of the study products, provide the blood, biopsies and cervicovaginal samples associated with that dose, and keep your follow-up appointment on time, you will receive $700 because this represents one-half of the study.

You may be required to provide your Social Security number to be paid. If your payment for study participation exceeds $600 per year, this information must be reported to the Internal Revenue Service.

10. Can you leave the study early?

- You can agree to be in the study now and change your mind later.
- If you wish to stop, please tell us right away.
- Leaving this study early will not stop you from getting regular medical care.
- If you leave the study early, Johns Hopkins may use or give out your health information that it already has if the information is needed for this study or any follow-up activities.

11. Why might we take you out of the study early?

You may be taken out of the study if:

- Staying in the study would be harmful.
- You need treatment not allowed in the study.
- You fail to follow instructions.
- You become pregnant.
- The study is cancelled.
- There may be other reasons to take you out of the study that we do not know at this time.

If you are taken out of the study early, Johns Hopkins may use or give out your health information that it already has if the information is needed for this study or any follow-up activities.

12. How will your privacy be protected?

Johns Hopkins has rules to protect information about you. Federal and state laws also protect your privacy.

The research team working on the study will collect information about you. This includes things learned from the procedures described in this consent form. They may also collect other information including your name, address, date of birth, and other details.

Generally, only people on the research team will know your identity and that you are in the research study. However, sometimes other people at Johns Hopkins may see or give out your information. These include people who review research studies, their staff, lawyers, or other Johns Hopkins staff.

People outside of Johns Hopkins may need to see your information for this study. Examples include government groups (such as the Food and Drug Administration), safety monitors, other hospitals in the study and companies that sponsor the study.

We cannot do this study without your permission to use and give out your information. You do not have to give us this permission. If you do not, then you may not join this study.

We will use and disclose your information only as described in this form and in our Notice of Privacy Practices; however, people outside Hopkins who receive your information may not be covered by this promise. We try to make sure that everyone who needs to see your information keeps it confidential – but we cannot guarantee this.

The use and disclosure of your information has no time limit. You may cancel your permission to use and disclose your information at any time by notifying the Principal Investigator of this study by phone or in writing. If you contact the Principal Investigator by phone, you must follow-up with a written request that includes the study number and your contact information. The Principal Investigator's name, address, phone and fax information are on page one of this consent form.
If you do cancel your permission to use and disclose your information, your part in this study will end and no further information about you will be collected. Your cancellation would not affect information already collected in the study.

13. What treatment costs will be paid if you are injured in this study?

Johns Hopkins and the federal government do not have programs to pay you if you are hurt or have other bad results from being in the study. However, medical care at Johns Hopkins is open to you as it is to all sick or injured people.

- If you have health insurance: The costs for any treatment or hospital care you receive as the result of a study-related injury will be billed to your health insurer. Any costs that are not paid for by your health insurer will be billed to you.

- If you do not have health insurance: You will be billed for the costs of any treatment or hospital care you receive as the result of a study-related injury.

By signing this form you will not give up any rights you have to seek compensation for injury.

14. What other things should you know about this research study?

a. What is the Institutional Review Board (IRB) and how does it protect you?
The Johns Hopkins Medicine IRB is made up of:
- Doctors
- Nurses
- Ethicists
- Non-scientists
- People from the local community.

The IRB reviews human research studies. It protects the rights and welfare of the people taking part in those studies. You may contact the IRB if you have questions about your rights as a participant or if you think you have not been treated fairly. The IRB office number is 410-955-3008. You may also call this number for other questions, concerns or complaints about the research.

b. What do you do if you have questions about the study?
Call the study doctor, Dr. Craig Hendrix at 410-955-9707. If you wish, you may contact the study doctor by letter or by fax. The address and fax number are on page one of this consent form. If you cannot reach the study doctor or wish to talk to someone else, call the IRB office at 410-955-3008.

c. What should you do if you are injured or ill as a result of being in this study?
If you think you are injured or ill because of this study, call Dr. Craig Hendrix at 410-955-9707 during regular office hours

If you have an urgent medical problem related to your taking part in this study call Dr. Craig Hendrix at 410-955-9707 during regular office hours and at 410-375-4418 after hours and on weekends.

d. What happens to Data, Tissue, Blood and Specimens that are collected in the study?
Scientists at Johns Hopkins work to find the causes and cures of disease. The data, tissue, blood and specimens collected from you during this study are important to both this study and to future research.

If you join this study:

- You will not own the data, or the tissue, blood, or other specimens given by you to the investigators for this research.
- Both Johns Hopkins and any sponsor of this research may study your data and the tissue, blood or other specimens collected from you.
- If data, tissue, blood, or other specimens are in a form that identifies you, Johns Hopkins may use them for future research only with your consent and IRB approval.
- If data, tissue, blood or other specimens are in a form that we believe does not identify you, they may be shared with other academic medical centers, non-profit organizations, corporate sponsors and other commercial companies without your consent or IRB approval.
- You will not own any product or idea created by the researchers working on this study.
- You will not receive any financial benefit from the creation, use or sale of such a product or idea.

e. What are the Organizations that are part of Johns Hopkins?
Johns Hopkins includes the following:

- The Johns Hopkins University
- The Johns Hopkins Hospital
- Johns Hopkins Bayview Medical Center
- Howard County General Hospital
- Johns Hopkins Community Physicians.
- Suburban Hospital
- Sibley Memorial Hospital

15. What does your signature on this consent form mean?

Your signature on this form means that:

- you understand the information given to you in this form
- you accept the provisions in the form
- you agree to join the study

You will not give up any legal rights by signing this consent form.

WE WILL GIVE YOU A COPY OF THIS SIGNED AND DATED CONSENT FORM

_____

Signature of Participant Date/Time

_____

Signature of Person Obtaining Consent Date/Time

NOTE: A COPY OF THE SIGNED, DATED CONSENT FORM MUST BE KEPT BY THE PRINCIPAL INVESTIGATOR; A COPY MUST BE GIVEN TO THE PARTICIPANT; AND, IF APPROPRIATE A COPY OF THE CONSENT FORM MUST BE PLACED IN THE PARTICIPANT'S MEDICAL RECORD.

ONLY CONSENT FORMS THAT INCLUDE THE JOHNS HOPKINS MEDICINE LOGO CAN BE USED FOR CONSENTING RESEARCH PARTICIPANTS. IF THIS CONSENT FORM DOES NOT HAVE A JOHNS HOPKINS MEDICINE LOGO, DO NOT USE IT TO CONSENT RESEARCH PARTICIPANTS.

APPENDIX B: FAME 05 Study Documents

- Study Protocol
- Informed Consent Form

FAME 05 STUDY PROTOCOL

B.1 Objectives

Primary Objectives:

- To compare the levels of tenofovir and tenofovir diphosphate in the cervicovaginal fluid, genital tissue, rectal fluid and blood samples using single dose gel and film formulations of tenofovir microbicide.
- To compare the safety of single dose tenofovir gel and film formulations

Secondary Objective:

- To compare the effect of single dose tenofovir gel or film formulation on cervical tissue explant challenge with HIV.

B.2 Study Design

B.2.1 Identification of Study Design

This is a single site, randomized open-label crossover study. Five subjects will receive the treatment sequence of gel followed by film. Five other subjects will receive the treatment sequence of film followed by gel.

B.2.2 Description of Study Population

The study population will be HIV uninfected women who meet criteria as outlined in section 5.

B.2.3 Time to Complete Accrual

This project will take 3 months to accrue and 6 months to complete.

B.2.4 Study Groups

Subjects will receive tenofovir gel verses tenofovir film in a crossover design in a randomized sequence.

B.2.5 Site

The study will be carried out at the Johns Hopkins Drug Development Unit.

B.3 Study Population

B.3.1 Selection of Study Population

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

B.3.2 Recruitment

Participants will be recruited from a variety of sources. Participants will also be referred to the study from other local research projects. Recruitment materials will be approved by the Johns Hopkins Institutional Review Board

B.3.3 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 completing participation 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.

B.3.4 Inclusion Criteria

1. 18 to 45 years of age (inclusive) with a history of receptive vaginal intercourse.
2. HIV negative within 28 days of enrollment (Appendix 1). Note: Only non-reactive subjects will be enrolled. Individuals testing positive will be excluded, but confirmatory testing and referral for follow-up will be determined by the testing algorithm conducted by the Johns Hopkins Hospital Clinical Laboratory.
3. Understand and agree to local STI reporting requirements.
4. Able and willing to provide written informed consent to take part in the study.
5. Able and willing to provide adequate information for locator purposes.
6. Availability to return for all study visits, barring unforeseen circumstances.
7. Availability to return for the second formulation dosing at the same time in the subject's menstrual cycle as when the first formulation was administered, at least 10 days before menses.
8. Willing to abstain from vaginal intercourse and insertion of anything (e.g., drug, vaginal douche, personal lubricant or sex toy) in vagina for 72 hours before each study product exposure, and 10 days following study product dosing, comprising a total of 26 days of abstinence, no insertion of vaginal products/objects while participating in the study.
9. Willingness to have partner(s) use condoms (must not contain Nonoxynol-9) for the duration of the study.
10. Agree not to participate in other research studies involving drugs and/or medical devices.
11. Negative qualitative urine pregnancy test.
12. Per participant report, using an effective method of contraception at enrollment; hormonal method (except vaginal ring) used continuously for the past 30 days;

intrauterine device (IUD [copper or hormonal] inserted at least 30 days prior to enrollment) and willingness to use effective method of contraception until the completion of final scheduled study visit if enrolled. Female sterilization or sexual activity with vasectomized partner. Women abstinent from sexual activity with a male partner for the past 30 days and who intend to remain abstinent throughout their period of study participation may also be enrolled.

13. Willingness to remain in the research unit for up to 12 hours on each of two dosing days.

B.3.5 Exclusion Criteria

1. Current sexual partner known by participant to be HIV seropositive.
2. Individuals who, by history, engage in condom-less intercourse with HIV-infected partners, or partners that have unknown HIV serostatus, or women who exchange sex for money, shelter, or gifts.
3. Active sexually transmitted infection or documented treatment of sexually transmitted infections including, but not limited to: chlamydia, gonorrhea, syphilis, trichomonas, cervicitis or PID within 8 weeks prior to enrollment.
4. Individuals with active hepatitis B infection.
5. Known history of genital HSV (diagnosed by either clinical or laboratory test).
6. Symptomatic vaginal candidiasis or bacterial vaginosis.
7. Undiagnosed irregular uterine bleeding
8. Pathology of the female genital tract, defined as clinically apparent Grade 2 or higher pelvic examination finding (observed by study staff) at Screening and/or Enrollment (per the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events Version 1.0, December, 2004 (Clarification dated August 2009), Addendum 1

Female Genital Grading Table for Use in Microbicide Studies). (Note: Cervical bleeding associated with speculum insertion and/or specimen collection judged to be within the range of normal according to the clinical judgment of the IoR/designee is considered expected non-menstrual bleeding and is not exclusionary; Otherwise eligible participants with exclusionary pelvic and/or rectal examination findings may be enrolled/randomized after the findings have improved to a non-exclusionary severity grading or resolved. If improvement to a non-exclusionary grade or resolution is documented within 42 days of providing informed consent, the participant may be enrolled.)

9. Individuals who are status post hysterectomy.
10. History of any cervicovaginal procedure (i.e. colposcopy with cervical biopsy) within the past 2 months.
11. History of cone biopsy or extensive loop electrosurgical excision procedure (LEEP), which in the judgment of the investigator may affect permeability assessment.
12. Any known primary or secondary uro-genital malformations, which in the assessment of the investigator may interfere with the intended urine collection for PK studies.
13. Use of vaginally administered medications within 4 week of enrollment
14. Any active urinary tract infection
15. By history, subjects with irregular menstrual cycles.
16. At screening:
    a. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) greater than 1.5 X the site laboratory ULN (upper limit of normal)
    b. Hemoglobin less than 10.0 g/dL
    c. Platelet count less than 100,000/mm3

d. Other safety tests outside of the normal range that in the judgment of the investigator may interfere with conduct of the study.
e. Positive findings on urinalysis that are clinically significant in the opinion of the investigator

17. Estimated creatinine clearance < 60 ml/min based on established nomograms
18. Recent history (past 6 months) of injection drug use or, a level of alcohol use that, in the judgement of the Investigator of Record, may interfere with the conduct of this study.
19. Unwillingness to refrain from aspirin and NSAIDs product use for one week prior to and one week post study procedures.
20. Use of warfarin or heparin.
21. Use of systemic immunomodulatory medications within 4 weeks of enrollment.
22. Use of product containing nonoxynol-9 within 4 weeks of enrollment.
23. Use of any investigational products within 4 weeks of enrollment.
24. Any other medical conditions deemed not safe for participation by the investigator.
25. Any individual that is pregnant or is actively breast feeding.
26. Post-menopausal defined as 12 months of amenorrhea.

B.4 Study Product

B.4.1 Regimen

Subjects will be randomized to receive one of the two following sequences:

- Single dose of Tenofovir 1% gel (equivalent to 40 mg of Tenofovir in 4 mL's of gel), followed by single dose Tenofovir film (40 mg/film)
- Single dose of Tenofovir film (40 mg/film) followed by single dose Tenofovir 1% gel

B.4.2 Administration

A study clinician will administer the product with the aid of a speculum for consistency of placement in the region between the mid vagina and anterior fornix.

B.4.3 Study Product Formulation

Tenofovir Films: TFV film contains 40 mg of TFV per film

Tenofovir Gel: The 1% tenofovir gel contains 1 gm/100 mL of PMPA (9-R-2-phosphonomethoxypropyl adenine monohydrate), an acyclic nucleotide analogue with activity in vitro against retroviruses, including HIV-1 and HIV-2, as well as hepadnaviruses.(19)

B.4.4 Study Product Supply and Accountability

CONRAD is the IND holder for tenofovir and will ensure that packaged study product is provided to the Johns Hopkins Investigational Drug Service (IDS) pharmacy. The University of Pittsburgh Magee Women's Research Institute (MWRI) will provide the tenofovir film product to the IDS. All study products will be available to the study staff through the IDS Pharmacy. The IDS staff will maintain complete records of all study products received for this protocol and dispensed to participants. These records will not be available to other members of the research staff. Additional documentation will be required for study gel 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 after the study is completed or terminated.

B.4.5 Study Product Dispensing

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

B.4.6 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. Vaginal douching and vaginal application of personal lubricants will be prohibited for the ten days following dosing with a tenofovir formulation. Condoms provided by study staff will not be coated with any type of spermicide.

B.5 Study Procedures

This section describes visit-specific study procedures.

B.5.1 Visit 1: Screening Visit (within 28 days prior to Formulation #1 Dosing)

All subjects will undergo screening procedures prior to enrollment in the protocol. Screening procedures may occur over several visits. Informed consent will be obtained at screening, prior to the procedures listed in Table 4 above. This will be done by an interactive question and answer process where the potential subject will be asked specific questions to determine her understanding of the purpose, procedures, risks and benefits in participating in the trial. If in the judgment of the investigator the subject cannot demonstrate sufficient understanding, then that subject will be excluded from participation in the protocol.

Screening procedures will include:

- Complete history and general physical examination to determine eligibility for the study.
- A record of concomitant medications will be obtained
- Complete GYN examination including swab collection to rule out STI's
    - One cervical swab: to test for gonorrhea, chlamydia, and trichomoniasis.
    - Vaginal pH if indicated
    - Vaginal swabs for wet prep, if indicated
    - Pap smear if indicated
- Phlebotomy to obtain blood for pregnancy test, complete metabolic panel and complete blood count, HIV testing, hepatitis B surface antigen and syphilis treponemal testing.
- Urinalysis to rule out urinary tract infection.
- Vital signs, weight, and height will be obtained.
- Assessment for adverse events that occur over the screening period.
- Subjects will be advised to abstain from vaginal intercourse, vaginal products, vaginal douche or vaginal insertion of sex toys at least 72 hours prior to dosing of formulation # 1 and formulation #2, and 10 days following each dosing (with the exception of study dosings and procedures)
- Pregnancy testing will be repeated prior to administration of formulation #1.

Subjects who meet the inclusion and exclusion criteria following the Screening Visit will be enrolled and randomized to a formulation sequence (gel-film or film-gel). As part of scheduling, care will be taken to attempt scheduling the visit at a time when the participant is not expecting to be actively bleeding or anticipating her menses within 10 days.

Subjects with active vaginal bleeding at the dosing visit will not be randomized/enrolled at that time. Subjects found to have vaginal bleeding on pelvic exam may be rescheduled if vaginal bleeding resolves before 30 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.

B.5.2 Visits 2 & 8: Formulation Dosing Visits

The dose formulation will be vaginally administered by a study clinician.

Study procedures and follow-up will then proceed as outlined in the Schedule of Events.:

Dosing Visit Hour-by-Hour (same for gel and film)

Day 0 (within 28 days of all screening evaluations)

- Subject arrives at the research unit
- Urine pregnancy test performed, required negative to continue with dose.
- Vital signs will be obtained.
- Medical history and concomitant medications will be reviewed and updated.
- Symptom-directed exam will be performed.
- Assessment for adverse events as noted from the history, and physical examination and as defined in section 9.3.

-1 hour

- Insert saline lock for PK's
- Pre-dose blood collection for drug concentration assessment.

0 hour

- Dose tenofovir formulation.
- Begin blood collections for drug concentration

- Plasma at following times: 0, 0.5, 1, 2, 4, 5, 8, 12 hours after dosing.
- PBMC at following times: 0, 2, 4, 8 hours after dosing. The saline lock will be removed following the 12 hour pharmacokinetic time point.

5 hours (+/- 1 hour)

- Cervicovaginal samples (fluid sampling with an absorptive device from mid-vagina, fornices, exocervix,) and cervical biopsies (three biopsies for homogenate drug concentration and explant challenge) are being collected.
- Rectal fluid collection

During each study visit, participants will be queried for adverse events and 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. Study participants will be counseled to collect and return to the study staff any film product that comes out of the vagina after dosing.

24 hours (Visits 3 and 9)

- Blood collection for tenofovir plasma and PBMC.
- Vital signs will be obtained.
- Medical history and concomitant medications will be reviewed and updated.
- Adverse event assessment
- Symptom-directed physical exam, if needed.
- Subject instructions including counseling to avoid the use of spermicide and other non-study vaginal products (other than tampons during menstruation and female condoms) while participating on the study. Subjects will be counseled to abstain from sexual

intercourse and all other insertive vaginal practices for 10 days following each administered dose (or 7 days after the last cervicovaginal sampling at 72 hours).

48 hours (Visits 4 and 10)

- Blood collection for Complete Metabolic Panel, Complete Blood Count, urinalysis (if clinically indicated) and tenofovir plasma concentrations.
- Vital signs will be obtained.
- Medical history and concomitant medications will be reviewed and updated.
- Assessment for adverse events.
- Symptom-directed physical exam, if needed.
- Subject instructions including counseling to avoid the use of spermicide and other non-study vaginal products (other than tampons during menstruation and female condoms) while participating on the study. Subjects will be counseled to abstain from sexual intercourse and all other insertive vaginal practices for 10 days following each administered dose (or 7 days after the last cervicovaginal sampling at 72 hours).

72 hours (Visits 5 and 11)

- Blood collection for tenofovir plasma concentrations.
- Vital signs will be obtained.
- Medical history and concomitant medications will be reviewed and updated.
- Assessment for adverse events.
- Symptom-directed physical exam, if needed.
- Cervicovaginal samples (vaginal fluid sampling from mid-vagina, fornices, exocervix,) and cervical biopsy (three biopsies for tissue drug concentrations and explant challenge) are being collected.

- Rectal fluid collection
- Subject instructions including counseling to avoid the use of spermicide and other non-study vaginal products (other than tampons during menstruation and female condoms) while participating on the study. Subjects will be counseled to abstain from sexual intercourse and all other insertive vaginal practices for 10 days following each administered dose (or 7 days after the last cervicovaginal sampling at 72 hours).

<u>168 hours (Visits 6 and 12)</u>

- Blood collection for tenofovir plasma.
- Vital signs will be obtained.
- Medical history and concomitant medications will be reviewed and updated.
- Assessment for adverse events.
- Symptom-directed physical exam, if needed.
- Cervicovaginal fluid (vaginal fluid sampling from mid-vagina, fornices, exocervix).
- Rectal fluid collection.
- Safety labs, including a complete metabolic panel and complete blood count and a urinalysis (if clinically indicated).
- Subject instructions including counseling to avoid the use of spermicide and other non-study vaginal products (other than tampons during menstruation and female condoms) while participating on the study. Subjects will be counseled to abstain from sexual intercourse and all other insertive vaginal practices for 10 days following each administered dose (or 7 days after the last cervicovaginal sampling at 72 hours).

B.5.3. Visits 7 & 13: Two Week Post-Dose Follow-Up Visit

Subjects will return to the research clinic at Day 14 (Visit 7) to assess their state of health post-study. This evaluation will include a review of medical history and concomitant medications, a symptom-directed examination and vital signs will be obtained. Safety labs, including a complete metabolic panel, complete blood count, urinalysis, HIV testing, and a qualitative urine pregnancy test will be performed.

Subjects are released to home with a scheduled Dosing Visit (Visit #8) appointment planned to coincide within one week of the time in the menstrual cycle associated with the first dosing visit. The first Follow-up Visit (visit 7) should follow the first Dosing Visit at 14 days (+ 2 days) The final Follow-up Visit (Visit 13) will occur 14 days (+2 days) after Dosing Visit #2(Visit 12). The events for the second Follow-up visit (Visit #13) are the same as for the first Follow-up visit (Visit 7).

B.5.4 Clinical Evaluations and Procedures

Physical exams will include the following assessments:

- Vital signs:
    - Blood pressure
    - Pulse
    - Temperature
    - Weight (at Screening only)
    - Height (at Screening only)
- Clinical assessments of:
    - Complete history and physical examination at screening.
    - Review of concomitant medications at screening and each study visit.
    - Adverse events assessment at each study visit.

- Interim history, including menstrual history
- Symptom-directed examination including the following systems:
  - Genitourinary
  - Gastrointestinal
  - Systemic as needed

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.

B.5.5 Laboratory Evaluation

- Pregnancy test
- Urinalysis, if clinically indicated
- GC/CT/Trichomonas Nucleic Acid Amplification Test
- Syphilis Chemiluminescence Assay
- HIV Testing
- Complete metabolic panel
  - Sodium
  - Potassium
  - Chloride
  - Carbon Dioxide
  - Glucose
  - Urea Nitrogen (BUN)
  - Creatinine
  - Calcium
  - Total Protein

    - Albumin
    - Total Bilirubin
    - Alkaline Phosphatase
    - AST
    - ALT
- Complete Blood Count
    - WBC
    - RBC
    - Hemoglobin
    - Hematocrit
    - Indices
    - RDW
    - Platelets
    - MPV
- Tenofovir and metabolite drug levels in cervical tissue, cervicovaginal fluid, rectal fluid, plasma, and PBMCs. Metabolite drug levels to be measured in tissue and PBMC's only.

B.5.6 Specimen Collection and Processing

Since samples from up to 6 compartments will be collected, standardized protocols previously established for sample collection, isolation of mucosal mononuclear cells from tissue (above), cell separation and counting, will be reviewed and amended as needed for this investigational protocol.

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 safety laboratory results are not available due to administrative or laboratory error, sites are permitted to re-draw specimens.

B.5.7 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 to the Hopkins University 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

B.6 Assessment of Safety and Clinical Management

B.6.1 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, the CONRAD 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, CONRAD Medical Officer, and the DAIDS Medical Officers.

B.6.2 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 Johns Hopkins HIV Microbicide 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 External Safety Monitor, the DAIDS Medical Officers and CONRAD Medical Officer on a monthly basis. These data summaries will include adverse event, type, grade and association with the study products, accrual and retention data. The External Safety Monitor will evaluate adverse event data independently as well to determine whether or not the study protocol should continue as originally designed, should be changed, or should be terminated.

Approximately once a month the PSRT will convene via teleconference to review adverse event data. If more urgent safety matters arise, these calls can occur more frequently.

The IRB will be notified of any serious and unexpected adverse events according to the policies of the Johns Hopkins Medicine IRB.

The following information will be submitted to the 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 Protocol Safety Review;

- 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.

B.6.3 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. The term "investigational product" for this study refers to both the gel and the film, as well as the study gel applicator.

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 are instructed to seek immediate emergency care. Where feasible and medically appropriate, participants will be encouraged to seek evaluation at Johns Hopkins Hospital, where the study clinicians are based, and to request that a study clinician be contacted upon their arrival. With appropriate permission of the participant, whenever possible, records from all non-

study medical providers related to untoward medical occurrences will be obtained for review. All participants reporting an untoward medical occurrence will be followed clinically 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.

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 1.0, December 2004 (Clarification dated August 2009) and the Female Genital Grading Table for Use in Microbicide Studies (Addendum 1 to the DAIDS Table for Grading Adult and Pediatric Adverse Events, Version 1.0, December 2004 [Clarification dated August 2009]), 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 1.0, December 2004 (Clarification dated August 2009). 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 AEs reported on CRFs will be assessed based on the Manual for Expedited Reporting of Adverse Events to DAIDS, the Investigators Brochures, and the clinical judgment of the PI/designee. The study products that must be considered when AE relationships are assigned are tenofovir gel, tenofovir gel applicator, and tenofovir 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 Officers. At a minimum, the AE must be re-assessed by study staff at least 2 weeks after the participant's study exit visit; 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. 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.

B.6.4 Expedited Adverse Event Reporting Requirements

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 RSC website at http://rsc.tech-res.com/safetyandpharmacovigilance/. 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.

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 CONRAD and 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 by CONRAD and DAIDS Medical Officers. The RSC Safety Office will then prepare the final report which will go to CONRAD for signature and submission to the FDA. Copies of this final report will be filed with CONRAD and RSC. Additionally, the RSC Safety Office will distribute safety reports to all DAIDS sites that use products under investigation in this study. 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 agents that must be considered in determining relationships of AEs requiring expedited reporting to DAIDS are: tenofovir gel, tenofovir film, and the study gel applicator.

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).

Participants Who Become Pregnant

Urine pregnancy tests will be performed during the investigational protocol. Participants who become pregnant in follow-up will be referred for obstetric care. The participant will be permanently discontinued and the following procedures will be performed at discontinuation:

complete metabolic panel and complete blood count. Pelvic exams will only be performed to evaluate a participant's reported symptom. No genital specimens will be collected in a pregnant participant. Staff will continue follow-up contact with the participant to obtain pregnancy outcome data. Of note, the participant will be encouraged to continue in the study so that safety data might be collected.

B.6.5 Clinical Management of Adverse Events

By definition, an adverse event can be either a 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 report 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 during the safety contact telephone call and in-clinic follow-up visits. Referral to appropriate care will be offered to participants as needed. Vaginally applied medications should not be used if possible.

B.6.5.1 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. Product will be permanently discontinued for this participant. If a participant experiences a grade 1 or 2 adverse event following product use, site investigators have the discretion to hold any subsequent product dosing until evaluation can take place in the clinic. The study product will continued to be held at investigator discretion until the condition resolves and/or the investigator deems it safe

to resume product use. If a participant experiences a grade 3 or 4 event following product dosing, the site will temporarily hold any subsequent study product dosings, notify the PSRT, and await its recommendation regarding further product dosing. Participants who discontinue study product will be encouraged to remain in the study for follow up evaluations for safety. With the exception of pregnancy and HIV seroconversion, as outlined in Section 8.4 above, since these are single dose administrations of study product formulations, the study team will attempt to complete all evaluations associated with that dosing formulation according to the study protocol, so long as there is no increased risk for the study participant.

B.6.6 Criteria for Early Termination of Study Participation

Participants may voluntarily withdraw from the study for any reason at any time. Participants will be withdrawn from the study if they test positive for HIV antibody or RNA, or become pregnant during the study. Any participant who tests positive for HIV while enrolled in the study will be immediately removed from the study and referred to the appropriate care provider for additional counseling and treatment as necessary. HIV 4th generation EIA and RNA test results will be available prior to any subsequent study intervention.

No additional doses of study drug will be given. Patients who become pregnant will not receive additional doses of study drug, and will be discontinued from the study, as described in Section B.6.4. Pregnancy outcomes will be collected for all study participants by contacting these women soon after anticipated parturition. After the participant's final study contact, any pregnancy outcomes that meet criteria for SAE reporting as described above (e.g., congenital anomalies) occurring among participants will continue to be expeditiously reported.

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.

B.7 Statistical Considerations

B.7.1 Review of Study Design

The primary aim of this study is to directly compare data among women receiving active microbicide in the form of a water-based gel, the delivery method most widely used in microbicide research currently, and novel thin film formulations of the microbicide. Urogenital, systemic and menstrual symptoms will be collected via symptom review with participants. Testing for sexually transmitted infections will occur at the screening visit to rule out other potential causes of reproductive tract inflammation and epithelial disruption. PK measures will assess local and systemic absorption of tenofovir following single exposure. PD measures include ex vivo HIV explant challenge. Primary outcomes will be comparison of PK and PD readouts of film compared to gel formulation. Similar to the design of FAME 02, FAME 02B, and FAME 04, we do not plan a placebo condition for PD assessments and, there is no need for a placebo for PK assessments. FAME 04 will provide placebo v. active arm data for multiple dosing of the TFV film and gel to provide some comparator data for safety outcomes, but this is not critical in this small, single dose PK/PD focused study.

B.7.2 Sample Size and Accrual

Sample size of 10 will allow us to exclude differences as large as 1.2 standard deviation units relative to the mean when comparing film to gel results with 80% power and 5% 2-sided Type I error. In our experience with these intensive PK designs, this sample size has been more than adequate to the exploratory nature of these studies. Research participants without complete key data, for whatever reason, may be replaced. Key data includes plasma, cervical biopsies, and cervicovaginal fluid from 80% of planned collection times, each. Research participants will be recruited from among prior research participants in similar types of studies. It is expected to take 3 months to accrue and 6 months to complete.

B.7.3 Study Endpoints

Primary Endpoint

Pharmacokinetic (PK)

- Systemic absorption of tenofovir
    - Systemic absorption of tenofovir will be determined following exposure as detected in plasma and PBMC samples as outlined in the study SOP's.
- Local absorption of tenofovir
    - Local absorption of tenofovir and metabolites will be determined following exposure as detected in genital tissue biopsy samples as outlined in the study SOP's.
- Persistence of tenofovir in cervicovaginal and rectal fluid
    - Persistence of tenofovir will be determined as detected levels of tenofovir in cervicovaginal and rectal fluid collected as outlined in the study SOPs.

Secondary Endpoints

Pharmacodynamic (PD)

- Ex vivo explant challenge with HIV
    - Cervical tissue will be challenged ex vivo with HIV and supernatant aliquots will be sampled over 14 days to determine p24 antigen concentration.
- Safety
    - Descriptive data describing adverse clinical and laboratory events will be categorized by treatment formulation.

B.7.4 Blinding

Complete concealment of allocation will not be feasible in this study. Participants and study staff will not be blinded to drug formulation.

B.7.5 Random Assignments

Johns Hopkins IDS will prepare a randomization sequence (either gel, then film or film, then gel) for subjects and dispense product according to that schedule.

B.7.6 Data Monitoring and Analysis

B.7.6.1 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 a double locked room. The research staff, under the direction of the primary investigator, will create and maintain an electronic database on the computers in the Drug Development Unit research offices. The database will be backed up every night onto the appropriate server's back-up system. Appropriate firewall and virus scanning software are installed and updated routinely by the information technology staff.

Case report forms will be used as the first point of data entry for this protocol. Study data management staff will manually review the forms for completeness and accuracy. If queries or

discrepancies are noted, the staff person will speak with the clinician in question as soon as possible to resolve the problem. The investigator will review the case report forms for completeness and accuracy at the end of the study.

B.7.6.2 Safety Assessment

Safety endpoints will be determined using all history, physical, and laboratory data prior to and following each of the product doses. The number of adverse events in each treatment 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. Differences in the proportion of participants experiencing adverse events among the treatment arms will be compared using Fisher's exact tests. However, this Phase I study may not have an adequate sample size to detect significant differences between treatment arms.

B.7.6.3 Analysis of PK Data

Blood levels of TFV and TFV-DP will be evaluated after vaginal administration. PK parameters for plasma and PBMC TFV and TFV-DP will be estimated (e.g., Cmax, Tmax, AUC, elimination rate constant) using WinNonlin (Pharsight, Inc., Cary, NC). All PK parameters for plasma and PBMC as well as paired assessments of other matrices will be compared between formulations using a multi-level analysis (STATA/IC 11.2 for Windows software, StataCorp LP, College Station, TX) to assess formulation, sequence, and individual effects.

B.7.6.4 Analysis of PD Data

HIV explant challenge will be assessed using cumulative HIV p24 measured in culture media supernatant. These values will be compared between formulations using a multi-level analysis to assess product, sequence, and subject effects. Concentration-response relationships will be

evaluated for PK-PD data using sigmoid Emax modeling among other pharmacometric methods (WinNonlin, Pharsight, Inc. Cary, NC).

B.8 Human Subject Considerations

The investigators will make every effort 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 and the protocol will have been submitted to the FDA. The investigators will permit audits by the NIH or the FDA or any of their appointed agents.

B.8.1 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.

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 because protocol-specified guidelines for abstinence and condom use are expected to protect male partners from exposure to the study product. In addition, based on both preclinical and clinical data, no toxicity is anticipated from the study product.

Children

The NIH has mandated that children be included in research trials when appropriate. This study will enroll women aged 18 and above who are able to give informed consent. This study meets

“Justifications for Exclusion” criteria for 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.

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.

Pregnant women

Pregnancy is an exclusion criterion because there are no current recommendations for the use of tenofovir during pregnancy. Prior to administration of study product, a urine pregnancy test will be performed on all women. During the informed consent process, women will be informed that tenofovir is not known to prevent pregnancy and that the effect of tenofovir on a developing human fetus is unknown. All potential participants will be required to use a reliable method of contraception as outlined in the Inclusion Criteria. Women who become pregnant during the study period following randomization and exposure to study product will not be excluded from analysis. Subjects will be followed during and after pregnancy to determine outcomes. Subjects found to be pregnant during the study will not receive any further tenofovir formulation dosing.

B.8.2. 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 encouraged to ask questions and to exchange information freely with the study team. Only listed research staff 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.

B.8.3 Risk/Benefit Statement

Risks

It is not expected that this trial will expose human subjects to unreasonable risk.

1% Tenofovir Gel

Administration of tenofovir gel intravaginally at 0.3% and 1% concentrations in the HPTN 050 Phase I study resulted in minimal local irritation and little or no systemic AEs were identified. Although 92% of participants reported at least 1 AE, 87% of those reported AEs were mild, and 70% of the AEs were limited to the genitourinary tract. Four severe AEs were reported, with only one, lower abdominal pain, thought to be product-related. The risks associated with tenofovir gel are believed to be less than those identified for systemic use. Some of the possible side effects of the study gel are dryness, itching, burning, or pain in the genital area.

In the HPTN 050 Phase I study of tenofovir gel, serum PK analysis in a subset of participants demonstrated that there is no clinically significant systemic toxicity. Fourteen of 25 women with PK results had low, but detectable, serum drug levels. Given that Phase I data demonstrate measurable plasma concentrations of tenofovir in some participants, participants with hepatitis B infection might be at risk for development of tenofovir resistant hepatitis B. However, participants with known hepatitis B infection will not be eligible for enrollment. It is not known what effect tenofovir gel could have on the HIV virus or HIV disease progression in HIV-

infected participants or their partners. There is a theoretical risk that tenofovir absorbed systemically from tenofovir disoproxil fumarate or vaginal tenofovir gel could result in mutations of the HIV virus in participants who become infected with HIV during the study, or their partner, if the partner is infected with HIV. Limited resistance data from HPTN 050 show no new resistance mutations in plasma or cervicovaginal lavage specimens after 14 days of tenofovir gel use. No participant had high level tenofovir mutations (e.g., K65R).

In a male tolerance study of 1% tenofovir gel, there were few genital findings observed after product use and all findings were classified as mild, small in size and required no treatment. The most common symptoms included mild pain (burning, irritation, discomfort) and pruritus. All reported urogenital symptoms were felt to be mild.

In CAPRISA 004, there were no SAEs deemed related to the use of study product. No renal disorders were observed in the study. Mild, self-limiting diarrhea was more common among women who used tenofovir gel (16.9%) compared to women who used the placebo gel (11.0%). No tenofovir resistance was observed among the women who became infected with HIV in the tenofovir group. No increase in hepatic flares was observed in participants infected with the hepatitis B virus. There were no safety concerns in the 54 pregnancies observed in the trial.

Tenofovir Film

This is the second human study of TFV film and the first study is just recently underway, therefore, no human safety data is available. In studies of a 40 mg and 20 mg film in macaques, the films were inserted into pigtailed macaques daily for five days one week, followed by four days the next week. Safety of repeated, daily exposures was measured by repeated colposcopic assessment, vaginal pH, vaginal smear and microbiology tracking. Colposcopy revealed similar tissue appearance in both TFV film arms compared to placebo. There were no indications of

product induced tissue disruption to vaginal or cervical mucosal surfaces. Vaginal microbiology assessments revealed similar shifts in flora prevalence across both the tenofovir formulations and the placebo study arm. Vaginal pH fluctuated similarly across all three study arms. Polymorphonuclear cell counts determined from Gram stained vaginal smears increased somewhat with exposure to the higher dose tenofovir formulation compared to the lower dose tenofovir and the placebo arms.

Collection of genital tissue by biopsy may cause discomfort and spotting. On rare occasions, chemical cauterization and or suturing may be required if hemorrhaging occurs. Phlebotomy or the intravenous catheter may lead to discomfort which may persist for the duration of the indwelling lock, 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. Confinement to the clinical research unit for a 12 hour period may cause boredom or discomfort.

B.8.4 Minimization of Risks

To minimize the risk of a product being dosed to the wrong subject, study personnel will confirm the IDS dispensed drug with the study subject identity, per hospital protocol. The clinical investigation sheet checklist will be signed off in real time to assure there is no redundant sampling outside the requirements of sampling called for by randomization.

B.8.5 Benefits

Research participants will derive no direct benefit from this single dose study. The risks of the study are reasonable in light of the anticipated future benefits of a TFV film product. The anti-

HSV-2 and anti-HIV-1 activity of 1% tenofovir vaginal gel observed in CAPRISA 004, 51% and 39% respectively, holds forth the prospect of a potential benefit for FAME-04 participants who are randomized to receive the tenofovir gel. Further research is anticipated to test whether CAPRISA 004 results will be confirmed. Participants and others may benefit in the future from information learned from this study. Specifically, information learned in this study may lead to the development of safe and effective interventions to prevent HIV transmission. Participants also may appreciate the opportunity to contribute to the field of HIV prevention.

Participants will receive HIV/STI risk reduction counseling, HIV and STI testing, physical exam, pelvic exam, and routine laboratory testing related to blood, liver, and kidney function. Participants may be provided or referred for STI treatment in accordance with CDC guidelines. For other medical conditions identified as part of the study screening and/or follow-up procedures, participants will be referred to other sources of care available in their community. Some volunteers may have the opportunity to access expedient treatment and decreased morbidity due to early diagnosis and treatment of abnormalities in serology, blood count, liver or kidney function tests. 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.

B.8.6 Compensation

Volunteers will not be charged for any of the study visits, study supplies or examinations. There are no costs to participants in this study, including overnight housing and transport provided by the study team. Using the Johns Hopkins Drug Development unit remuneration schedule, women will be compensated for their time and inconvenience while participating in the protocol.

This remuneration schedule is based on standardized payments related to the study visits and study-specific procedures.

B.8.7 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 double locked area. All computer information about study volunteers is kept on a computer with log-on passwords. The data management and clinical staff are the only personnel with access to the protected health information of study volunteers. 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. The computers used by the data groups are connected to the Johns Hopkins School of Medicine network, which is redundantly firewalled against penetration from the outside. Study-specific files are accessible only to staff of the Division of Clinical Pharmacology via a password-protected server. All research records will be kept for a minimum of five years following closure of this study.

B.8.8 Communicable Disease Reporting

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

B.8.9 Access to HIV-Related Care

The investigators do not expect a screening population at high risk for HIV infection. However, trained clinical staff will refer subjects who test positive or indeterminate via the HIV antibody screen test to a physician for follow-up testing and care. Participants who have positive or

indeterminate results will have standard post-test counseling as well as limited follow-up confirmatory testing provided by the study. 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. Various local resources are available for medical care for HIV positive patients, including the Moore Clinic at Johns Hopkins University. Subjects will also be offered Maryland Community Services Locator referral information. In addition to referral information, subjects will be provided with information for local resources for support groups and other services.

B.8.10 Study Discontinuation

NIAID, the International Partnership for Microbicides, the US FDA, other government or regulatory authorities, or the Johns Hopkins Medicine 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 gel or applicator, or any other condition deemed as an emergency event by the study staff, the External Safety Monitor will contact the Principal Investigator to initiate a temporary hold on further enrollment.

B.9 Laboratory Specimens and Biohazard Containment

B.9.1 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 7 indicates the designated laboratory for sample testing.

Table B1. Designated Labs for Testing

Test	Method	Laboratory
Urine	HCG, urinalysis	JHH Clinical Laboratory
Cervicovaginal fluid	Tenofovir concentration	CPAL* (non-diagnostic laboratory) Baltimore, MD
Cervical swab	Nucleic Acid Amplification test	JHH Clinical Laboratory
Plasma	Tenofovir concentration	CPAL (non-diagnostic laboratory) Baltimore, MD
PBMC	TFV-DP level	CPAL (non-diagnostic laboratory) Baltimore, MD
Rectal fluid	Tenofovir	CPAL (non-diagnostic laboratory) Baltimore, MD
Blood	Complete Metabolic Panel Complete Blood Count HCG, serum hepatitis B surface antigen	JHH Clinical Laboratory

	HIV infection screening (Appendix II) Syphilis Chemiluminesence assay (with confirmatory RPR; FTAABS performed on RPR negative samples)	
Cervical tissue homogenate	Tenofovir and TFV-DP concentration	CPAL (non-diagnostic laboratory) Baltimore, MD
Cervical Biopsy	HIV explant challenge	CPAL (non-diagnostic laboratory) Baltimore, MD

*CPAL, Clinical Pharmacology Analytical Laboratory, Division of Clinical Pharmacology, Johns Hopkins University

B.9.2 Urine Samples

A urinalysis will be used to screen for possible urinary tract infection when clinically indicated. Urine will be tested for qualitative HCG.

B.9.3 Cervical Samples

Biopsies to measure tenofovir drug concentrations and tissue explant susceptibility to HIV infection.

B.9.4 Cervical Swabs

C. trachomatis, N. gonorrhoeae, and trichomonas will be detected using an amplified DNA assay.

B.9.5 Cervicovaginal Fluid Samples

Using Standardized Operating Procedures for cervicovaginal fluid collection previously established by the Johns Hopkins Clinical Pharmacology Analytical Laboratory, samples will be obtained to measure tenofovir concentrations.

B.9.6 Rectal Fluid Samples

Using Standardized Operating Procedures (SOP's) previously established by the Johns Hopkins Clinical Pharmacology Analytical Laboratory, rectal fluid samples will be obtained via anoscopy to measure tenofovir concentrations.

B.9.7 Plasma Samples

Plasma samples will be sent to the Hopkins CPAL for assay of tenofovir concentration.

B.9.8 Quality Control and Quality Assurance Procedures

The Johns Hopkins Drug Development Unit and CPAL have a wealth of experience from taking part in several clinical studies of microbicides. Thus, all testing done in this research laboratory is performed with the same level of quality control as required in a licensed clinical laboratory. Because all of the proposed studies to be conducted in this project will be using an investigational product, the studies will be conducted under IND and additional measures will be undertaken to ensure that all protocols will be conducted under good laboratory practice. Tissue and fluid samples will be collected under previously established SOP's developed by the Johns Hopkins Clinical Pharmacology Analytical Laboratory (CPAL).

B.9.9 Specimen Storage and Possible Future Research Testing

Participants will be consented for future use of vaginal and cervical specimens and blood samples. Any leftover samples will be stored at CPAL for an indefinite period of time. The principal investigator will assume primary responsibility for control of this area.

Any results from research done on leftover specimens will not be placed in health records and will be kept confidential. Informed consent will give participants the option to withdraw their consent for use of their specimens for future research. The language and format employed in the screening and enrollment consents 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. When all laboratory assays have been completed and the study has been closed, the PI for the study will notify the clinical site Laboratory Manager to discard all samples from volunteers in the study who chose to have their samples destroyed at the end of the study. These samples will be discarded in the appropriate manner and the disposition will be documented.

Biohazard Containment

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

B.10 Administrative Procedures

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

B.10.1 Study Coordination

Study implementation will follow this protocol, which may not be amended without prior written approval from the Sponsor and DAIDS Medical Officer. Close coordination between protocol team members is necessary to track study progress, respond to queries about proper study

implementation, and address other issues in a timely manner. Rates of accrual, retention, follow-up, and AE incidence will be monitored closely by the team.

B.10.2 Study Monitoring

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 source document verification to ensure the accuracy and completeness of study data
- 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 of the IND application.

B.10.3 Protocol Compliance

Amendments to the protocol will require prior written approval from the principal investigators. All protocol amendments will be submitted for DAIDS review and approval facilitated by the DAIDS Regulatory Support Center (RSC). Once approval has been provided by the RSC, such amendments will be submitted to the Johns Hopkins Medicine Institutional Review Board (IRB) for final review and approval. Once the Johns Hopkins Medicine IRB has given final approval, the amendment can be implemented.

B.10.4 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 DAIDS. Applicable records include source documents, site registration documents and reports, correspondence, informed consent forms, and notations of all contacts with the participant.

B.10.5 Use of Information and Publications

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

If you are using Epic for this study, fax a copy of the signed consent form to 410-367-7382.

Patient I.D. plate

RESEARCH PARTICIPANT INFORMED CONSENT AND PRIVACY AUTHORIZATION FORM

Protocol Title: **Comparison of the Pharmacokinetics and Pharmacodynamics of Single Dose Tenofovir Vaginal Gel and Film Formulation (FAME-05) Version 1.0 (Dated July 31, 2015)**

Application No: **IRB00046617**

Sponsor: **Division of AIDS/ National Institute Of Allergy & Infectious Disease (NIAID)/ National Institutes of Health (NIH)**

Principal Investigator: **Craig W. Hendrix, MD**
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Baltimore, MD 21287
Phone: 410-955-9707
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1. What you should know about this study:

- You are being asked to join a research study. This consent form explains the research study and your part in it. Please read it carefully and take as much time as you need. Ask your study doctor or the study team to explain any words or information that you do not understand.
- You are a volunteer. If you join the study, you can change your mind later. There will be no penalty or loss of benefits if you decide to quit the study.
- During the study, we will tell you if we learn any new information that might affect whether you wish to continue to be in the study.
- If we think your participation in this study may affect your clinical care, information about your study participation will be included in your medical record, which is used throughout Johns Hopkins. Doctors outside of Johns Hopkins may not have access to this information. You can ask the research team to send this information to any of your doctors.
- When Johns Hopkins is used in this consent form, it includes The Johns Hopkins University, The Johns Hopkins Hospital, Johns Hopkins Bayview Medical Center, Howard County General Hospital, Johns Hopkins Community Physicians, Suburban Hospital, Sibley Memorial Hospital and All Children's Hospital.
- Biospecimens will be collected in this study. Biospecimens may include any of the following: blood, tissue, saliva, urine, bone marrow, cells, etc. Most biospecimens contain DNA, which is the genetic code for each person.

- A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.
- If you would like to review the information for this study, or a summary of the results, ask the study team doctor for the ClinicalTrials.gov study registration number.

2. Why is this research being done?

This research is being done to help develop a microbicide, which is a drug placed in the vagina to help prevent HIV transmission. This study will look to see how two vaginally-applied forms of an investigational drug called tenofovir gel and tenofovir film spreads into blood, cervical tissue (the tissue between the vagina and uterus or womb), and cervicovaginal fluid (a mixture of fluids from the vagina and cervix), and to gather information about the safety of the gel and film.

Tenofovir is an FDA approved drug that is currently used, in pill form, to treat HIV and to prevent HIV infection. Tenofovir gel and tenofovir film are called investigational drugs because tenofovir has not been approved for use in the gel or film form. The use of tenofovir gel and tenofovir film in this research study is investigational. The word "investigational" means that tenofovir gel and tenofovir film are not approved for marketing by the Food and Drug Administration (FDA). The FDA is allowing the use of tenofovir gel and tenofovir film in this study.

Tenofovir gel has previously been tested in humans, we have less information about testing of the tenofovir in the film form in humans. Importantly, all of the ingredients that make up the tenofovir film have been tested in humans and have been found to be safe.

Tenofovir works by preventing HIV from making copies of itself, thereby stopping the replication of HIV. This study is not testing to see if the study drugs prevent you from getting infected with HIV. Researchers do not yet know if these drugs when used in the vagina will work in humans to protect against HIV. The best way to protect against getting HIV infection during sex is to use a condom every time you have sex.

We are asking women to join this study who:

- Are at least 18 years of age
- Have had vaginal sex at least once
- Do not have HIV
- Are using a form of birth control (This may include: hormonal methods such as oral contraceptives or hormonal injections or implants used continuously for the past 30 days; intrauterine device (IUD [copper or hormonal] inserted at least 30 days prior to joining the study), female sterilization, or sexual activity with vasectomized partner. Women abstinent from sexual activity with a male partner for the past 30 days and who intend to remain abstinent throughout their period of study participation may also be enrolled.
- Have a womb (uterus)
- Are in general good health

The study will be done at the Drug Development Unit (DDU) of The Johns Hopkins Hospital.

How many people will be in this study?

Up to 10 women will enroll in and complete the study.

3. What will happen if you join this study?

If you agree to be in this study, we will ask you to do the following things:

Screening Visit (Visit 1):

This visit will last about 2 hours. It is performed to make sure that you do not have any health problems that might make some parts of the study more uncomfortable or dangerous for you. The following procedures will be performed during this visit:

- Complete medical history and general physical examination, including a review of any medications you may be taking and a review of your sexual practices.
- Complete GYN exam (pelvic exam) using a speculum (a device used to open the vagina). This will include collecting a sample of vaginal fluid with a swab from the opening of your womb (cervix) to be tested for the sexually transmitted infections called gonorrhea, chlamydia, and trichomoniasis. If you have one of these infections you can get treatment from your primary care doctor or another clinic and then you can be re-screened for the study. We will not provide treatment nor pay for treatment of these infections. The law requires us to report positive chlamydia and gonorrhea tests to the health department.
- Vital signs (including blood pressure, heart rate, temperature, height and weight)
- Collect urine for a urinalysis for routine safety testing.
- Collect blood (a little more than 1 tablespoon) for routine safety tests. The blood will be used to make sure that your blood count is good and that your blood clots normally. It will also be tested for liver and kidney function tests. As part of being in this study, you will have a test for syphilis and for HIV virus (the virus that causes AIDS). You will be given the State of Maryland HIV consent form as part of that process. If this test is positive, you will be referred for proper medical care and counseling. The law requires us to report positive tests to the health department.

If the screening tests show that it is safe for you to take part in the study, we will ask you to come back to start the study.

If you join the study, you must not have vaginal sex, nor insert any vaginal products or objects, including sex toys into your vagina for at least 3 days prior to the study visits. You will also be instructed to not have vaginal sex or put any vaginal products or objects into your vagina for 10 days after the gel or film dosing. Since there is one tenofovir film dose and one tenofovir gel dose, this means that you will need to avoid vaginal sex and not put any products or objects in your vagina for a total of 26 days while you are participating in the study.

During the study you will receive calls or emails from the study staff reminding you of your visits. We ask that you keep track of all medications you use during the study and bring these to every visit.

There will be two full day visits for the study. Depending on bed availability at the hospital, you may be required to stay overnight for the dosing visits. If beds are not available, each dosing visit will last about 13 hours. Each full day visit will be followed by five follow up visits.

Full Day Visits 2 and 8 (Day 0):

We will ask you to either, come to the hospital the night before your dosing visit and stay until the following evening or come to the research unit early in the morning and stay for about 13 hours.

The following procedures will be performed during this visit:

- You may have a physical exam
- Tell us about any changes in your health or medications.
- Pregnancy test
- Vital signs (blood pressure, heart rate and temperature)
- Intravenous (IV) catheter will be inserted into a vein and kept in place until you are discharged. This is done to help in the collection of blood during the visit. If necessary, the IV catheter may be removed prior to discharge and you may have your blood drawn with a needle instead.
- You will have your blood collected periodically throughout the day to measure the amount of study drug in your blood. Each blood sample will be about 1- 1½ teaspoons. There will be a total of 16 samples collected for a total of about 7 tablespoons of blood.
- You will receive the tenofovir film first (Visit 2). At your second dosing visit (Visit 8), you will receive the tenofovir gel.
- A study doctor will then insert a speculum and put the study drug into your vagina.
- A study doctor will insert a speculum and you will have cervicovaginal samples taken (a sample taken from the vagina and cervix using an absorptive device like a swab or sponge), and cervical biopsies (samples of tissue from your cervix) about 5 hours after the dose of study drug. This procedure usually takes about 15 minutes.
- You will also have a clear, plastic, lubricated tube gently inserted into the rectum (no more than 4 inches) in order to collect the rectal fluid with a sponge.
- You will be advised to avoid the use of spermicide and other non-study vaginal products (with the exception of tampons and female condoms) during the study.
- You will be asked to not insert anything into the vagina for up to 10 days after you receive the study drug.
- It is possible that you may see the tenofovir film coming out of your vagina. If you notice this, you should not try to put the film back in your vagina. If you notice any tenofovir film coming out of your vagina or in your underwear, place the film in a plastic Ziploc bag and return this bag to the study staff at your next clinic visit.

24 Hours (Day 1, Visits 3 and 9):

The following procedures will be performed during this visit:

- Vital signs, including blood pressure, heart beat, and temperature
- Blood collections (about 1 ½ tablespoons) to measure the amount of study drug in your blood.
- Tell us about any changes in your health or medications.
- You will be advised to avoid the use of spermicide and other non-study vaginal products (with the exception of tampons and female condoms) during the study.
- You will be asked to not insert anything in the vagina for up to 10 days after you receive the study drug.

48 Hours (Day 2, Visits 4 and 10):

The following procedures will be performed during this visit:

- Vital signs (blood pressure, heart rate and temperature)
- Blood collections (about 3 teaspoons) to measure the amount of study drug in your blood and to assess the health of your blood, liver and kidneys. You may have more blood taken if you are experiencing any problems.
- A urine sample may be collected to look for bladder or urinary tract infections.
- Tell us about any changes in your health or medications.
- You will be advised to avoid the use of spermicide and other non-study vaginal products (with the exception of tampons and female condoms) during the study.

- You will be asked to not insert anything in the vagina for up to 10 days after you receive the study drug.

72 Hours (Day 3, Visits 5 and 11):

The following procedures will be performed during this visit:

- Vital signs (blood pressure, heart rate and temperature)
- Tell us about any changes in your health or medications.
- Blood collections (about 1 teaspoon) to measure the amount of study drug in your blood.
- A study doctor will insert a speculum into your vagina and you will have cervicovaginal fluid samples and cervical biopsies taken as on Day 0.
- You will also have a clear, plastic, lubricated tube gently inserted into the rectum (no more than 4 inches) in order to collect the rectal fluid with a sponge.
- You will be advised to avoid the use of spermicide and other non-study vaginal products (with the exception of tampons and female condoms) during the study.
- You will be asked to not insert anything into the vagina for up to 10 days after you receive the study drug.

168 Hours (Day 7, Visits 6 and 12):

The following procedures will be performed during this visit:

- Vital signs (blood pressure, heart rate and temperature)
- Blood collections (about 3 teaspoons) to measure the amount of study drug in your blood and to assess the health of your blood, liver, and kidneys. You may have more blood taken if you are experiencing any problems.
- A study doctor will insert a speculum into your vagina and you will have cervicovaginal fluid samples taken as on Day 0 and Day 3. No biopsies are planned for this visit.
- You will also have a clear, plastic, lubricated tube gently inserted into the rectum (no more than 4 inches) in order to collect the rectal fluid with a sponge.
- A urine sample may be collected to look for bladder or urinary tract infections.
- Tell us about any changes in your health or medications.
- You will be advised to avoid the use of spermicide and other non-study vaginal products (with the exception of tampons and female condoms) during the study.
- You will be asked to not insert anything in the vagina for up to 10 days after you receive the study drug.

Follow-Up Visits (Visits 7 and 13):

About one week following Day 7, you will return to the clinic and the following procedures will be performed:

- You will tell us about any changes in your health and medications.
- You may have a physical exam.
- Vital signs, including blood pressure, heart beat, and temperature.
- Blood collections (about 3 teaspoons) to measure the amount of study drug in your blood and to assess the health of your blood, liver, and kidneys. You may have more blood taken if you are experiencing any problems. Your blood will again be tested for the HIV virus (the virus that causes AIDS). You will be given the State of Maryland HIV consent form as part of that process. If this test is positive, you will be referred for proper medical care and counseling. The law requires us to report positive tests to the health department.
- A urine sample will be collected for a pregnancy test and safety testing to identify bladder or urinary tract infections.

- You will be advised to avoid the use of spermicide and other non-study vaginal products (with the exception of tampons and female condoms) during the study.
- You will be asked to not insert anything in the vagina for up to 10 days after you receive the study product.

Request to collect and store biospecimens for future research

As part of this research study, we would like to ask you to let us store your biospecimens and health information for future research. This research could include other diseases, but will not include gene sequencing or the creation of cell lines.

If, in the future, we decide to analyze the samples, we will ask the IRB (a research review board that protects the rights and welfare of study participants) for permission first. There is no time limit on how long the samples will be stored, but if, in the future, we decide to analyze the samples, we will ask IRB permission first.

The study doctor can provide you with additional information if you have questions. Also, further information about our use of your biospecimens can be found in this consent document under the heading "*What happens to Data and Biospecimens that are collected in the study".*

You can still be in the study, even if you do not want your samples to be stored for future studies.

Please indicate below if you agree to allow us to store the biospecimens we collect for this study for use in future research*?*

YES _____ _____
Signature of Participant Date

NO _____ _____
Signature of Participant Date

How long will you be in the study?

You will be in this study for about 3 months.

Future Contact:

We would like your permission to contact you about other studies you may be eligible for in the future.

Please indicate below if you agree to be contacted for future research.

YES _____ _____
Signature of Participant Date

NO _____ _____
Signature of Participant Date

4. What are the risks or discomforts of the study?

Risks from study gel and film: The study products can cause some side effects. We do not yet know all the side effects of the film. Some, but not all women who used gel in other studies have had discharge from the vagina and irritation and discomfort.

Risks from the study drug:
Side effects reported from women in previous tenofovir gel studies include:

- Dryness, itching, burning feeling, irritation or pain in the genital area
- Lower abdominal pain
- Mild diarrhea

Risks of blood draws: Blood drawing may cause discomfort. You may feel dizzy or faint, or develop a bruise, swelling or infections where the needle is inserted. The total amount of blood collected during the entire study is less than 1 cup (about 13 tablespoons)

Risks of pelvic (GYN) exam and cervicovaginal fluid sampling: During pelvic exams and the cervicovaginal fluid sampling, you may feel discomfort or pressure in your vagina and/or pelvis. From the pelvic exam you may also have vaginal bleeding or spotting.

Risk of rectal sponge sampling: When rectal fluid is collected via insertion of the plastic tube into the anus, on rare occasion you may experience mild discomfort or have pain (should you have another condition that is already causing pain in the area)

Risks from STD Testing: You may become embarrassed, worried, or anxious when receiving STD counseling. You also may become worried or anxious while waiting for your test results or after receiving positive test results. It may also cause sadness or depression. HIV positive results have been associated with depression, suicidal thoughts, denial, and social isolation. Trained counselors will be available to help you deal with these feelings. Although the study site will make every effort to protect your privacy and confidentiality, it is possible that your involvement in the study could become known to others, and that social harms may result (i.e., because you could become known as "high risk" for HIV infection). For example, you could be treated unfairly or discriminated against, or could have problems being accepted by your family and/or community. In addition to referring you to treatment services, we can refer you to health or mental health services if you wish. However, Johns Hopkins and the NIH do not have funds to pay for treatment once you are referred.

By law, positive results from some STD tests have to be reported to local health authorities. This reporting may result in a potential loss of confidentiality.

Risks from intravenous (IV) catheters: Risks include pain from the needle stick, bruising, and bleeding, but these are usually mild. Sometimes a person can feel dizzy or faint when the IV catheter is being inserted. Other risks include infection, damage to the vein, blood clot, or stroke (if air enters the vein), but these are rare.

Risks from cervical biopsies: You may feel slight to moderate pain at the time of the biopsy (like being pinched) which usually resolves quickly, but could last a few hours. You may have spotting (small amounts of vaginal bleeding) for 1 – 2 days. There is a small risk of the biopsy area becoming infected or having bleeding that is heavier than spotting. It is important for you to know your body is healing for 24-48 hours after the biopsy is collected. However, if you have bleeding heavier than your usual menstrual

period, notice a foul odor or a heavier vaginal discharge (more than usual), you should contact the study clinic right away. To ensure that the biopsy sites heal, we will ask you to avoid vaginal intercourse and putting anything into your vagina (such as tampon, sex toy) for 7 days after the biopsy is collected (10 days after receiving the study product).

There may be side effects and discomforts that are not yet known.

5. Are there risks related to pregnancy?

Pregnant women cannot take part in this study. There are no current recommendations for the use of tenofovir during pregnancy. Tenofovir is not known to prevent pregnancy and the effect of tenofovir on the developing human fetus is unknown. In order to reduce these risks, you will be tested to determine if you are pregnant at the beginning of the study and this test will be repeated the day you receive the tenofovir gel or film. If any of these tests show that you are pregnant, you will not be allowed to continue in the study. It is important for you to let the study doctor know if you become pregnant or suspect that you are pregnant while taking part in this study.

This research may hurt an embryo or fetus in ways we do not currently know.

6. Are there benefits to being in the study?

There is no direct benefit to you from being in this study. If you take part in this study, you may help others in the future.

7. What are your options if you do not want to be in the study?

You do not have to join this study. If you do not join, your care at Johns Hopkins will not be affected.

8. Will it cost you anything to be in this study?

No.

9. Will you be paid if you join this study?

You will receive money for the time and inconvenience of being in the study. The amount you are paid will be based on the number of study visits, study doses, blood collections, cervicovaginal samples, and cervical biopsies.

You will receive $1,400 total for your participation in the study if you keep all appointments and follow the instructions of the study team. $30 of the total is a bonus which may be deducted in part or whole if you fail to keep your appointments on time or fail to follow instruction. All payments are made by check at the end of the study. If you withdraw before the study is completed, you will be paid only a portion of the $1,400 based on how much of the study you complete. For example, if you only receive one of the study products, provide the blood, biopsies and cervicovaginal samples associated with that dose and keep your follow-up appointment, you will receive $700, because this represents one-half of the study.

You may be required to provide your social security number to be paid for taking part in this study. Federal tax law requires that you report your research payments when you file your taxes. If your total payments from Johns Hopkins exceed $600 per year, Johns Hopkins will report these payments to the Internal Revenue Service and you will receive a 1099-MISC form from us.

10. Can you leave the study early?

- You can agree to be in the study now and change your mind later.
- If you wish to stop, please tell us right away.
- Leaving this study early will not stop you from getting regular medical care.

If you leave the study early, Johns Hopkins may use or give out your health information that it has already collected if the information is needed for this study or any follow-up activities.

11. Why might we take you out of the study early?

You may be taken out of the study if:

- Staying in the study would be harmful.
- You need treatment not allowed in the study.
- You fail to follow instructions.
- You become pregnant.
- The study is cancelled.
- There may be other reasons to take you out of the study that we do not know at this time.

If you are taken out of the study early, Johns Hopkins may use or give out your health information that it has already collected if the information is needed for this study or any follow-up activities.

If you become pregnant during the study, we will collect blood to evaluate your blood cell counts and liver and kidney function. We may also perform a pelvic examination if you report any symptoms at the time.

If we withdraw you from the study because further participation will be harmful, we will want to perform a physical evaluation of your condition for your safety. If you test positive for HIV during the study, we will counsel you regarding the results of the test, and will refer you to appropriate care providers for further counseling, follow-up and treatment if necessary.

12. How will your privacy be protected?

We have rules to protect information about you. Federal and state laws and the federal medical Privacy Rule also protect your privacy. By signing this form you provide your permission, called your "authorization," for the use and disclosure of information protected by the Privacy Rule.

The research team working on the study will collect information about you. This includes things learned from the procedures described in this consent form. They may also collect other information including your name, address, date of birth, and information from your medical records (which may include information about HIV status, drug, alcohol or STD treatment, genetic test results, or mental health treatment).

The research team will know your identity and that you are in the research study. Other people at Johns Hopkins, particularly your doctors, may also see or give out your information. We make this information available to your doctors for your safety.

People outside of Johns Hopkins may need to see or receive your information for this study. Examples include government agencies (such as the Food and Drug Administration), safety monitors, other sites in the study and companies that sponsor the study.

We cannot do this study without your authorization to use and give out your information. You do not have to give us this authorization. If you do not, then you may not join this study.

We will use and disclose your information only as described in this form and in our Notice of Privacy Practices; however, people outside Johns Hopkins who receive your information may not be covered by this promise or by the federal Privacy Rule. We try to make sure that everyone who needs to see your information keeps it confidential – but we cannot guarantee that your information will not be re-disclosed.

The use and disclosure of your information has no time limit. You may revoke (cancel) your permission to use and disclose your information at any time by notifying the Principal Investigator of this study by phone or in writing. If you contact the Principal Investigator by phone, you must follow-up with a written request that includes the study number and your contact information. The Principal Investigator's name, address, phone and fax information are on page one of this consent form.

If you do cancel your authorization to use and disclose your information, your part in this study will end and no further information about you will be collected. Your revocation (cancellation) would not affect information already collected in the study, or information we disclosed before you wrote to the Principal Investigator to cancel your authorization.

13. Will the study require any of your other health care providers to share your health information with the researchers of this study?

As a part of this study, the researchers may ask to see your health care records from your other health care providers.

14. What treatment costs will be paid if you are injured in this study?

Johns Hopkins and the federal government do not have programs to pay you if you are hurt or have other bad results from being in the study. However, medical care at Johns Hopkins is open to you as it is to all sick or injured people.

The costs for any treatment or hospital care you receive as the result of a study-related injury that are not covered by a health insurer will be billed to you.

By signing this form you will not give up any rights you have to seek compensation for injury.

15. What other things should you know about this research study?

a. What is the Institutional Review Board (IRB) and how does it protect you?

The Johns Hopkins Medicine IRB is made up of:

- Doctors
- Nurses
- Ethicists
- Non-scientists
- and people from the local community.

The IRB reviews human research studies. It protects the rights and welfare of the people taking part in those studies. You may contact the IRB if you have questions about your rights as a participant or if you think you have not been treated fairly. The IRB office number is 410-955-3008. You may also call this number for other questions, concerns or complaints about the research.

When the Johns Hopkins School of Medicine Institutional Review Board (IRB) reviews a study at another site, that site (institution) is solely responsible for the safe conduct of the study and for following the protocol approved by the Johns Hopkins IRB.

b. What do you do if you have questions about the study?
Call the principal investigator, Dr. Craig Hendrix, at 410-955-9707. If you wish, you may contact the principal investigator by letter or by fax. The address and fax number are on page one of this consent form. If you cannot reach the principal investigator or wish to talk to someone else, call the IRB office at 410-955-3008.

c. What should you do if you are injured or ill as a result of being in this study?
If you think you are injured or ill because of this study, call Ed Fuchs at pager 410-283-1075 during regular office hours.

If you have an urgent medical problem related to your taking part in this study, call Ed Fuchs at pager 410-283-1075 during regular office hours and after hours or on weekends.

After the tone, enter the phone number where you can be called, press the # key, and hang up.

d. What happens to Data and Biospecimens that are collected in the study?
Johns Hopkins and our research partners work to understand and cure diseases. The biospecimens and/or data you provide are important to this effort.

If you join this study, you should understand that you will not own your biospecimens or data, and should researchers use them to create a new product or idea, you will not benefit financially.

With appropriate protections for privacy, Johns Hopkins may share your biospecimens and information with our research sponsors and partners.

16. What does your signature on this consent form mean?

Your signature on this form means that:

- you understand the information given to you in this form
- you accept the provisions in the form
- you agree to join the study

You will not give up any legal rights by signing this consent form.

WE WILL GIVE YOU A COPY OF THIS SIGNED AND DATED CONSENT FORM

Signature of Participant	(Print Name)	Date/Time
Signature of Person Obtaining Consent	(Print Name)	Date/Time
Signature of Witness to Consent Procedures (optional unless IRB or Sponsor required)	(Print Name)	Date/Time

NOTE: A COPY OF THE SIGNED, DATED CONSENT FORM MUST BE KEPT BY THE PRINCIPAL INVESTIGATOR; A COPY MUST BE GIVEN TO THE PARTICIPANT; IF YOU ARE USING EPIC FOR THIS STUDY A COPY MUST BE FAXED TO 410-367-7382; IF YOU ARE NOT USING EPIC A COPY MUST BE PLACED IN THE PARTICIPANT'S MEDICAL RECORD (UNLESS NO MEDICAL RECORD EXISTS OR WILL BE CREATED).

ONLY CONSENT FORMS THAT INCLUDE THE JOHNS HOPKINS MEDICINE LOGO CAN BE USED TO OBTAIN THE CONSENT OF RESEARCH PARTICIPANTS.

APPENDIX C: Permissions to include published studies

FAME 02b Permission

FAME 05 Permission

FAME 02B Permission

RE: use of published manuscript in thesis

Ballen, Karen <KBallen@liebertpub.com>

Tue 2/27/2018 5:23 PM

To: Jenny Robinson <jrobin87@jhmi.edu>;

Dear Jennifer:

As you are the author of the original article, you do not need to request Copyright Permission.

Kind regards,

Karen Ballen
Manager

From: Jenny Robinson [mailto:jrobin87@jhmi.edu]
Sent: Tuesday, February 27, 2018 11:11 AM
To: Ballen, Karen <KBallen@liebertpub.com>
Subject: use of published manuscript in thesis

Good morning,

I authored the following manuscript which was published in the April 2017 edition of AIDS Research and Human Retroviruses. I am writing for more information about obtaining permission from the journal to include this manuscript in my PhD thesis dissertation at the Johns Hopkins Bloomberg School of Public Health. Based on the information I submitted to the Permissions webpage, it did not require additional permission but I would like to make sure I interpreted that correctly.

Manuscript in question: Robinson JA et al. Comparison of dapivirine vaginal gel and film formulation pharmacokinetics and pharmacodynamics (FAME 02B). AIDS Res Hum Retro; 2017:33(4):339-346.

Thank you in advance for your help.

Sincerely,

Jennifer Robinson

Jenny Robinson, MD, MPH, FACOG
Assistant Professor, Division of Family Planning
Department of Gynecology and Obstetrics
Johns Hopkins School of Medicine
jrobin87@jhmi.edu

FAME 05 Permission

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Title: Comparison of the Pharmacokinetics and Pharmacodynamics of Single-Dose Tenofovir Vaginal Film and Gel Formulation (FAME 05)

Author: Jennifer Robinson, Mark Marzinke, Edward Fuchs, et al

Publication: JAIDS: Journal of Acquired Immune Deficiency Syndrome

Publisher: Wolters Kluwer Health, Inc.

Date: Feb 1, 2018

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concept profiles of Multipurpose Prevention Technologies (MPTs) in
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85. Benson LS, Martins SL, Whitaker AK. Correlates of Heterosexual Anal Intercourse among Women in the 2006-2010 National Survey of Family Growth. *The journal of sexual medicine* 2015; **12**(8): 1746-52.
86. Waki K, Sugawara Y. Implications of integrase inhibitors for HIV-infected transplantation recipients: Raltegravir and dolutegravir (S/GSK 1349572). *BioScience Trends* 2011; **5**(5): 3.

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DEMOGRAPHIC AND PERSONAL INFORMATION

Born in Manila, Philippines in 1979

Current Appointments

University

2014-present Assistant Professor, Division of Family Planning, Department of Gynecology and Obstetrics, Johns Hopkins University School of Medicine

2014-present Assistant Professor, Division of Clinical Pharmacology, Department of Medicine, Johns Hopkins University School of Medicine

Hospital

2010-present Attending Physician, Johns Hopkins Bayview Medical Center

Personal Data

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Department of Gynecology and Obstetrics
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Baltimore, MD 21224
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Education and Training

Undergraduate

1997-2001 B.A. Biology and English Literature, University of Virginia, Charlottesville, VA

Doctoral/graduate

2002-2006 M.D., Tulane University School of Medicine, New Orleans, LA
M.P.H.T.M., Tulane University School of Public Health and Tropical Medicine, New Orleans, LA

Postdoctoral

2006-2007 Intern, Obstetrics and Gynecology, Drexel University/Hahnemann University Hospital, Philadelphia, PA

2007-2010 Resident, Obstetrics and Gynecology, Drexel University/Hahnemann University Hospital, Philadelphia, PA

2010-2012 Fellowship, Family Planning, Johns Hopkins University Bayview Medical Center, Baltimore, MD
2011-present PhD Candidate, Graduate Training Program in Clinical Investigation, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
2012-2014 Fellowship, Clinical Pharmacology, Johns Hopkins University School of Medicine, Baltimore, MD

Professional Experience
2011-2015 Staff physician, Planned Parenthood Maryland, Baltimore MD
2012-2015 Staff physician, Gynemed Surgical Center, Baltimore MD

PUBLICATIONS
Original Research
1. Nyirjesy P, **Robinson J**, Mathew L, Lev-Sagie A, Reyes I, Culhane JF. Alternative therapies in women with chronic vaginitis. Obstetrics and Gynecology. 2011;177(4):856-861.
2. Coleman JS, Fuchs E, Aung WS, Marzinke MA, Bakshi RP, Spiegel HM, **Robinson J**, Hendrix CW. Feasibility of radiolabeled small molecule permeability as a quantitative measure of microbicide candidate toxicity. Contraception. 2016;93(4):331-336.
3. **Robinson JA**, Marzinke MA, Bakshi RP, Fuchs EJ, Radebaugh CL, Aung W, Spiegel HM, Coleman JS, Rohan LC, Hendrix CW. Comparison of dapivirine vaginal gel and film formulation pharmacokinetics and pharmacodynamics (FAME 02B). AIDS Res Hum Retroviruses. 2016 Dec 13. doi: 10.1089/AID.2016.0040.
4. **Robinson JA,** Marzinke MA, Fuchs EJ, Bakshi RP, Spiegel HML, Coleman JS, Rohan LC, Hendrix CW. Comparison of the pharmacokinetics and pharmacodynamics of single-dose tenofovir vaginal film and gel formulation (FAME 05). J Acquir Immune Defic Syndr. 2018 Feb 1;77(2):175-182.

Review Articles
1. **Robinson JA**, Jamshidi R, Burke AE. Contraception for the HIV-positive woman: a review of interactions between hormonal contraception and antiretroviral therapy. Infectious Diseases in Obstetrics and Gynecology, 2012.doi:10.1155/2012/890160.
2. **Robinson JA**, Burke AE. Obesity and hormonal contraceptive efficacy. Womens Health. 2013;9(5):453-66.
3. Nanda K, Stuart GS, **Robinson J**, Gray AL, Tepper NK, Gaffield ME. Drug interactions between hormonal contraceptives and antiretrovirals: a systematic review. AIDS. 2017 Apr 24;31(7):917-952.
4. Rubin SL, **Robinson JA.** Bleeding associated with hormonal contraceptives: understanding and managing a common problem. Current Obstetrics and Gynecology Reports. 2017 July 22 (first online). DOI 10.1007/s13669-017-0219-x.

Other Media
1. Informational video entitled “Female Birth Control Options” on Johns Hopkins Bayview Medical Center webpage for Division of Family Planning.

FUNDING

Extramural Sponsorship

Research Extramural Funding – Current

6/29/06-11/30/20 A5338: An open-label, non-randomized study of pharmacokinetic interactions among depot medroxyprogesterone acetate (DMPA), rifampicin (RIF), and efavirenz (EFV) in women co-infected with human immunodeficiency virus (HIV) and tuberculosis (TB)
UM1AI068636
NIH/NIAID
PI: Rosie Mngqibisa
Role: Protocol Vice Chair

1/1/14-11/30/20 Laboratory Center (LC): Microbicide Trials Network
1-UM1AI106707-01, I/O 90057531
NIH/NIAID
Total direct cost per year: $222,282
PI: Craig Hendrix
Role: Co-I, 7%; I am involved in protocol development for several studies investigating novel products to prevent HIV transmission.

Research Extramural Funding – Pending – n/a

Research Extramural Funding – Previous

2011-2013 Assessing the contraceptive needs of HIV-positive adolescent females compared to an HIV-negative cohort
IRB Number NA_00050109
Society of Family Planning
Total direct costs: $66,420
Role: PI, 50% effort

2013-2017 Pharmacokinetics and pharmacodynamics of the etonogestrel implant when co-administered with efavirenz
IRB Number NA_00087585
American College of Obstetricians and Gynecologists/Bayer HealthCare Pharmaceuticals Research Fellowship in Long Acting Reversible Contraception
Total direct cost: $19,510
Role: PI

6/15/10-5/31/15 FAME (Film Antiretroviral Microbicide Evaluation)
U19AI0882639-03, JHU Subaward 4485
NIH/NIAID
Total direct costs: $2,809,788
PI: Craig Hendrix
Role: Co-I

10/1/15-9/30/17 Post Fellowship Salary Support
I/O 90065363
Susan Thompson Buffett Foundation
Total direct cost per year: $37,710
Role: PI, 20% effort; This funding provides salary support

Educational Extramural Funding – Previous

7/12-6/14 5T32GM066691-12 and 2T32GM066691-11
Postdoctoral Fellowship in Clinical Pharmacology, Johns Hopkins University SOM
NIGMS
Total direct costs: $251,742 and $235,590
PI: Theresa Shapiro, MD, PhD
Role: Fellow, 100% effort

Clinical Extramural Funding – n/a
System Innovation or Quality Improvement Extramural Funding – n/a
Other Extramural Funding – n/a

Intramural Funding

Research Intramural Funding – n/a
Educational Intramural Funding – Current

2015-present Genes to Society: Reproductive Health
JHU SOM
Role: Course Coordinator, 12% effort

Educational Intramural Funding – Previous – n/a
Clinical Intramural Funding – n/a
System Innovation or Quality Improvement Intramural Funding – n/a

CLINICAL ACTIVITIES

Clinical Focus – I am one of three attending physicians providing abortion and complex contraception care at JHBMC and JHH in the Division of Family Planning. We are a regional referral center for women seeking medical and surgical abortions, as well as reversible and permanent contraception. I also provide generalist OB/Gyn care in both inpatient and outpatient settings.

Certification

Medical, other state/government licensure

2010-present Maryland medical license #D0070561, expiration 9/30/19
2010-present DEA license #FR1880586, expiration 4/30/19
2010-present CDS license #M70770, expiration 4/30/19

Boards, other specialty certification

2010 American Board of Obstetrics and Gynecology Written Exam
2014 American Board of Obstetrics and Gynecology Oral Exam

Clinical (Service) Responsibilities

2010-present Johns Hopkins Bayview Medical Center – inpatient attending (Labor and Delivery, Gyn consult coverage, direct care and resident supervision, 2-3 days per month)

2011-present Johns Hopkins OB/Gyn at White Marsh – outpatient attending (4-6 days per month)
2012-present Women's Center for Family Planning, Johns Hopkins Bayview Medical Center – outpatient attending (3-5 days per month)
2014-present Johns Hopkins OB/Gyn at Bayview Medical Center – outpatient attending (~1 days per month)

Clinical Productivity (for FY18)
- In-Patient OR Volume: 2 in-state, 1 out-of-state
- Out-Patient OR Volume: 61 in-state, 2 out-of-state
- Out-Patient Office Visits
    - Bayview: 45 in-state, 1 out-of-state
    - Women's Center for Family Planning: 115 in-state, 1 out-of-state
    - White Marsh: 166 in-state, 4 out-of-state
- Deliveries: 49
- RVUs: 2450

Clinical Draw – n/a
Membership in or examiner for specialty board – n/a

Clinical Program Building / Leadership
2015-present Ryan Program Director, Johns Hopkins Bayview Medical Center – supervise resident training in contraception and abortion, with focus on postpartum and post-abortion contraception

Clinical Demonstration Activities
2011-present Presented didactics and hands-on training in manual vacuum aspiration and IUD insertion to medical and nursing students 1-2 times per year, JHU SOM
2012 Led a manual vacuum aspiration training, Edward Via College of Osteopathic Medicine, Blacksburg VA

Development of nationally/internationally recognized clinical standard of care – n/a

EDUCATIONAL ACTIVITIES
Educational Focus – I have been involved in direct teaching and training of medical students and residents in various aspects of contraception and abortion care, as well as general OB/Gyn. Since taking over as course director for the Reproductive Sciences block of the Genes to Society undergraduate medical school curriculum, I am now responsible for the medical students' foundational knowledge of the reproductive system. I am involved in teaching during the OB/Gyn clerkship, both in a classroom setting and in conjunction with direct patient care. I have also done lectures and demonstrations on topics related to women's health and family planning at the Johns Hopkins Bloomberg School of Public Health and School of Nursing.

Teaching
Classroom instruction

JHMI/Regional

2011-present Lecturer, medical student lectures on contraception and abortion (given once during every 8-week rotation), JHU SOM, Baltimore MD

2/3/12 Guest Lecturer, Clinical Aspects of Reproductive Health – "Abortion", JHSPH, Baltimore MD

3/3/14, 4/3/15 Guest Lecturer, Adolescent Sexual and Reproductive Health – "Writing for Advocacy", JHSPH, Baltimore MD

11/4/14 Small Group Preceptor, SFM Pharmacology, JHU SOM, Baltimore MD

12/2/14 Journal Club Preceptor, SFM Pharmacology, JHU SOM, Baltimore MD

2016-2018 Guest Lecturer, Family Planning Policies and Programs (SPH 380.655.01) – "Contraception: Today and Tomorrow", JHSPH, Baltimore MD

1/16-present Course Director, Genes to Society – Reproductive Sciences, JHU SOM, Baltimore MD
Lectured 2nd year medical students on a variety of female reproductive health topics, led small group and team-based learning sessions, and coordinated the overall course

7/11/17 Guest Lecturer, Advanced Practice in Women's Health (NR 110.569) – "Benign Pelvic Masses", JHU SON, Baltimore MD

5/10/18, 5/14/18 Guest Lecturer, Organ Physiology (ME 360.720), Department of Physiology – "Female Reproductive Physiology" and "Contraception and Assisted Reproductive Technology", JHU SOM, Baltimore MD

National None
International None

Clinical instruction

JHMI/Regional

7/10-6/12 Fellow; residents and 3rd and 4th year medical students. Inpatient and outpatient OB/Gyn and Family Planning services. Johns Hopkins Bayview Medical Center

7/12-present Attending; fellows, residents, and 3rd and 4th year medical students. Inpatient and outpatient OB/Gyn and Family Planning. Johns Hopkins Bayview Medical Center

2/14-present Transition to the Wards: led sessions on proper surgical scrub technique for medical students entering clinical clerkships; JHU SOM

6/14-present Selective Director for Medical Student Selective in Family Planning; JHU SOM

National - n/a
International – n/a

CME instruction

JHMI/Regional

10/20/14 Guest Lecturer, "We've Got You Covered: Immediate Postpartum Long-Acting Reversible Contraception", Medstar St. Mary's Hospital, Leonardtown MD

4/13/15 Presenter, "Updates in Contraception for Women Living with HIV", 25th Annual Clinical Care of the Patient with HIV Infection and Care of the Patients with Viral Infections, Baltimore MD

National

01/18 Association of Professors in Gynecology and Obstetrics Faculty Development Seminar, Manalapan FL
"Knowing What I Live By: Learn to Lead a Values Clarification"
Led a workshop instructing OB/Gyn educators on how to facilitate a values clarification with a variety of learners (medical students, residents, office staff, nurses, etc.)

Workshops/seminars – n/a

Mentoring

Pre-doctoral Advisees / Mentees

2015-present Maya Seigel, Johns Hopkins School of Medicine Class of 2019

Post-doctoral Advisees / Mentees

7/15-present Stacy Sun, MD, Gynecology and Obstetrics Class of 2018, JHU SOM
7/15-present Sunitha Suresh, MD, Gynecology and Obstetrics Class of 2019, JHU SOM
7/18-present Kristen Lee, MD, Gynecology and Obstetrics Class of 2022

Advisees – n/a
Thesis committees – n/a

Educational Program Building/Leadership

07/14-present Selective Director, Selective in Family Planning, OB/Gyn Clerkship, JHU SOM
Organize and coordinate a 2-week selective for 3rd year medical students as part of their OB/Gyn clerkship. In addition to organizing the students' activities while on the selective, I also provide direct clinical instruction.

11/15-present Course Director (12% effort), Reproductive Sciences, Genes to Society, JHU SOM
As course director, I am responsible for the overall management of this 4-week course that addresses male and female reproductive systems. This involves developing the overall curriculum related to reproductive health, managing the course schedule, and coordinating presenters. During the course itself, I am responsible for delivering several lectures on a variety of topics, leading small group discussions and interactive sessions, and proctoring quizzes and exams.

06/18-present Site Director, Visiting Resident Rotation for OB/Gyn Residents from Walter Reed National Military Medical Center

09/18-present Member, Education Policy and Curriculum Committee, Johns Hopkins School of Medicine
Represent Genes to Society, Year 2 curriculum

Educational Demonstration Activities to external audience – n/a

RESEARCH ACTIVITIES

Research Focus

- Contraception for women living with HIV

- Drug interactions
- Clinical pharmacology
- Microbicides

Research Program Building / Leadership – n/a
Research Demonstration Activities – n/a
Inventions, Patents, Copyrights – n/a
Technology Transfer Activities – n/a

SYSTEM INNOVATION AND QUALITY IMPROVEMENT ACTIVITIES
System Innovation Focus – n/a

System Innovation and Quality Improvement efforts within JHMI:
2016-2017 Beta Team member, Gyn/OB Departmental effort to improve processes for managing patients with early pregnancy failure or pregnancies of unknown location

ORGANIZATIONAL ACTIVITIES
Institutional Administrative Appointments – n/a

Editorial Activities
Editorial Board Appointments – n/a

Journal peer review activities
2014-present Obstetrics and Gynecology
5/15-present PLoS ONE

Other peer review activities – n/a

Advisory Committees, Review Groups/Study Sections
10/14-present Member, AIDS Clinical Trials Group Clinical Pharmacology Advisory Group

Professional Societies
2006-present American College of Obstetricians and Gynecologists
2006-2014 Junior Fellow
2014-present Fellow
2009-present Member, Association of Reproductive Health Professionals
2010-present Junior Fellow, Society of Family Planning
2011-present Member, National Abortion Federation
2011-present Member, Physicians for Reproductive Health
2017-present Member, Association of Professors of Gynecology and Obstetrics

Conference Organizer – n/a
Session Chair – n/a

Consultantships

7/2015-4/2017 Member, World Health Organization Technical Consultation on drug interactions between hormonal contraceptives and antiretroviral medications

RECOGNITION

Awards, Honors

2016 Nominated for W. Barry Wood Jr. Award for Excellence in Teaching
This award is given annually to two faculty members from the pre-clinical medical school curriculum who are recognized as being the most inspirational and/or effective teachers by students.

2017 Semi-Finalist for the Distinguished Teaching Society of JHUSOM

2018 Nominated for George J Stuart Award for Outstanding Clinical Teaching
This award is given annually by the senior medical school class to an outstanding clinical teacher in the school of medicine.

2018 Semi-Finalist for the Distinguished Teaching Society of JHUSOM

2018 Excellence in Teaching and Mentorship Award from the Gyn/OB Residency Class of 2018, JHUSOM

2018 APGO Excellence in Teaching Award, Department of Gynecology and Obstetrics, JHUSOM

2019 Excellence in Teaching and Mentorship Award from the Gyn/OB Residency Class of 2019, JHUSOM

Invited Talks

JHMI/Regional

6/14/11 Grand Rounds Speaker, Drexel University College of Medicine/Hahnemann University Hospital Department of Obstetrics and Gynecology, "Emergency Contraception Update", Philadelphia PA

12/10/11 Speaker and trainer, Student National Medical Association Regional Conference, "Long-acting Reversible Contraceptives", Baltimore MD

2/1/14 Speaker, Law Students for Reproductive Justice Mid-Atlantic/Northeast Regional Conference, "Being an Abortion Provider", Washington DC

7/29/14 Speaker, Urban Health Residency Noon Conference, Johns Hopkins University SOM, "Politics and Reproductive Health in the United States: A Sobering Update", Baltimore MD

9/3/14 Speaker, Internal Medicine Resident Education, Johns Hopkins Bayview Medical Center, "Contraceptive Methods"

4/13/15 Presentation at regional HIV conference

4/3/18 Grand Rounds Speaker, Drexel University College of Medicine/Hahnemann University Hospital Department of Obstetrics and Gynecology, "Multipurpose Prevention Technologies: New solutions for old problems", Philadelphia PA

10/11/18 Grand Rounds Speaker, Johns Hopkins University School of Medicine, Department of Gynecology and Obstetrics, "Family Planning Updates", Baltimore MD

10/25/18 Grand Rounds Speaker, Sinai Hospital, Department of Obstetrics and Gynecology, "Family Planning Updates", Baltimore MD

National

11/5/11 Medical Students for Choice National Conference, Baltimore MD
Panelist, "Being an Abortion Provider
Trainer, "Manual Vacuum Aspiration Workshop"
10/25/14 Presenter, Doctors for America National Leadership Conference, "Advocacy Skills for Abortion Providers", Baltimore MD

International
11/30/11 International Conference on Family Planning, Dakar, Senegal
Trainer, Contraceptive Implant Insertion and Removal, Clinical Training in Long-Acting and Permanent Methods and Injectables
Panelist, post-abortion provision of long-acting reversible contraceptive methods
11/14/13 International Conference on Family Planning, Addis Ababa, Ethiopia
Presenter, Contraception for Special Populations
Trainer, Contraceptive Implant Insertion and Removal

OTHER PROFESSIONAL ACCOMPLISHMENTS

Posters
4/28/14 Robinson JA, Fox MC, Jamshidi R, Trent M, Anderson J, Burke AE. Contraceptive needs of HIV-infected adolescent women: a qualitative analysis. American College of Obstetricians and Gynecologists Annual Clinical Meeting, Chicago IL
4/28/14 Robinson, JA, Fox MC, Jamshidi R, Trent M, Anderson J, Burke AE. Contraceptive needs of HIV-positive adolescent women compared to an HIV-negative cohort. American College of Obstetricians and Gynecologists Annual Clinical Meeting, Chicago IL

Community Services
Oct. 2014-March 2015 Volunteered on Reproductive Health Van, Baltimore City Health Department, Baltimore MD

Humanitarian Activities
May-June 2012 Volunteer with American Refugee Committee, Tak Province, Thailand

2011-2012 Leadership Training Academy, Physicians for Reproductive Health, Washington DC and New York NY