MATRIX-001

A Phase I Randomized, Placebo-Controlled, Double-Blind Study to Assess Safety, Pharmacokinetics, and Modeled Pharmacodynamics of a Vaginal Insert Containing Tenofovir Alafenamide and Elvitegravir

MATRIX: A USAID Project to Advance the Research and Development of Innovative HIV Prevention Products for Women

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4 May 2023

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MATRIX
Advancing R&D of Innovative
HIV Prevention Products for Women

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LIST OF ABBREVIATIONS AND ACRONYMS

ACRO	African Clinical Research Organisation
AE	Adverse event
AIDS	Acquired immunodeficiency syndrome
API	Active pharmaceutical ingredient
bNAb	Broadly neutralizing antibodies
BV	Bacterial vaginosis
BXV	Vaginal biopsy
BXC	Ectocervical biopsy
CBC	Complete blood count
CDC	Centers for Disease Control and Prevention (US)
CDM	Clinical Data Manager
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Act
CM	Concomitant medication
CRA	Clinical Research Associate
CRF	Case report form
CRM	Clinical Research Manager
CT	*Chlamydia trachomatis*
CV	Cervicovaginal
CVF	Cervicovaginal fluid
CVL	Cervicovaginal lavage
CVT	Cervicovaginal tissue
DAIDS	Division of AIDS
DRA	Drug regulatory authority
D2D	Design to Delivery
eCRF	Electronic case report form
EVG	Elvitegravir (Vitekta®)
EVMS	Eastern Virginia Medical School
FDA	U.S. Food and Drug Administration

FDC	Fixed dose combination
FTC	Emtricitabine (Emtriva®)
FWA	Federal-wide assurance
GC	*Neisseria gonorrhea*
GCP	Good Clinical Practice
GDP	Good Documentation Practice
GI	Gastrointestinal
GMP	Good Manufacturing Practice
HBsAg	Hepatitis B surface antigen
HIPAA	The Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
IB	Investigator's Brochure
ICH	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
IHC	Immunohistochemistry
IND	Investigational New Drug
INSTI	Integrase strand transfer inhibitor
IRB	Institutional Review Board
ISP	Independent Safety Physician
IUD	Intrauterine device
KOH	Saline/potassium hydroxide
KPPB	Kenya Pharmacy and Poisons Board
LDMS	Laboratory Data Management System
LTFU	Loss-to-follow-up
MPA	Multiple Project Assurance
NDA	New Drug Application
NIH	National Institutes of Health
NSAID	Nonsteroidal Anti-inflammatory Drug
NOAEL	*No-observed-adverse-effect-level*
PBMC	Peripheral blood mononuclear cells
PD	Pharmacodynamics
PEP	Post-exposure prophylaxis
PI	Principal Investigator
PK	Pharmacokinetics

PrEP	Pre-exposure prophylaxis
QA	Quality assurance
QC	Quality control
RF	Rectal fluid
RTI	Reproductive tract infection
SAE	Serious adverse event
SAHPRA	South African Health Products Regulatory Authority
SAP	Statistical analysis plan
SOP	Standard operating procedures
SSP	Study specific procedures
STI	Sexually transmitted infection
TAF	Tenofovir alafenamide (Vemlidy®)
TAM	Thymidine analog mutation
TDF	Tenofovir disoproxil fumarate (Viread®)
TEAE	Treatment-emergent adverse effect
TESAE	Treatment-emergent serious adverse effect
TFV	Tenofovir
TV	*Trichomonas vaginalis*
UPT	Urine pregnancy test
UTI	Urinary tract infection
VF	Vaginal fluid

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A Phase I Randomized, Placebo-Controlled, Double-Blind Study to Assess Safety, Pharmacokinetics, and Modeled Pharmacodynamics of a Vaginal Insert Containing Tenofovir Alafenamide and Elvitegravir

INVESTIGATOR SIGNATURE FORM

Version 1.0; 4 May 2023

A Study of the Microbicide R&D to Advance HIV Prevention Technologies through Responsive Innovation and eXcellence (MATRIX) Collaborative

Funded by:
US Agency for International Development (USAID)

IND Sponsor:
CONRAD

I, the Investigator of Record, agree to conduct this study in full accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (US) Health and Human Service regulations (45 CFR 46); USAID regulations (2 CFR 200 and 22 CFR 225); applicable U.S. Food and Drug Administration regulations; standards of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice Guidance (E6); Institutional Review Board/Ethics Committee determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (e.g., USAID) and institutional policies.

I agree to maintain all study documentation for at least two years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least two years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the sponsor. CONRAD will inform the investigator/institution as to when these documents no longer need to be retained.

I have read and understand the information in this protocol and in the Investigator's Brochure(s), including the potential risks and side effects of the products under investigation, and will ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about the obligations incurred by their contribution to the study.

_____
Name of Investigator of Record (print)

_____ _____
Signature of Investigator of Record Date

MATRIX-001

A Phase I Randomized, Placebo-Controlled, Double-Blind Study to Assess Safety, Pharmacokinetics, and Modeled Pharmacodynamics of a Vaginal Insert Containing Tenofovir Alafenamide and Elvitegravir

PROTOCOL SUMMARY

Short Title: Safety and PK study of Tenofovir Alafenamide (TAF) and Elvitegravir (EVG) Vaginal Insert

Clinical Phase: Phase 1

IND Sponsor: CONRAD

Funders: USAID

Protocol Co-Chair: Leila E. Mansoor, B.Pharm, PhD

Protocol Co-Chair: Nelly R. Mugo, MBChB, MMed, MPH

Sample Size: MATRIX-001 will enroll approximately 60 evaluable participants

Study Population: Healthy, non-pregnant, HIV-uninfected women aged 18-50 years at low risk for HIV acquisition

Study Sites: Three sites in the United States, South Africa and Kenya:
Eastern Virginia Medical School (EVMS);
Centre for the AIDS Programme of Research in South Africa (CAPRISA); and Kenya Medical Research Institute (KEMRI).

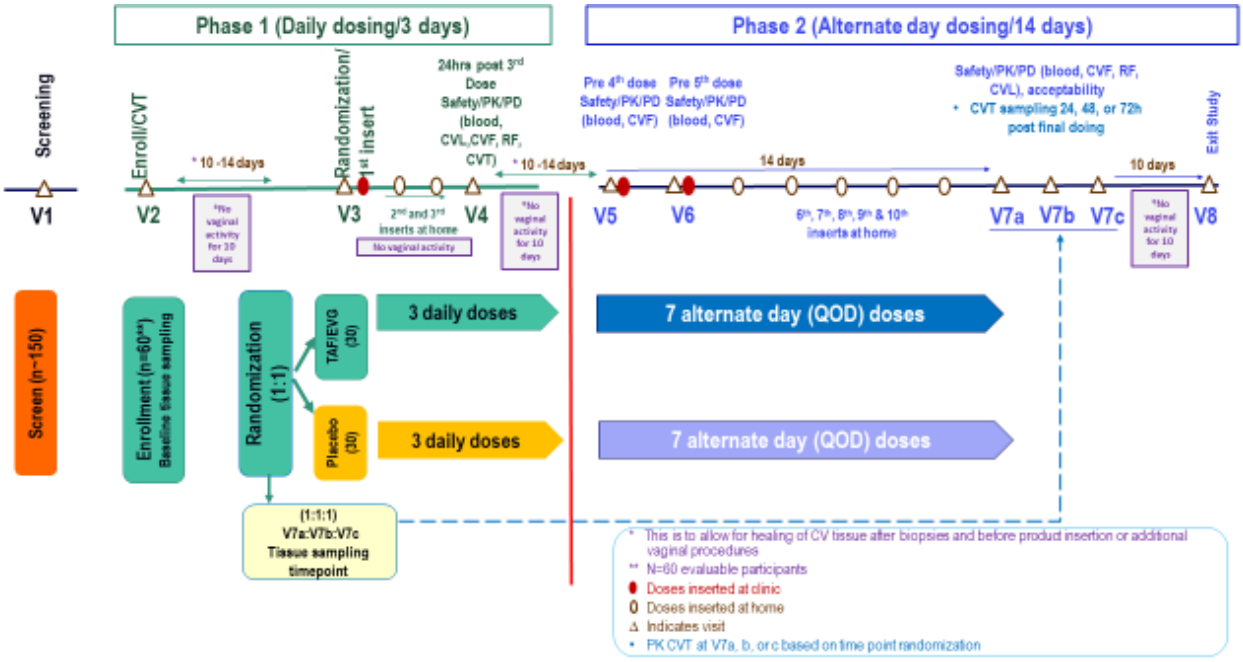
Study Design: Phase 1, multi-site, randomized, placebo-controlled, double-blinded study of vaginal administration of the TAF/EVG insert

Study Duration: The total duration of the study will be approximately 12 months. Accrual will occur over approximately 8 months, with approximately 2-3 months of follow-up per participant.

Study Products: Vaginal insert containing TAF and EVG, 20/16 mg
Placebo vaginal insert

Study Regimen: Participants will be randomized (1:1) to apply either a placebo or TAF/EVG insert vaginally once daily for 3 consecutive days and every other day (QOD) for 14 days. Participants will also be randomized (1:1:1) to 3 different tissue sampling time points post-treatment (24hr, 48hr and 72hr after the last dose).

Figure 1: Study Visit Schedule



Primary Objective:

Safety

- To evaluate the urogenital and systemic safety of the TAF/EVG insert (20/16 mg) administered vaginally in multiple doses, with emphasis on adverse events developing in the cervicovaginal tract

Primary Endpoints:

Safety

- Changes after dosing:
  - Any treatment-emergent adverse events (TEAE), Grade 2 or higher

Secondary Objectives:

Pharmacokinetics (PK)

- To characterize the multi-compartmental PK profile of the TAF/EVG insert administered vaginally in multiple doses

Modeled *in vitro* Pharmacodynamics (PD)

- To characterize the anti-HIV activity of the TAF/EVG insert in cervicovaginal fluid (CVF) administered vaginally in multiple doses
- To characterize the anti-herpes simplex virus (HSV) activity of the TAF/EVG insert in CVF administered vaginally in multiple doses

Acceptability

- To explore user experiences and identify product attributes considered likely to challenge and/or facilitate future sustained use of the TAF/EVG insert applied vaginally.

Subclinical Safety and Vaginal Microbiome

- To characterize changes from randomization in soluble and cellular cervicovaginal (CV) mucosal markers after multiple dose vaginal administration of the TAF/EVG insert
- To characterize changes from baseline in the vaginal microbiota after multiple dose vaginal administration of the TAF/EVG insert

Secondary Endpoints:

Pharmacokinetics (PK)

- Concentrations of tenofovir (TFV), TAF and EVG in plasma after dosing
- Concentrations of TFV, TAF and EVG in CVF and rectal fluid (RF) after dosing
- Concentrations of TFV, tenofovir diphosphate (TFV-DP), TAF and EVG in cervicovaginal tissue (CVT) after dosing

Modeled *in vitro* PD

- Anti-HIV activity in CVF obtained at baseline and after dosing
- Anti-HSV activity in CVF obtained at baseline and after dosing

Acceptability

- Responses to key questions on acceptability (e.g., satisfaction, comfort with insertion, willingness to use) at enrollment and after dosing

Subclinical Safety and Vaginal Microbiome

- Changes in HIV-1 target immune cell phenotype (e.g., CD45, CD68, CD3, and CD1a) and HIV-1 activation/proliferation markers (e.g., HLA-DR) in CVT
- Changes in soluble markers of innate mucosal immunity and inflammatory response in CVF (e.g., IL-1α, IL-6, IL-10, TNFα, RANTES, MIP-1α, IP-10, GM-CSF, IL-8, IL-1RA, SLPI, and BD2)
- Changes in the Nugent Score, relative abundance of vaginal microbial species and community state type (CST) measured by 16S RNA sequencing

Exploratory Objective:

Modeled Tissue PD

- To characterize the anti-HIV activity of the TAF/EVG insert in CVT administered vaginally in multiple doses

Exploratory Endpoints:

Modeled Tissue PD

- p24 antigen production in CVT infected with HIV-1 *ex vivo*, at baseline and after dosing

1 KEY ROLES

1.1 Protocol Identification

Protocol Title:	A Phase I Randomized, Placebo-Controlled, Double-Blind Study to Assess Safety, Pharmacokinetics, and Modeled Pharmacodynamics of a Vaginal Insert Containing Tenofovir Alafenamide and Elvitegravir
Protocol Number:	MATRIX-001
Short Title:	Safety and PK study of TAF/EVG Vaginal Insert
Date:	4 May 2023

1.2 Funding Agency, IND Sponsor and Monitor Identification

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2 INTRODUCTION

2.1 Background

Adolescent girls and young women (AGYW) bear the burden of the HIV-1 epidemic, with more than 59% of new infections occurring in women in sub-Saharan Africa (SSA).[1] The daily dosing requirement and systemic side effects, primarily gastrointestinal (GI), have made it difficult, particularly for AGYW, to adhere to daily tenofovir disoproxil fumarate (TDF)/emtricitabine (FTC) for HIV-1 prevention.[2, 3]

Although long-acting pre-exposure prophylaxis (PrEP) regimens, such as injectables and implants, will likely improve adherence over daily pill ingestion, there is clear evidence that many individuals do not want to use continuous systemically administered products and want discreet, **on demand, user-controlled** products. In SSA, adolescents and young people desire and even prefer on-demand HIV prevention options.[4] Across multiple end-user studies a subset of women consistently indicate a preference for on-demand HIV and multipurpose prevention technologies (MPT) over daily use and even longer-acting products.[5, 6] In recent stakeholder consultations conducted by the MATRIX Design to Delivery (D2D) Hub in Zimbabwe, Kenya, and South Africa, more than 80 percent of the respondents, mostly young women, indicated their support for on-demand HIV prevention products of the kind MATRIX is developing. Specifically, more than 90% of the stakeholders in all three countries expressed support for the vaginal insert to be tested in MATRIX 001. Similarly, recent acceptability data from the contraceptive field, among AGYW in both high- and low-income countries, indicate that many prefer an on-demand, female controlled, peri-coital contraceptive,[7-12] rather than a daily regimen or even a long-acting product.

Topical inserts have the potential for increased adherence as they are easy to store and transport, discreet, and convenient and easy to use. Vaginal or rectal administration of HIV prevention products provides local absorption of drug, enhanced bioavailability, high concentration at portals of virus entry, decreased systemic side effects, and reduced dosing frequency, all of which would increase adherence and persistence.[13-17]

While there are several US Food and Drug Administration (FDA) approved antivirals to reduce HSV-2 shedding from an infected individual's genital tract and reduce the duration of painful HSV-2 flare ups, *there is no approved HSV-2 primary prevention product.* HSV-2 is the most common cause of genital ulcers and is the most prevalent viral sexually transmitted infection (STI) in the US and world-wide, with an estimated 417 million people worldwide living with HSV-2.[18, 19] SSA is the most severely affected region of the world with up to 80% of sexually active women infected with HSV-2 by age 35 years.[20] There are substantial data that HSV-2 and HIV-1 infections are synergistic, with asymptomatic shedding of HSV-2 causing increased susceptibility to HIV-1 and increased shedding of HSV-2 among HIV-1 infected individuals (reviewed in [21]). The development of an HIV-1 and HSV-2 primary prevention MPT would have a significant public health impact, especially for AGYW.

Through a robust product development process incorporating iterative end-user and human-centered design research and pre-clinical and clinical data supporting vaginal and rectal safety, PK, and protection against HIV (and HSV), CONRAD has developed a dual-compartment MPT insert containing two potent and synergistic antiretrovirals, tenofovir alafenamide (TAF) and

elvitegravir (EVG) for the prevention of HIV and HSV acquisition. This fast-dissolve insert is ready for advanced clinical development through an extended safety and PK clinical study as proposed herein. MATRIX-001 is a multi-dose vaginal safety and PK study of this novel, on-demand, TAF/EVG insert.

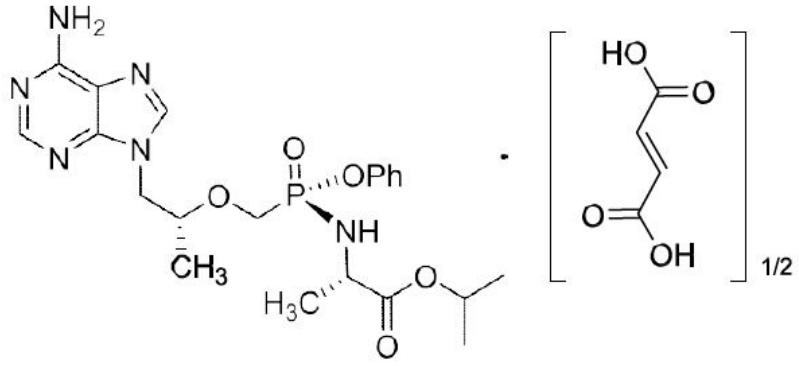
2.2 TAF/EVG Vaginal Insert

2.2.1 Description

Tenofovir alafenamide (TAF)

Tenofovir alafenamide (TAF) is a nucleotide reverse transcriptase inhibitor and a prodrug of tenofovir. TAF is converted *in vivo* to tenofovir (TFV), an acyclic nucleoside phosphonate (nucleotide) analog of adenosine 5′-monophosphate, which in turn is converted to the active metabolite, TFV-diphosphate, intracellularly.[22]

The chemical name of tenofovir alafenamide fumarate drug substance is L-alanine, *N*- [(*S*)-[[(1*R*)-2-(6-amino-9*H*-purin-9-yl)-1-methylethoxy]methyl]phenoxyphosphinyl]-, 1- methylethyl ester, (2*E*)-2-butenedioate (2:1). It has an empirical formula of C21H29O5N6P•½(C4H4O4) and a formula weight of 534.50. It has the following structural formula:

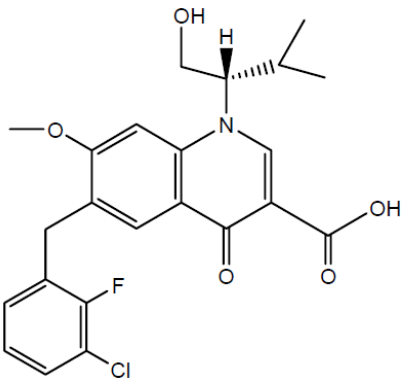


Tenofovir alafenamide fumarate is a white to off-white or tan powder with a solubility of 4.7 mg per mL in water at 20 °C.[22]

Elvitegravir (EVG)

Elvitegravir (EVG) is a 2nd generation human immunodeficiency virus (HIV) integrase inhibitor. EVG is a white crystalline irregularly shaped material and has an acidic pKa of 6.6 and Log D of 4.5 at pH 6.8. EVG is a poorly water soluble and highly permeable drug. It is considered a BCS class-2 compound. EVG (anhydrous crystalline form γ) is chemically stable in the solid state when exposed to heat, humidity and light.

The chemical name of EVG is (S)-6-(3-chloro-2-fluorobenzyl)-1-(1-hydroxy-3-methylbutan-2-yl)-7methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid. It has a molecular formula of $C_{23}H_{23}ClFNO_5$ and a molecular weight of 447.9 (free acid). It has the following structural formula:



EVG is a white to off-white crystalline powder with a solubility of > 35µg/mL in aqueous buffers at pH 9.0. EVG is stable for at least three years at 30°C.

2.2.2 Mechanism of Action

TAF

TAF is a phosphonamidate prodrug of tenofovir (2'-deoxyadenosine monophosphate analog). Plasma exposure to TAF allows for permeation into cells and then TAF is intracellularly converted to tenofovir through hydrolysis by cathepsin A. Tenofovir is subsequently phosphorylated by cellular kinases to the active metabolite tenofovir diphosphate (TFV-DP). TFV-DP inhibits HIV-1 replication through incorporation into viral DNA by the HIV reverse transcriptase, which results in DNA chain-termination.

TFV has activity that is specific to human immunodeficiency virus (HIV) and hepatitis B virus (HBV), and herpes simplex virus (HSV). Cell culture studies have shown that tenofovir can be fully phosphorylated when combined in cells. TFV-DP is a weak inhibitor of mammalian DNA polymerases that include mitochondrial DNA polymerase γ and there is no evidence of mitochondrial toxicity in cell culture based on several assays including mitochondrial DNA analyses.[23]

EVG

EVG is an HIV-1 integrase strand transfer inhibitor (INSTI). Integrase is an HIV-1 encoded enzyme that is required for viral replication. Inhibition of integrase prevents the integration of HIV-1 DNA into host genomic DNA, which happens around 8-11 hours after infection, blocking the formation of the HIV-1 provirus and propagation of the viral infection. EVG does not inhibit human topoisomerases I or II.[24]

2.2.3 Strength of Study Product

Tenofovir alafenamide and elvitegravir (TAF/EVG) inserts have been formulated to contain 20 mg of tenofovir alafenamide free base (equivalent to 22.40 mg tenofovir alafenamide fumarate salt form) and 16 mg of elvitegravir.

2.3 Non-Clinical Studies of TAF and EVG

2.3.1 Anti-HIV-1 Activity

TAF

The antiviral activity of TAF against laboratory and clinical isolates of HIV-1 subtype B was assessed in lymphoblastoid cell lines, peripheral blood mononuclear cells (PBMCs), primary monocyte/macrophage cells and CD4-T lymphocytes. The EC50 values for TAF ranged from 2.0 to 14.7 nM. TAF displayed antiviral activity in cell culture against all HIV-1 groups (M, N, O), including sub-types A, B, C, D, E, F, and G (EC50 values ranged from 0.10 to 12.0 nM) and strain specific activity against HIV-2 (EC50 values ranged from 0.91 to 2.63 nM).[23] TAF also inhibits HSV-2 clinical isolates such as KW strain with an EC50 of 424 nM.[25]

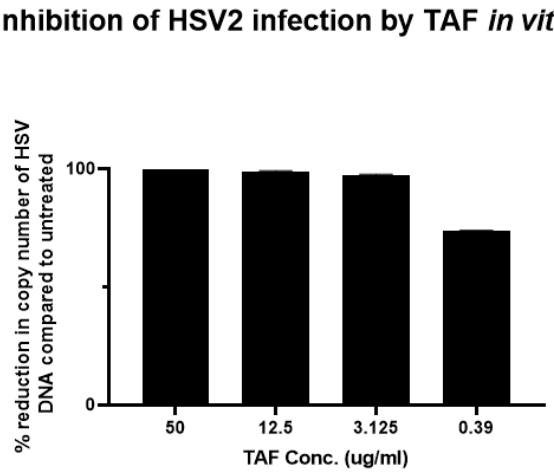
EVG

The antiviral activity of EVG against laboratory and clinical isolates of HIV-1 was assessed in T lymphoblastoid cells, monocyte/macrophage cells, and primary peripheral blood lymphocytes. The 50% effective concentration (EC_{50}) values ranged from 0.02 to 1.7 nM. EVG displayed antiviral activity in cell culture against HIV-1 clades A, B, C, D, E, F, G, and O (EC_{50} values ranged from 0.1 to 1.3 nM) and activity against HIV-2 (EC_{50} value of 0.53 nM). The antiviral activity of EVG with antiretroviral drugs in two-drug combination studies was not antagonistic when combined with the INSTI raltegravir, NNRTIs (efavirenz, etravirine, or nevirapine), NRTIs (abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, or zidovudine), Pis (amprenavir, atazanavir, darunavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, or tipranavir), the fusion inhibitor enfuvirtide, or the CCR5 co-receptor antagonist maraviroc. EVG did not show inhibition of replication of HBV or HCV in cell culture.[24]

The antiviral activity of EVG drug substance prepared at varying doses in suspension with insert formulation components was tested in a cervicovaginal tissue (CVT) explant model. Complete protection against HIV-1_{BAL} infection was observed when EVG tissue concentrations were in the range of ~10^3-10^4 ng/g, whereas only partial protection was observed at ~10^2 ng/g. These data suggest that EVG tissue concentration of at least 1000 ng/g will be efficacious prophylactically against HIV infection of CVT and therefore were set as the target benchmark for drug development.[26]

2.3.2 Anti-HSV-2 Activity

To assess in vitro Anti-HSV-2 activity, HEC1A cells were seeded in 48 well plates and the following day were treated for a total of 6 hours with different concentrations of TAF in duplicates. In the last hour of incubation cells were infected with about 1X10-3 multiplicity of infection (MOI) per well HSV-2(G) isolate (ATCC, VR-734) for an hour. The treatment and inoculum were removed and fresh media was added and cells were incubated for 5 days. HSV-2 DNA was evaluated by a quantitative RT-PCR amplification of cell culture supernatants on day 5 using SYBR-green (Roche, Basel, Switzerland). Supernatants (6 µl) were amplified using the forward primer 50-TCGCCAGCACAAACTCAT -30 and the reverse primer 50- CCACCGACCTCAAGTACAAC -30 targeting glyprotein B of HSV-g isolate. The amount of HSV-2 DNA in all treated wells was compared to untreated control. Results are presented below as percent reduction in the copy number of HSV-2 DNA compared to untreated control.[27]



2.3.3 Resistance (HIV)

HIV-1 isolates with reduced susceptibility to TAF have been selected in cell culture. HIV-1 isolates selected by TAF expressed a K65R substitution in HIV-1 RT, sometimes in the presence of S68N or L429I substitutions; in addition, a K70E substitution in HIV-1 RT was observed.[23]

HIV-1 isolates with reduced susceptibility to EVG were selected in cell culture. Reduced susceptibility to EVG was associated with the primary integrase substitutions T66A/I, E92G/Q, S147G, and Q148R. Additional integrase substitutions observed in cell culture selection included D10E, S17N, H51Y, F121Y, S153F/Y, E157Q, D232N, R263K, and V281M.[24]

2.3.4 Cross-resistance (HIV)

TFV resistance substitutions, K65R and K70E, result in reduced susceptibility to abacavir, didanosine, emtricitabine, lamivudine, and tenofovir. HIV-1 with multiple thymidine analog mutations (TAM) (M41L, D67N, K70R, L210W, T215F/Y, K219Q/E/N/R), or multinucleoside resistant HIV-1 with a T69S double insertion mutation or with a Q151M mutation complex including K65R, showed reduced susceptibility to TAF in cell culture.[23]

EVG-resistant viruses showed varying degrees of cross-resistance in cell culture to raltegravir in the INSTI class depending on the type and number of substitutions in HIV-1 integrase.[24]

2.3.5 Mutagenicity and Carcinogenesis

EVG was not genotoxic in the reverse mutation bacterial test (Ames test) and the rat micronucleus assay. In an in vitro chromosomal aberration test, EVG was negative with metabolic activation; however, an equivocal response was observed without activation.[24]

2.3.6 In Vitro Metabolism

TAF is reportedly hydrolyzed within cells to form TFV, which is phosphorylated to the active metabolite, TFV-DP. *In vitro,* TAF is reportedly metabolized to TFV by carboxylestrase 1 in hepatocytes, by cathepsin A in PBMCs and macrophages, with minimal metabolism via CYP3A.[28]

The metabolism of EVG is mediated primarily via intestinal and hepatic cytochrome P450 (CYP) 3A enzymes.[24]

2.3.7 Mutagenicity and Carcinogenesis

Since TAF is rapidly converted to tenofovir and a lower tenofovir exposure in rats and mice is observed after TAF administration compared to TDF administration, carcinogenicity studies were conducted only with TDF. Long-term oral carcinogenicity studies of TDF in mice and rats were carried out at exposures up to approximately 10 times (mice) and 4 times (rats) those observed in humans at the 300 mg therapeutic dose of TDF for HIV-1 infection. The TFV exposure in these studies was approximately 167 times (mice) and 55 times (rat) those observed in humans after administration of Genvoya treatment. At the high dose in female mice, liver adenomas were increased at tenofovir exposures 10 times (300 mg TDF) and 167 times (10 mg TAF in Genvoya) that in humans. In rats, the study was negative for carcinogenic findings.[23]

TAF was not genotoxic in the reverse mutation bacterial test (Ames test), mouse lymphoma or rat micronucleus assays.

EVG was not genotoxic in the rat micronucleus assay. Long-term carcinogenicity studies of oral EVG were carried out in mice (104 weeks) and in rats (up to 88 weeks in males and 90 weeks in females). No drug-related increases in tumor incidence were found in mice at doses up to 2000 mg per kg per day alone or in combination with 25 mg per kg per day ritonavir at exposures 3- and 14-fold, respectively, the human systemic exposure at the recommended daily dose of 150 mg. No drug-related increases in tumor incidence were found in rats at doses up to 2000 mg per kg per day at exposures 12- to 27-fold, respectively, in male and female, the human systemic exposure.[24]

2.3.8 Pregnancy, Teratogenic Effects, and Lactation

Oral EVG studies in animals have shown no evidence of teratogenicity or an effect on reproductive function. In offspring from rat and rabbit dams treated with EVG during pregnancy, there were no toxicologically significant effects on developmental endpoints. The exposures (AUC) at the embryo-fetal No Observed Adverse Effects Levels (NOAELs) in rats and rabbits were respectively 23 and 0.2 times higher than the exposure in humans at the recommended daily dose of 150 mg.[24] Studies in rats have demonstrated that EVG is secreted in milk.[24]

Oral TAF studies in animals have shown no adverse embryo-fetal effects at similar exposures (rats) and approximately 53 times higher exposures (rabbits) to those in the recommended daily dose for humans. Doses of up to 600 mg/kg/day were administered through lactation; no adverse effects were observed in the offspring on gestation day 7 at exposures approximate 14 times higher than the exposures recommended for humans. Studies in rats and monkeys have demonstrated that TAF is secreted in milk. [23]

Prospective pregnancy data from the Antiretroviral Pregnancy Registry (APR) are not sufficient to adequately assess the risk of birth defects or miscarriage in humans. However EVG and TAF use during pregnancy have been evaluated in a limited number of individuals and available data show no statistically significant difference in the overall risk of major birth defects. [23]

2.3.9 Reproductive Toxicity

There were no effects on fertility, mating performance or early embryonic development when TAF was administered to male rats at a dose equivalent to 155 times the human dose based on body surface area comparisons for 28 days prior to mating and to female rats for 14 days prior to mating through Day 7 of gestation.[23]

Oral EVG did not affect fertility in male and female rats at approximately 16- and 30-fold higher exposures (AUC), respectively, than in humans at the therapeutic 150 mg daily dose. Fertility was normal in the offspring of rats exposed daily from before birth (in utero) through sexual maturity at daily exposures (AUC) of approximately 18-fold higher than human exposures at the recommended 150 mg daily dose.[24]

2.4 Animal Studies of TAF/EVG Insert

2.4.1 Anti-HIV-1 Activity

Macaque efficacy studies performed in collaboration with the US Centers for Disease Control and Prevention (CDC) have demonstrated high protection (90-100%) conferred by TAF/EVG inserts against vaginal SHIV infection when administered as PrEP or post-exposure prophylaxis (PEP) 4h before or 4-8h after virus exposure.[29] PEP administration 24h after exposure showed >70% protection. TAF/EVG inserts applied rectally showed high level of protection, as well.

2.4.2 Pharmacokinetics

Macaque Study

CONRAD study 2708 evaluated the PK of TAF and EVG following administration of vaginal inserts containing a combination of TAF/EVG in female pigtailed macaques. Vaginal inserts containing 10/8 mg, 20/16 mg or 40/24 mg of TAF/EVG were administered to groups of 4 female macaques. One insert was administered vaginally to each macaque 5 times over an 8-week period with at least 1 week between doses. Animals were dosed on a single occasion at the 24h PK interval and on two separate occasions for both 2 and 4h PK intervals. Plasma, vaginal fluid (VF) and vaginal tissue biopsy samples were collected at 0.5 (blood only), 2, 4, and 24 hours post-dose. These samples were analyzed for concentrations of TAF, TFV and EVG. Vaginal biopsies were also evaluated for TFV-DP concentrations. The TAF/EVG insert did not result in adverse behavioral changes, physical changes in the vaginal vault or measurable systemic exposure to TAF, TFV or EVG in the macaques.[28] There was minimal dose proportionality between inserts for VF or tissue concentrations. TFV and EVG in VF TAF/EVG (20/16 mg) showed the most favorable PK and resulted in TFV-DP and EVG tissue levels in range with those shown to provide in vivo protection against vaginal SHIV infection in macaques.[30]

Rabbit Study

CONRAD Study 1645-116 evaluated the PK of TAF/EVG following once daily intravaginal administration of TAF/EVG formulated drug substance in female NZW SPF rabbits for 14 consecutive days. 5/4, 15/12 or 25/20 mg TAF/EVG formulated drug substance were administered to 5 groups of 4 female rabbits. Mean Cmax and AUC0-24hr values for TFV appeared to increase with increasing dose in an approximately dose proportional manner across the dose range on Days 1 and 13. Mean TFV maximal concentrations (Cmax) were 264 ng/mL on Day 1 and 354

ng/mL following the highest dose of EVG/TAF (20/25 mg). Systemic exposure (AUC0-24hr) to EVG and TFV did not appear to consistently change following repeated administration of EVG in combination with TAF. TFV mean systemic exposure was 861 hr*ng/mL on Day 1 and 1400 hr*ng/mL following the highest dose of EVG/TAF (20/25 mg).

2.4.3 Toxicology

Vaginal Irritation Study in Rabbits

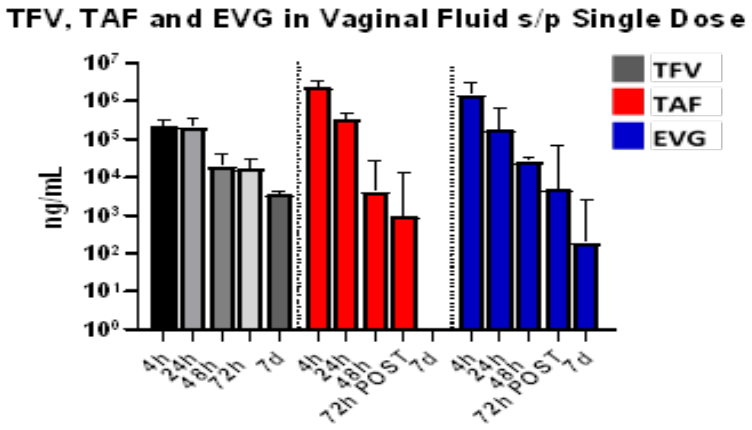
CONRAD Study 1645-116 evaluated potential local irritation and determined the PK following once daily intravaginal administration of EVG and TAF to female rabbits for 14 consecutive days. 24 female rabbits were randomized to 6 groups: vehicle control, placebo control, reference control (N-9), or 4/5, 12/15, or 20/25 mg EVG/TAF. The assessment of toxicity in this study was made based on mortality, clinical observations, body weights, vaginal irritation scoring, micro and macroscopic pathology of reproductive tissues. There were no adverse test article-related effects observed for the following parameters evaluated: bodyweights, clinical observations, vaginal irritation, macroscopic or microscopic pathology of reproductive tissues. Test article-related microscopic observations were present in the vagina and cervix at ≥12/15 mg EVG/TAF, however were not considered adverse due to the reduced severity in comparison with the reference control (Nonoxynol-9). Based upon the results, a no-observable-adverse-effect level (NOAEL) of 20 mg of EVG and 25 mg of TAF per animal administered daily for 14 days (highest dose tested) was established.

2.5 Clinical Studies of TAF/EVG Insert

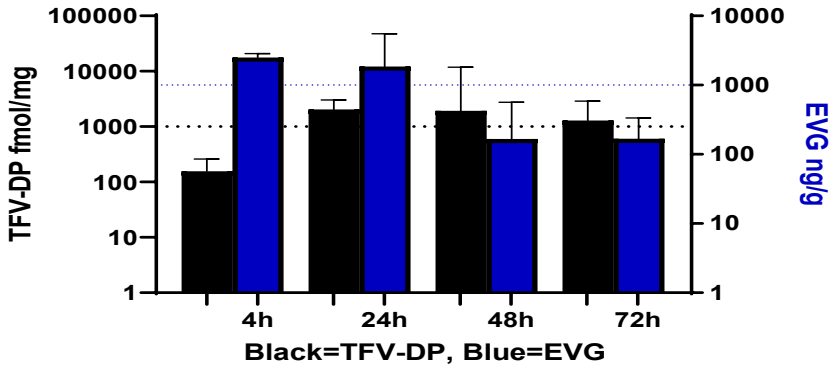
2.5.1 Pharmacokinetics

In the first-in-woman study (CONRAD 146 ClinicalTrials.gov NCT03762772; manuscript under review),[27] US women (n=16, healthy, 18-50 years old, HIV-uninfected) used one TAF/EVG (20/16mg) vaginal insert and were randomized (1:1) to sample collection time groups for up to 7 days post dosing. In Group 1, participants were randomized to have tissue sampling obtained at 4 and 48 hours versus 24 and 72 hours post insert use for Group 2. All participants had plasma, VF and vaginal tissue collected for PK of TAF, EVG, TFV and TFV-diphosphate (TFV-DP).

Systemic plasma exposure was low, consistent with topical delivery. Mucosal concentrations were high and prolonged, with mean and median TFV VF concentrations exceeding 200,000 ng/mL and 1,000 ng/mL for up to 24 hours and 7 days post dosing, respectively. All participants had vaginal tissue EVG concentrations of > 1 ng/mg at 4 and 24 hours post dosing. The majority had tissue TFV-DP concentrations exceeding 1000 fmol/mg by 24 – 72 hours post dosing.



TFV-DP and EVG concentrations in CV tissue after single dose



2.5.2 Safety

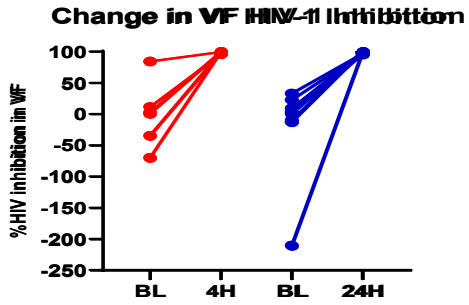
In CONRAD-146, a study conducted in the US, a single dose of TAF/EVG insert administered vaginally was safe and well-tolerated (manuscript under review).[27] There were no product related treatment-emergent adverse events (TEAE). There were 8 TEAEs reported by 7 participants (43.8%). The most common TEAE was increased blood glucose, occurring in 3 (18.8%) participants overall. No other TEAE occurred in more than 1 participant. All TEAEs were mild, and none were related to study treatment. TEAEs considered related to study procedure occurred in 2 (12.5%) participants overall. All events of increased blood glucose and events related to the study procedure occurred in Group 1 (4 hour sample collection) (3 [37.5%] and 2 [25.0%], respectively) and did not occur in Group 2 (24 hour sample collection). No treatment-emergent serious adverse effects (TESAE), deaths or TEAEs led to dose interruption, discontinuation of study treatment, or premature withdrawal from the study.

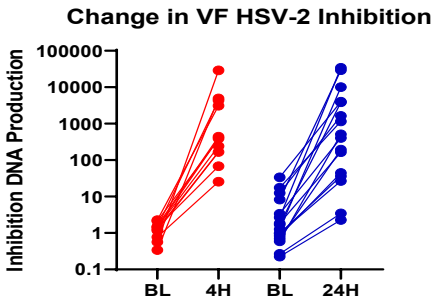
In CONRAD 134, an open-label study conducted in the US that assessed in-vivo disintegration time, safety, and product acceptability of 4 placebo vaginal inserts in 32 women aged 18-50 who were not at risk of pregnancy, placebo inserts were found to be safe, with one mild AE reported that was subsequently assessed as being unrelated to study drug. The optimized insert prototypes were found to disintegrate faster and have higher acceptability over first generation inserts.[26, 31]

CONRAD 117, conducted in the US, was the first-in-human study of vaginal microbicide inserts using TFV and/or FTC containing vaginal inserts. TEAEs were minimal; only one TEAE met the pre-specified criterion for the safety endpoint, but this event was in the placebo group. Colposcopy and physical exam findings were minimal, as were changes in microflora and systemic laboratory values.[26]

2.5.3 Pharmacodynamic Assessment

In CONRAD 146, participants in Group 1 had their 4 hour ectocervical tissue biopsies assessed for in vitro p24 antigen production after ex vivo HIV-1BaL infection, while participants in Group 2 had their 24 hour post dosing ectocervical tissue assessed for HSV-2 DNA production after ex vivo HSV-2 infection. PD was then modeled in vitro by quantifying the change in inhibitory activity of VF and vaginal tissue against human immunodeficiency virus (HIV) and herpes simplex virus type 2 (HSV-2) from baseline to after treatment. VF inhibition of HIV and HSV-2 *in vitro* significantly increased from baseline and was similarly high at 4 and 24 hours post dosing. Consistent with high tissue TFV-DP concentrations (described above), p24 antigen production from ectocervical tissues infected ex vivo with HIV significantly decreased from baseline at 4 hours post dosing. HSV-2 production from tissue also decreased post treatment.





2.5.4 In Vivo Disintegration

In CONRAD 146, a single dose of TAF/EVG insert administered vaginally to 16 women was completely dissolved and absorbed into the mucosal tissues, with no remnants visible, for 12 (75%) participants at the earliest assessment (4 or 24 hours). For the remaining 4 participants, the insert was dissolved but there were small gel like areas of spreading observed. No participant had an intact vaginal insert at the first dissolution assessment.

2.5.5 Acceptability

In CONRAD 146, a single vaginal dose of the TAF/EVG insert was found to be acceptable overall. At baseline, most participants were very comfortable with inserting the product (13 [81.3%]), despite the fact that most had not previously used vaginal suppositories (12 [75.0%]). All participants expressed either no preference or liked how the insert looked (16 [100%]). Most participants were somewhat or very interested in using the product in the future if it protected against HIV-1 and HSV-2 (14 [87.5%]). At the post treatment visit, most participants found the insert very or somewhat acceptable in size (16 [100%]), ease of insertion (16 [100%]), comfort after insertion (15 [93.8%]), dissolvability (15 [93.8%]), residue (13 [81.3%]), leakage (14 [87.5%]), scent (15 [93.8%]), color (16 [100%]), and discreetness (15 [93.8%]). Just under

half of participants overall (7 [43.8%]) reported that using an applicator would make insertion easier. Overall, participants generally were either unsure of the time it took for the insert to dissolve (7 [43.8%]) or reported less than 2 hours (7 [43.8%]). Seven participants overall noticed abnormal leakage/discharge either less than an hour (3 [18.8%]) or 1 to 4 hours after dosing (4 [25.0%]) by feeling it in their underwear (6 [37.5%]). All participants (16 [100%]) reported it was possible to use the insert without their partner's knowledge. Most participants (15 [93.8%]) reported not feeling anything once the insert was in the vagina.

2.6 Study Hypothesis and Rationale for Study Design

2.6.1 Primary Study Hypothesis

It is hypothesized that there will be no clinically significant change in safety endpoints from baseline when the TAF/EVG Insert, 20/16 mg, is administered vaginally once daily for 3 consecutive days and every other day (QOD) for 14 days by healthy, non-pregnant, HIV-uninfected adult women at low risk for HIV acquisition.

2.6.2 Rationale for Study Design

MATRIX-001 will examine the safety, PK, modeled PD, and acceptability of inserts containing the combination of TAF and EVG applied vaginally, daily for 3 days, and QOD for 14 days. The inserts are ultimately intended to be the basis of an event-driven, on-demand method for prevention of HIV and HSV sexual infection.

3 OBJECTIVES

3.1 Primary Objective

Safety

- To evaluate the urogenital and systemic safety of the TAF/EVG insert (20/16 mg) administered vaginally in multiple doses, with emphasis on adverse events developing in the cervicovaginal tract

3.2 Secondary Objectives

Pharmacokinetics (PK)

- To characterize the multi-compartmental PK profile of the TAF/EVG insert administered vaginally in multiple doses

Modeled *in vitro* PD

- To characterize the anti-HIV activity of the TAF/EVG insert in CVF administered vaginally in multiple doses
- To characterize the anti-HSV activity of the TAF/EVG insert in CVF administered vaginally in multiple doses

Acceptability

- To explore user experiences and identify product attributes considered likely to challenge and/or facilitate future sustained use of the TAF/EVG insert applied vaginally

Subclinical Safety and Vaginal Microbiome

- To characterize changes from baseline in soluble and cellular CV mucosal markers after multiple dose vaginal administration of the TAF/EVG insert
- To characterize changes from baseline in the vaginal microbiota after multiple dose vaginal administration of the TAF/EVG insert

3.3 Exploratory Objective

Modeled *in vitro* PD

- To characterize the anti-HIV activity of the TAF/EVG insert in CVT administered vaginally in multiple doses.

4 STUDY DESIGN

4.1 Identification of Study Design

MATRIX-001 is a Phase 1, multi-site, randomized, placebo-controlled, double-blinded study of vaginal administration of the TAF/EVG insert, 20/16 mg, that are administered daily for 3 consecutive days and QOD for 14 days.

4.2 Summary of Major Endpoints

Primary Endpoints:

Safety

- Changes after dosing:
    - Any TEAE, Grade 2 or higher

Secondary Endpoints:

Pharmacokinetics (PK)

- Concentrations of TFV, TAF and EVG in plasma after dosing
- Concentrations of TFV, TAF and EVG in CVF and RF after dosing
- Concentrations of TFV, TFV-DP, TAF and EVG in CVT after dosing

Modeled *in vitro* PD

- Anti-HIV activity in CVF obtained at baseline and after dosing
- Anti-HSV activity in CVF obtained at baseline and after dosing

Acceptability

- Responses to key questions on acceptability (e.g., satisfaction, comfort with insertion, willingness to use) at enrollment and after dosing

Subclinical Safety and Vaginal Microbiome

- Changes in HIV-1 target immune cell phenotype (e.g., CD45, CD68, CD3, and CD1a) and HIV-1 activation/proliferation markers (e.g., HLA-DR) in CVT
- Changes in soluble markers of innate mucosal immunity and inflammatory response in CVF (e.g., IL-1α, IL-6, IL-10, TNFα, RANTES, MIP-1α, IP-10, GM-CSF, IL-8, IL-1RA, SLPI, and BD2)
- Changes in the Nugent Score, relative abundance of vaginal microbial species and community state type (CST) measured by 16S RNA sequencing

Exploratory Endpoints:

Modeled Tissue PD

- p24 antigen production in CVT infected with HIV-1 *ex vivo*, at baseline and after dosing

4.3 Description of Study Population

The study population will consist of healthy, non-pregnant, HIV-uninfected women aged 18-50 years at low risk for HIV acquisition who meet the criteria outlined in Sections 5.2 and 5.3.

4.4 Time to Complete Accrual

The time to complete accrual at all sites is anticipated to be approximately 8 months.

4.5 Study Groups

MATRIX-001 will complete approximately 60 evaluable participants, randomized (1:1) into either placebo or active (TAF/EVG) group. Participants will also be randomized to biopsy sample timepoint collection at the end of product use. See Section 10.6 for additional details.

4.6 Expected Duration of Participation

Once randomized to placebo or active group, participants will complete approximately 2-3 months of follow-up. The total duration of the study will be approximately 12 months.

4.7 Sites

The study will be conducted at 3 sites, one site each in the United States (EVMS), South Africa (CAPRISA) and Kenya (KEMRI).

5 STUDY POPULATION

5.1 Selection of the Study Population

The inclusion and exclusion criteria in Sections 5.2 and 5.3 will be used to ensure the appropriate selection of study participants.

5.1.1 Recruitment

Participants will be recruited from a variety of sources across sites including, but not limited to, existing databases (as permitted by local guidelines on protected health information [PHI]), outpatient clinics, universities, and community-based locations. Recruitment materials will be approved by site Institutional Review Boards/Independent Ethics Committees (IRB/IEC) prior to use per local requirements. Community education strategies, including group sessions, may be employed as part of participant/partner outreach. Sites will develop and implement local standard operating procedures (SOP) to target potential participants for recruitment.

5.1.2 Retention

Once a participant is enrolled in MATRIX-001, study sites will make every effort to retain the participant in follow-up to minimize possible bias associated with loss-to-follow-up (LTFU). An average retention rate of 95% will be targeted across sites. Sites will develop and implement local SOPs to target and ensure high rates of retention.

5.2 Inclusion Criteria

Individuals must meet all the following criteria prior to baseline sampling at Visit 2 (Enrollment), unless otherwise specified:

1) Aged 18 to 50 years (inclusive) at Screening.

2) Assigned female sex at birth.

3) Able and willing to provide written informed consent to be screened for and enrolled in MATRIX-001 in one of the study languages (as specified in site SOP).

4) General good health (by volunteer history) without any evidence of clinically significant systemic disease (as determined by Investigator of Record [IoR] or designee).

5) Has had vaginal sex and has an intact uterus and cervix.

6) Has a regular and/or predictable bleeding pattern based on the opinion of the investigator, or is oligomenorrheic or amenorrhoeic.

7) HIV-uninfected based on testing performed at Screening and Enrollment (per protocol algorithms in Appendix II).

8) Negative urine pregnancy test at Screening and Enrollment.

9) Protected from pregnancy by an effective contraceptive method as confirmed by site SOP; effective methods include:
    - minimum of 3 months of use of a combined hormonal contraceptive method (except vaginal rings)
    - minimum of 6 months of use of a progestin only contraceptive method or copper IUD
    - Sterilization of participant or partner
    - Correct and consistent condom use (for US site only)
    - Abstinence from penile-vaginal intercourse (for US site only)

10) Participants over the age of 21 (inclusive) must have documentation of a Grade 0 Pap smear within the past 3 years prior to Enrollment, per the Female Genital Grading Table for Use in Microbicide Studies Addendum 1 (Dated November 2007) to the DAIDS Table for Grading Adult and Pediatric Adverse Events, Corrected Version 2.1, July 2017, or Grade 1 Pap smear at Screening with no treatment required.

11) Normal cervicovaginal mucosa (as defined in MATRIX-001 Study Specific Procedures [SSP] manual).

12) Willing and able to comply with protocol requirements, including abstaining from vaginal activity and product use at specified times (see Section 6.7.2).

13) Per participant report, if in a relationship, must be in a mutually monogamous relationship with a partner who is not known to be HIV positive or to currently have an STI.

5.3 Exclusion Criteria

Individuals must not meet any of the following criteria prior to baseline sampling at Visit 2 (Enrollment), unless otherwise specified:

1) Per participant report, intends to do any of the following during the study participation period:
    - Become pregnant.
    - Breastfeed.
    - Relocate away from the study site.
    - Travel away from the study site for a time period that would interfere with product resupply and/or study participation.

2) Currently breastfeeding.

3) Positive HIV test at Screening or Enrollment.

4) History of sensitivity/allergy to any component of the study product, topical anesthetic, cellulose based thrombogenic material, or to both silver nitrate and Monsel's solution.

5) Positive test for *Trichomonas vaginalis* (TV), *Neisseria gonorrhea* (GC), *Chlamydia trachomatis* (CT), *Treponema pallidum* (Syphilis), or Hepatitis B surface antigen (HBsAg) at Screening or (per participant report) treated for GC, CT, TV, HBsAg or syphilis in the past 12 months.

6) Chronic or acute vulvar, vaginal or cervical symptoms (pain, irritation, spotting/bleeding other than what would be expected from contraceptive use, discharge, etc.).

7) Known bleeding/clotting disorder, including use of anti-coagulation.

8) Need for continued use of any contraindicated concomitant medications (as listed in Appendix III).

9) Participation in any other trial with use of an investigational drug/device within the last 30 days or planned participation in any other investigational trial with use of a drug/device during the study.

10) Participants who previously received an HIV vaccine or HIV broadly neutralizing antibody (bNAb) are not eligible. Individuals may be eligible if they participated in an HIV vaccine or bNAb study but have documentation that they did not receive active product (e.g., placebo recipients).

11) Prior use of PEP or oral PrEP (including FTC/TDF) in the past 4 weeks or any prior use of long-acting systemic PrEP (including cabotegravir or islatravir).

12) Grade 2 or higher pelvic finding or laboratory abnormality, per the DAIDS Table for Grading Adult and Pediatric Adverse Events, Corrected Version 2.1, July 2017 and/or Addenda 1 (Female Genital Grading Tables for Use in Microbicide Studies [Dated November 2007]) or clinically significant laboratory abnormality as determined by the clinician.

13) Use of any of the following in the past 12 months: stimulants (cocaine [including crack], methamphetamine, or non-physician prescribed pharmaceutical-grade stimulants), or inhaled nitrates, or illicit injection drug use of any kind.

14) Has any other condition that, based on the opinion of the IoR or designee, would preclude provision of informed consent, make participation in the study unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the study objectives.

5.4 Co-enrollment Guidelines

As indicated in Section 5.3, participants must not take part in other research studies involving drugs, medical devices, rectal and genital products, or vaccines within 30 days of Enrollment and while taking part in MATRIX-001, unless they can provide documentation that they did not receive active product (e.g., placebo recipients) or if approved by the Protocol Safety Review Team (PSRT). Should any participant report concurrent participation in contraindicated studies (may include observational / cohort studies) after enrolling in MATRIX-001, the IoR/designee will consult the PSRT regarding ongoing product use and other potential safety considerations associated with co-enrollment.

6 STUDY PRODUCT

6.1 Regimen

The product being used in this study is the TAF/EVG Vaginal Insert 20/16 mg. Each insert for vaginal administration contains 20 mg tenofovir alafenamide (TAF) and 16 mg elvitegravir (EVG). At each of the study sites, participants will be randomized (1:1) to receive either TAF/EVG vaginal insert or placebo. Each participant will receive a total of 10 doses of vaginal insert (3 doses inserted as one dose per day and 7 doses inserted as one dose on alternating days for 14 days).

Figure 2: Study Product Regimen

See Figure 1: Study Visit Schedule.

6.2 Administration

Each participant will self-administer the first, 4th, and 5th doses of the vaginal insert in the clinic at Visits 3, 5 and 6. The rest of the doses will be inserted at home by the participants. The vaginal insert(s) will be placed deep (approximately 4-5 cm) in the vagina.

6.2.1 TAF/EVG Vaginal Insert

Product Name:	Combination vaginal insert containing tenofovir alafenamide (TAF) and elvitegravir (EVG)
Dosage Form:	Vaginal Insert
Unit Dose:	20 mg/16 mg (TAF/EVG)
Route of Administration:	Intravaginal
Physical Description:	White to off-white uncoated bullet shaped inserts
Manufacturer:	Patheon Pharma Services (part of Thermo Fisher Scientific, Whitby, ON, Canada

6.2.2 Matching Placebo Vaginal Insert

Product Name:	Matching placebo vaginal insert
Dosage Form:	Vaginal Insert
Unit Dose:	0mg
Route of Administration:	Intravaginal
Physical Description:	White to off-white uncoated bullet shaped inserts
Manufacturer:	Patheon Pharma Services (part of Thermo Fisher Scientific), Whitby, ON, Canada

6.3 Study Product Formulation and Storage

Inserts will be packaged in 20 cc, round, white, high-density polyethylene (HDPE) bottles with a child-resistant closure and aluminum, induction-sealed foil liner. Each bottle will contain 20 vaginal inserts and be labeled as per local requirements. Study product will be dispensed for the number of inserts needed for each phase.

Study products should be stored in white induction-sealed HDPE bottle along with polyester coil and a desiccant at room temperature (15° - 30°C) (59° - 86°F) in a locked cabinet or secure area in the clinic prior to dispensing. Consideration should always be given to measures that minimize contact during handling, preparation, and disposal procedures.

6.3.1 TAF/EVG Vaginal Insert

The product being used in this study is the TAF/EVG Vaginal Insert 20/16 mg. Each insert for vaginal administration contains 20 mg tenofovir alafenamide (TAF) and 16 mg elvitegravir (EVG). TAF is a nucleotide reverse transcriptase inhibitor (NRTI) and a prodrug of tenofovir and EVG is an integrase inhibitor. Both of the active pharmaceutical ingredients (APIs) are supplied by Gilead Sciences, Inc.

In addition to the APIs, the insert contains the following inactive excipients: Povidone, magnesium stearate, Polaxomer (also known as Kolliphor), polyethylene glycol, mannitol, and lactose anhydrous. The insert dimensions are: length: 1.5 cm, width: 0.7 cm, height: 0.6 cm. Each insert is approximately 500 mg in weight. The insert formulation is manufactured under current Good Manufacturing Practice (cGMP) at Patheon Pharma Services (part of Thermo Fisher Scientific), Whitby, ON, Canada.

6.3.2 Matching Placebo Vaginal Insert

The placebo vaginal insert will be similar in appearance to the TAF/EVG insert. The composition of the insert includes: lactose anhydrous, mannitol, polyethylene glycol, Povidone, Polaxomer (also known as Kolliphor), and magnesium stearate. The insert dimensions are: length: 1.5 cm, width: 0.7 cm, height: 0.6 cm. Each insert is approximately 500 mg in weight. The insert formulation is manufactured under current Good Manufacturing Practice (cGMP) at Patheon Pharma Services (part of Thermo Fisher Scientific), Whitby, ON, Canada.

6.4 Supply and Accountability

CONRAD will oversee the manufacture and analysis/release (Patheon Pharma Services (part of Thermo Fisher Scientific), Whitby, ON, Canada) of the study product under current Good Manufacturing Practices (cGMP).

6.4.1 Study Product Supply

The site will manage the distribution of the study product according to the protocol. The site will be provided sufficient study product for its designated number of participants. Supplies will be distributed by CONRAD or CONRAD designee.

6.4.2 Study Product Dispensing

TAF/EVG Inserts will be dispensed to clinic staff on behalf of the participant or directly to the participant, upon receipt of a written prescription from an authorized prescriber. An authorized prescriber includes the IoR or a licensed clinician(s) directly responsible to the IoR as noted on the Form FDA 1572.

6.4.3 Study Product Accountability

Each Clinical Research Site (CRS) Pharmacist of Record (PoR) is required to maintain a complete record of all TAF/EVG inserts received and subsequently dispensed. All unused TAF/EVG inserts

must be returned to the IND Sponsor, CONRAD, after the study is completed or terminated unless otherwise instructed by the IND Sponsor.

6.4.4 Retrieval of Unused Study Products

All undispensed study product remaining at the end of the study will be recorded by the site and reconciled by the study monitor(s). After reconciliation, the sponsor will provide instruction regarding disposal, storage, or return of product.

6.5 Ancillary Study Supplies

Clinic staff will offer male condoms to all participants.

6.6 Concomitant Medications

All concomitant medications will be recorded on CRFs for participants starting at Screening. Participants may use acetaminophen (paracetamol) on an as-needed basis during the study. Concomitant medications include all prescription medications, over-the-counter preparations, vitamins, nutritional supplements, recreational drugs, and herbal preparations.

6.7 Prohibited Medications, Products and Practices

6.7.1 Prohibited Medications and Products

Participants should not use the prohibited medications and products as listed in Appendix III during the study unless instructed by a clinician in which case they should inform study staff.

6.7.2 Prohibited Practices

Participants will be asked to abstain from any vaginal and anal activity, douching, and use of all intravaginal objects and products at the times specified below.

- Beginning 48 hours before enrollment visit, during daily dosing phase and approximately 48 hours before and 10 days after CVT biopsy collections:
    - Vaginal and/or anal intercourse
    - Finger stimulation
    - Insertion of fingers or any objects into the vagina or rectum including
        - Sex toys, female condoms, diaphragms, or other vaginal barrier methods
        - Menstrual cups and tampons (acceptable to use outside of dosing phases)
- 72 hours before and after CVT biopsy collections:
    - Use of aspirin greater than 81 mg
- For the duration of the study:
    - Use of any drugs which could prolong bleeding and/or clotting or otherwise interfere with study results per study criteria (i.e., daily NSAID use, St. John's Wort, blood thinners, etc.)
    - Use of vaginal products including spermicides, lubricants, douches, medications, moisturizers, or contraceptive vaginal rings

7 STUDY PROCEDURES

An overview of the study visits and evaluations schedule is provided in Appendix I. Presented in this section is additional information on visit-specific study procedures. Detailed instructions to guide and standardize procedures across sites as well as to specify the visit windows are provided in the MATRIX-001 SSP Manual.

Figure 3: Study Visit Schedule

See Figure 1: Study Visit Schedule.

7.1 Screening Visit

Potential participants will provide written informed consent for screening and enrollment prior to any study related procedures. Eligibility determination will start at Visit 1 and be confirmed in Visit 2. This will include baseline medical history, physical and pelvic examinations, urine pregnancy test (UPT), HIV/STI screening, and blood baseline safety tests at Visit 1. Menstruating participants will be scheduled after menstrual flow ceases and ideally in the luteal phase of the menstrual cycle. Note that if the participant is not able to complete or needs to repeat any of the study procedures conducted at this visit (e.g., blood draw), she may return prior to Visit 2. Labs may be repeated during the screening window per IoR/designee discretion.

Note: Potential participants who fail their first screening attempt may be re-screened once.

Table 1: Screening Visit

Screening Visit – Visit 1		
Component		**Procedures**
Administrative and Regulatory		• Informed consent process • Assess eligibility • Assign a unique Participant Identification (PTID) number • Collect locator information • Provide reimbursement • Schedule next visit/contact, if indicated
Behavioral/Counseling		• Protocol requirements counseling per Section 7.8 • Pre- and post-test HIV counseling, and risk reduction counselling • Collect demographic information
Clinical		• Collect medical and menstrual history • Assess concomitant medications (CM) • Pelvic (with bimanual) exam • Full physical exam • Treat or prescribe treatment for reproductive tract infections (RTI)/ urinary tract infections (UTI)/STIs, if indicated • Provide available test results
Laboratory	**Urine**	○ Pregnancy test ○ Urine dipstick/urinalysis
	Blood	○ HIV, HBsAg, HSV-2*, Syphilis, complete blood count (CBC) with platelets, Chemistries

Screening Visit – Visit 1		
Component		**Procedures**
	Genital	○ TV, GC, CT testing ○ Pap smear, if indicated ○ Saline/potassium hydroxide (KOH) wet mount for candidiasis and/or bacterial vaginosis (BV), if indicated

*HSV-2 results will not be provided to participants

7.2 Baseline Visits

7.2.1 Enrollment Visit – Visit 2

The enrollment visit should be scheduled within 8 weeks of Screening/Visit 1. Willing and eligible participants will be asked to complete a baseline acceptability questionnaire, provide a urine sample for a UPT and undergo HIV screening, provide a blood sample for plasma archive, and undergo baseline sample collection of CVF for PD, CVT for subclinical safety and PD, and cervicovaginal lavage (CVL) collection. Willing and eligible participants will be scheduled after menstrual flow ceases and ideally in the luteal phase of the menstrual cycle.

Table 2: Enrollment Visit

Enrollment Visit – Visit 2		
Component		**Procedures**
Administrative and Regulatory		• Confirm participation • Confirm eligibility • Review/update locator information • Provide reimbursement • Schedule next visit/contact, if indicated
Behavioral/Counseling		• Naïve product use assessment • Baseline behavioral questionnaire • Protocol requirements/prohibited vaginal and anal activity • Pre- and post-test HIV counseling, and risk reduction counselling
Clinical		• Review/update medical and menstrual history • Assess CM • Pelvic exam • Directed physical examination, if indicated • Vital signs and weight • Treat or prescribe treatment for RTI/UTI/STIs, if indicated • Provide available test results
Laboratory	**Urine**	○ Pregnancy test ○ Urine dipstick/urinalysis, if indicated
	Blood	○ HIV ○ Plasma archive
	Genital	○ PSA test for semen ○ Gram stain for Nugent score, pH ○ CVF for anti-HIV and anti-HSV ○ CVL for soluble markers ○ Vaginal biopsy (BXV) for immunohistochemistry (IHC) ○ Ectocervical biopsy (BXC) for HIV (EVMS only) ○ TV, GC, CT testing, if indicated ○ Saline/KOH wet mount for candidiasis and/or BV, if indicated

7.2.2 Randomization Visit – Visit 3

Visit 3 should be scheduled 10-14 days after Visit 2, not less than 10 days after biopsy. Visit 3 will be scheduled shortly after menstrual flow ceases for cycling participants to ensure the 3 days of product use do not occur during a menstrual cycle.

Participants will provide a urine sample for a UPT and undergo HIV screening prior to randomization. Participants will also complete a brief product use assessment and undergo baseline pre-insertion sample collection of CVL, CVF for PK, PD, and vaginal microbiota, and (if participant agrees) RF for PK. Provided they have negative HIV and pregnancy test results, participants will be randomized (1:1) to receive TAF/EVG versus placebo vaginal insert. Participants will also be randomized (1:1:1) into three time points for PK tissue sampling after the last dose (24, 48, or 72 hours post last dose).

Participants will then self-administer the first dose (single combination insert of TAF/EVG or placebo) in the clinic. Participant will be dispensed doses 2 and 3 for daily insertion at home.

Table 3: Randomization Visit

Randomization Visit – Visit 3		
Component		**Procedures**
Administrative and Regulatory		• Confirm participation • Conduct product assignment randomization (after HIV and pregnancy tests) • Conduct PK tissue sampling randomization (after HIV and pregnancy tests) • Review/update locator information • Provide reimbursement • Schedule next visit/contact
Behavioral/Counseling		• Protocol requirements/prohibited vaginal and anal activity • Pre- and post-test HIV counseling, and risk reduction counselling • Initial product use assessment
Clinical		• Review/update medical and menstrual history • Assess adverse events (AE) and CM • Pelvic exam pre-dosing • Directed physical examination, if indicated • Vital signs and weight • Treat or prescribe treatment for RTI/UTI/STIs, if indicated • Provide available test results
Laboratory	**Urine**	○ Pregnancy test ○ Urine dipstick/urinalysis, if indicated
	Blood	○ HIV
	Genital	○ PSA test ○ Gram stain for Nugent score, pH ○ CVF for PK and PD ○ CVL for soluble markers ○ Vaginal microbiota ○ TV, GC, CT testing, if indicated ○ Saline/KOH wet mount for candidiasis and/or BV, if indicated
	Anorectal	○ RF for PK (if participant agrees)

Randomization Visit – Visit 3	
Component	**Procedures**
Study Product/Supplies	• Self-insertion of 1st dose • Dispense doses 2-3 for home insertion

7.3 Follow-up Visits/Contacts

7.3.1 24-hour Post Third Dose Visit – Visit 4

Participants will undergo sample collection of CVL, blood for PK, CVF for PK, PD, and vaginal microbiota, CVT for PK and PD, (if participant agrees) RF for PK, and complete a brief product use assessment.

Table 4: 24-hour Post Third Dose Visit

24-hour Post Third Dose Visit – Visit 4		
Component		**Procedures**
Administrative and Regulatory		• Confirm participation • Review/update locator information • Provide reimbursement • Schedule next visit/contact
Behavioral/Counseling		• Protocol requirements/prohibited vaginal and anal activity • Product use assessment
Clinical		• Review/update medical and menstrual history • Assess for AE and CM • Pelvic exam • Directed physical examination, if indicated • Vital signs and weight • Treat or prescribe treatment for RTI/UTI/STIs, if indicated • Provide available test results
Laboratory	**Urine**	○ Pregnancy test, if indicated ○ Urine dipstick/urinalysis, if indicated
	Blood	○ Plasma for PK
	Genital	○ PSA test ○ Gram stain for Nugent score, pH ○ CVF for PK and PD ○ CVL for soluble markers ○ Vaginal microbiota ○ BXV for PK ○ BXC for HIV (EVMS only) ○ TV, GC, CT testing, if indicated ○ Saline/KOH wet mount for candidiasis and/or BV, if indicated
	Anorectal	○ RF for PK (if participant agrees)

7.3.2 Fourth Dose Visit – Visit 5

Visit 5 should be scheduled 10-14 days after Visit 4, not less than 10 days after biopsy. Visit 5 will be scheduled shortly after menstrual flow ceases for cycling participants to ensure the 14 days of product use do not occur during a menstrual cycle.

Participants will undergo pre-insertion sample collection of CVL, blood for PK, CVF for PK, PD, and vaginal microbiota, and (if participant agrees) RF for PK. Participants will then self-administer the fourth dose at the clinic.

Table 5: Fourth Dose Visit

Fourth Dose Visit – Visit 5		
Component		**Procedures**
Administrative and Regulatory		• Confirm participation • Review/update locator information • Provide reimbursement • Schedule next visit/contact
Behavioral/Counseling		• Protocol requirements/prohibited vaginal and anal activity
Clinical		• Review/update medical and menstrual history • Assess for AE and CM • Pelvic exam pre-dosing • Directed physical examination, if indicated • Vital signs and weight • Treat or prescribe treatment for RTI/UTI/STIs, if indicated • Provide available test results
Laboratory	**Urine**	○ Pregnancy test, if indicated ○ Urine dipstick/urinalysis, if indicated
	Blood	○ Plasma for PK
	Genital	○ PSA test ○ Gram stain for Nugent score, pH ○ CVF for PK and PD ○ CVL for soluble markers ○ Vaginal microbiota ○ TV, GC, CT testing, if indicated ○ Saline/KOH wet mount for candidiasis and/or BV, if indicated
	Anorectal	○ RF for PK (if participant agrees)
Study Product/Supplies		• Self-insertion of 4th dose

7.3.3 Fifth Dose Visit – Visit 6

Visit 6 will take place two days after Visit 5. Participants will undergo pre-insertion sample collection of CVL, blood for PK, CVF for PK, PD, and vaginal microbiota, and (if participant agrees) RF for PK. Participants will then self-administer the fifth dose at the clinic and receive their sixth to tenth doses for home insertion QOD.

Table 6: Fifth Dose Visit

Fifth Dose Visit – Visit 6	
Component	**Procedures**
Administrative and Regulatory	• Confirm participation • Review/update locator information • Provide reimbursement • Schedule next visit/contact
Behavioral/Counseling	• Protocol requirements/prohibited vaginal and anal activity

Fifth Dose Visit – Visit 6		
Component		**Procedures**
Clinical		• Review/update medical and menstrual history • Assess for AE and CM • Pelvic exam pre-dosing • Directed physical examination, if indicated • Vital signs and weight • Treat or prescribe treatment for RTI/UTI/STIs, if indicated • Provide available test results
Laboratory	**Urine**	○ Pregnancy test, if indicated ○ Urine dipstick/urinalysis, if indicated
	Blood	○ Plasma for PK
	Genital	○ PSA test ○ Gram stain for Nugent score, pH ○ CVF for PK and PD ○ CVL for soluble markers ○ Vaginal microbiota ○ TV, GC, CT testing, if indicated ○ Saline/KOH wet mount for candidiasis and/or BV, if indicated
	Anorectal	○ RF for PK (if participant agrees)
Study Product/Supplies		• Self-insertion of 5th dose • Dispense doses for home insertion

7.3.4 24/48/72-hours Post Last Dose Visit – Visit 7

Visit 7 will take place either 11, 12 or 13 days after Visit 6. Visit 7 will be split into three timepoints: a) 24-hours post last dose, b) 48-hours post last dose, and c) 72-hours post last dose. Only a third of participants will attend each timepoint, depending on randomization at Visit 3. Participants will provide a urine sample for a UPT, a blood sample for safety tests, and undergo HIV/STI testing. Participants will also undergo sample collection of CVL, blood for PK, CVF for PK, PD, and vaginal microbiota, CVT for subclinical safety, PK and PD, (if participant agrees) RF for PK, and complete an acceptability questionnaire.

Table 7: 24/48/72-hours Post Last Dose Visit

24-hr/48-hr/72-hr Post Last Dose – Visit 7	
Component	**Procedures**
Administrative and Regulatory	• Confirm participation • Review/update locator information • Provide reimbursement • Schedule next visit/contact
Behavioral/Counseling	• Comprehensive behavioral and product use questionnaire • Protocol requirements/prohibited vaginal and anal activity • Pre- and post-test HIV counseling, and risk reduction counselling
Clinical	• Review/update medical and menstrual history • Assess for AE and CM • Pelvic exam • Directed physical examination, if indicated • Vital signs and weight • Treat or prescribe treatment for RTI/UTI/STIs, if indicated • Provide available test results

24-hr/48-hr/72-hr Post Last Dose – Visit 7		
Component		**Procedures**
Laboratory	**Urine**	○ Pregnancy test ○ Urine dipstick/urinalysis, if indicated
	Blood	○ Plasma for PK ○ HIV, HSV-2*, Syphilis ○ CBC with platelets, Chemistries
	Genital	○ PSA test ○ Gram stain for Nugent score, pH ○ TV, GC, CT testing ○ CVF for PK and PD ○ CVL for soluble markers ○ Vaginal microbiota ○ BXV for IHC ○ BXV for PK ○ BXC for HIV (EVMS only) ○ Saline/KOH wet mount for candidiasis and/or BV, if indicated
	Anorectal	○ RF for PK (if participant agrees)

*HSV-2 results will not be provided to participants

7.3.5 Study Exit Visit (SEV) – Visit 8

Visit 8 should be scheduled 10 days after the participant's last biopsy at Visit 7. Participants will provide a urine sample for a UPT and undergo HIV testing. Participants will also undergo collection of CVL, blood for PK, CVF for PK, PD, and vaginal microbiota, and (if participant agrees) RF for PK. A subset of participants will also be asked to complete an in-depth interview (IDI) to further explore their experiences using the study product. This visit constitutes the Study Exit Visit (SEV).

Table 8: Study Exit Visit (SEV)

Study Exit Visit (SEV) – Visit 8		
Component		**Procedures**
Administrative and Regulatory		• Exit from study • Provide reimbursement
Behavioral/Counseling		• Pre- and post-test HIV counselling, and risk reduction counselling • IDI (subset)
Clinical		• Review/update medical history • Assess for AE and CM • Pelvic exam • Directed physical examination, if indicated • Vital signs and weight • Treat or prescribe treatment for RTI/UTI/STIs, if indicated • Provide available test results
Laboratory	**Urine**	○ Pregnancy test ○ Urine dipstick/urinalysis, if indicated
	Blood	○ HIV ○ Plasma for PK
	Genital	○ PSA test ○ Gram stain for Nugent score, pH ○ CVF for PK and PD ○ CVL for soluble markers ○ Vaginal microbiota ○ TV, GC, CT testing, if indicated ○ Saline/KOH wet mount for candidiasis and/or BV, if indicated
	Anorectal	○ RF for PK (if participant agrees)

7.4 Follow-up Procedures for Participants Who Temporarily Hold or Permanently Discontinue Study Product

7.4.1 Participants Who Become Infected with HIV

Participants who test positive for HIV prior to randomization will not be eligible to continue participating in the study. If a participant tests positive for HIV after randomization, the participant will be referred to local care and treatment services and may return to the research clinic for additional counseling and other support services, as needed. Continued study participation would be of no added benefit and thus follow-up visits will be discontinued, study product use will cease, and the participant will be considered terminated from the study. Participants who become infected after randomization may be offered additional laboratory testing (such as HIV RNA and HIV drug resistance testing), as clinically indicated per discussions between IoR/designee and MATRIX. Please reference the MATRIX-001 SSP Manual for additional details (www.matrix4prevention.org).

7.4.2 Participants Who Become Pregnant

Participants must be protected from pregnancy as described in Section 5.2. A pregnancy test will be performed at clinic visits per Appendix I. A pregnancy test will be done at other visits, if indicated. If pregnancy is confirmed, the participant will be referred for follow-up and discontinued from use of study product (if initiated). Every effort will be made to follow the pregnancy to outcome.

Any pregnancy that occurs while a participant is taking study drug product must be recorded in the Antiretroviral Pregnancy Registry (APR) online at http://www.apregistry.com/. The Investigator must register the pregnancy within fifteen (15) calendar days of first becoming aware of the pregnancy and maintained through to pregnancy outcome. A copy of the report will be provided to the sponsor and reported to the IRB/IEC, as needed, in accordance with local requirements.

7.4.3 Participants Who Temporarily Hold or Permanently Discontinue Study Product Use

Participants with a temporary product hold due to an AE or STI where short-term resolution is possible will continue their scheduled follow-up visits and have all protocol-specified study procedures except the following:

- Provision of vaginal insert, product use instructions, and product use adherence counseling.

The above study procedures are to resume at follow-up visits once study product use is resumed.

Participants who permanently discontinue study product use for any clinician-initiated reason other than HIV infection or pregnancy will discontinue study follow-up visits and procedures, as continued study participation would be of no added benefit. Participants will, however, be asked to complete all the procedures scheduled to occur at the SEV (Visit 8), if willing.

Note: The MATRIX-001 Management Team, in consultation with CONRAD, may provide guidance to the site regarding a modified study visit schedule to ensure PK samples are collected at the appropriate time points and/or omitted if the collection of samples would not be anticipated to yield analyzable data. Participants' duration of use and timing of study product hold/permanent discontinuation will be factored into a modified schedule. Refer to the MATRIX-001 SSP Manual for additional details.

Participants who permanently discontinue study product use due to an AE must continue to be followed in the study, if they are willing, until resolution (return to baseline) or stabilization of the AE is documented.

7.5 Interim Visits/Contacts

Interim visits may be performed at the participant's request or as deemed necessary by the investigator at any time during the study. The participant will be instructed to contact the site at any time during the study if she experiences moderate to severe urogenital symptoms (e.g., genital burning, irritation, stinging, pressure, rash, itching, discharge, urgency, dysuria, or hematuria), or if she experiences pelvic/lower abdominal pain, or moderate to heavy menstrual bleeding (more than she would have during a normal period). The participant will be asked to come in for an evaluation, as indicated.

Interim visits or procedures (e.g., pregnancy test, physical and/or pelvic examination, blood laboratory assessments, urine dipstick, microscopy, culture, STI testing, wet prep, and/or pH) may be performed as deemed necessary by the investigator. The participant will be asked to come in for an evaluation, as indicated. Interim visits after baseline sampling that require examination or interview due to symptoms will be recorded on CRFs.

When an interim visit occurs in response to an AE experienced by a study participant, study staff will assess the reported event clinically and provide or refer the participant to appropriate medical care, as necessary. All AEs will be evaluated and follow-up of any observed abnormalities will proceed according to Section 8.3.

7.6 Final Contact

Since participants' SEV include laboratory testing for HIV, additional contacts after the Visit 8 SEV may be required to provide them additional study test results, and post-test counseling, if needed. In addition, for participants who become pregnant during study participation, additional contacts may be required to ascertain the participant's pregnancy and infant outcome (see Section 7.4.2 for details). Study sites may complete these contacts at the study clinic or at community-based locations, depending on site capacities and site and participant preferences. All final contacts will be documented in participant study records.

7.7 Behavioral Evaluations

Participants will respond to interviewer-administered questionnaires at the Screening visit (Visit 1), Enrollment visit (Visit 2), and post-dose Visits 3, 4, and 7. The baseline assessments, done at Screening and Enrollment, will include, among other topics, demographics, questions on participants' prior experience and comfort using vaginal products as well as other vaginal hygiene

practices. Brief product use assessments between Enrollment and Visit 7 will explore reactions to study product and administration method. For example, we will ask about comfort with insertion, sensation with the product *in situ*, and acceptability of vaginal discharge. A follow-up behavioral, product use, and acceptability questionnaire will be administered at Visit 7 and will examine overall satisfaction with the product after using it, multiple components and correlates of acceptability, as well as relevant topics such as adherence and preference. The design of these assessments is informed by a recent systematic review of the Theoretical Framework of Acceptability (Sekhon),[32] published by Ortblad et al.,[33] and review of acceptability assessments in clinical trials research. These assessments will allow us to identify product attributes likely to challenge and/or facilitate future sustained use when applied vaginally by participants. Suggestions for product improvement will also be collected.

An in-depth interview is planned at study exit (Visit 8) with a sub-set of approximately 24 participants (~8 per site). These will be conducted using a semi-structured guide with, among other topics, questions on user acceptability of the product, user-centered suggestions for product design, packaging and delivery, and experiences with the direct application method. These IDIs will be conducted by a trained qualitative interviewer and will follow a semi-structured questionnaire guide. They are anticipated to last approximately 45-60 minutes. The interview notes, recording and transcripts from the in-depth interview will be considered as source documentation. The IDIs may be conducted in-person or remotely over a secure digital platform.

7.8 Protocol and Product Adherence Counseling

Contraception counseling will be provided to participants of childbearing potential at all visits beginning at the Screening Visit. Protocol adherence counseling will be provided at all visits beginning at the Screening Visit. Counseling will be provided by different site staff than those conducting the behavioral assessments in accordance with standard study methods and as specified in site SOPs.

Counseling also will include reminders regarding concomitant medication, sexual activity, intravaginal product use and practices, and behavioral restrictions during study participation. The participant will be asked to abstain from any vaginal and anal activity, douching, and use of all intravaginal objects and products at specified times (see Section 6.7.2). The participant will be asked if she followed the relevant study instructions and if she did not, will be reminded of protocol requirements and the visits may be rescheduled.

7.9 Clinical Evaluations and Procedures

Physical Examination and Medical History

The full physical examination will include general appearance, height, weight, vital signs, and evaluation of body systems as outlined in the MATRIX-001 SSP Manual. Participants will be asked about medications/therapies at every visit, including Visit 1. Vital signs and weight will be taken at all visits after Screening (after Visit 1). Study staff will ask about adverse events at all visits after baseline sampling (after visit 2). A full physical examination will be completed by the clinician at Screening. Thereafter, a directed physical examination will be performed to assess any complaints or symptoms as clinically indicated.

Pelvic Examination

Pelvic exams will be performed using visual inspection of external genitalia and using a speculum for examination of the cervix and vagina. Bimanual exam may be performed as needed (required at Screening Visit). Pelvic exams will be conducted at each visit for PK, PD and subclinical safety specimen collection and as indicated based on participant reports. CVF will be collected using swabs. Further specimen collection procedures will be detailed in the SSP. Biopsy areas will be checked for healing at applicable visits and as indicated.

Rectal Fluid Collection

For participants who consent to RF collection, this sample will be collected using a swab at certain visits to assess drug concentrations.

Cervicovaginal Tissue Collection

CVT will be obtained at Visits 2, 4 and 7 (per sampling timepoint randomization) to assess pharmacokinetics, pharmacodynamics, and anti-HIV activity.

The study site should carefully record the date and time of each collection of blood, CVF, and CVT biopsies. The time of sampling will be based on the time the first sample is taken (when multiple sample types are being collected at a time point, all samples should be collected within a short period). Samples collected outside the sampling windows below will be considered a protocol deviation:

- ± 4 hours outside of the 24 hour time point
- ± 8 hours outside of the 48 and 72 hour time points
- + 4 days outside of the 10 days time point

Note: Detailed information regarding the rectal and genital examination, as well as the associated procedures required for collecting specimens at each visit, can be found in the MATRIX-001 SSP Manual.

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

7.10 Laboratory Evaluations

Local Laboratory

- Urine
  - Pregnancy
  - Urine dipstick, with microscopy and culture if indicated (microscopy and culture done per local standard of care)

- Blood
  - HIV testing, with confirmatory testing as needed
  - HBsAg
  - Chemistries - AST, ALT, and Creatinine
  - CBC with platelets
  - Syphilis serology

- Genital
    - NAAT for GC/CT/TV
    - PSA
    - Pap smear (if indicated)
    - Gram stain for Nugent score, pH
    - Saline/KOH wet mount for candidiasis and/or BV (if indicated)

Designated Laboratory

- Blood
    - HSV-2 serology
    - Plasma for archive

- CVF
    - Microbiota

University of Colorado-Denver

- Blood
    - Plasma for PK for TAF, TFV, EVG

- CVF
    - CVF for PK for TAF, TFV, EVG

- RF
    - RF for PK for TAF, TFV, EVG

- CVT
    - BXV for PK for TAF, TFV, EVG

CONRAD Lab

- CVF/CVL
    - CVF for Anti-HSV2 and Anti-HIV
    - CVL for secreted soluble markers

- CVT
    - BXV for IHC
    - BXC for HIV (EVMS site only)

Only Local Laboratory test results will be provided to the participant. Once all required study analyses of collected specimens are complete, any remaining samples may be shipped to CONRAD for use in study-related quality assurance and quality control testing. If study samples will be used for assay validation or proficiency testing that is not study related, all participant identifiers (PTID) will be removed from the samples prior to use. Specimens obtained from participants who do not consent to long term storage will not be used for assay validation or proficiency testing purposes.

7.11 Pharmacokinetics (PK) and Pharmacodynamics (PD)

Table 9: Specimens Collected to Assess Safety, PK and Ex Vivo Antiviral Activity

Study Visit	Specimens collected for PK, PD and subclinical safety			
	Blood	CVF/CVL	RF	CVT
Visit 2		• CVF for PD • CVL for soluble markers		• 1 BXV for IHC • 2 BXC for HIV (EVMS only)
Visit 3		• CVF for PK • CVF for PD • CVL for soluble markers	• RF for PK (if participant agrees)	
Visit 4	• Plasma for PK	• CVF for PK • CVF for PD • CVL for soluble markers	• RF for PK (if participant agrees)	• 2 BXV for PK • 2 BXC for HIV (EVMS only)
Visits 5, 6	• Plasma for PK	• CVF for PK • CVF for PD • CVL for soluble markers	• RF for PK (if participant agrees)	
Visit 7a,b,c*	• Plasma for PK	• CVF for PK • CVF for PD • CVL for soluble markers	• RF for PK (if participant agrees)	• 2 BXV for PK • 1 BXV for IHC • 1-2 BXC for HIV (EVMS only)
Visit 8	• Plasma for PK	• CVF for PK • CVF for PD • CVL for soluble markers	• RF for PK (if participant agrees)	

* Only one third of the participants will have their biopsies at one visit 7, either a, b, or c, depending on sampling timepoint randomization

7.12 Specimen Management

Study sites will adhere to the standards of good clinical laboratory practice, in accordance with the MATRIX-001 SSP Manual and site SOP for proper collection, processing, labeling, transport, and storage of specimens. Specimen collection, testing, and storage at the site laboratories will be documented when applicable using the Laboratory Data Management System (LDMS). In cases where laboratory results are not available due to administrative or laboratory error, or out of range and the site IoR/designee determines reasonable to repeat, the site is permitted to re-draw specimens. No genetic testing (limited or genome-wide) is planned on leftover samples that are stored for the purposes of future research.

7.13 Laboratory Oversight

All laboratories participating in this study will adhere to MATRIX's Laboratory Policy (www.matrix4prevention.org).

7.14 Biohazard Containment

As the acquisition 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, National Institutes of Health (NIH), and all other applicable national regulatory authorities. All biological specimens will be transported using packaging mandated by Code of Federal Regulations (CFR) 42 Part 72. All dangerous goods materials, including diagnostic specimens and infectious substances, must be transported according to instructions detailed in the International Air Transport Association (IATA) Dangerous Goods Regulations. Biohazardous waste will be contained according to institutional, transportation/carrier, and all other applicable regulations.

8 ASSESSMENT OF SAFETY

8.1 Safety Monitoring

Site IoRs/designees 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 Co-Chairs, Protocol Safety Physician, Clinical Research Manager(s) (CRM), and CONRAD representatives will serve as the PSRT. The Clinical Data Manager(s) (CDM) prepares routine AE and clinical data reports (blinded to treatment assignment) for review by the PSRT, which meets via conference call approximately once per month or more frequently as needed throughout the period of study implementation to review safety data, discuss product use management, and address any potential safety concerns.

8.2 Clinical Data and Safety Review

A multi-tiered safety review process will be followed for the duration of this study. The site IoRs/designees are responsible for the initial evaluation and reporting of safety information at the participant level and for alerting the PSRT if unexpected concerns arise. Participant safety is also monitored at the project level through routine reviews conducted by the PSRT and study sponsors. Additional reviews may be conducted at each of these levels as dictated by the occurrence of certain events.

The CDM(s) will review incoming safety data on an ongoing basis. Events identified as questionable, inconsistent, or unexplained will be queried for verification. To ensure prompt review of AEs of concern (e.g., serious adverse events [SAE] and Related Grade 3+ AEs), such AE reports submitted in the clinical database can be forwarded to the PSRT for review within 48-72 hours of entry.

The PSRT will meet approximately every month, or as needed, via conference call to review clinical data reports generated by the CDM(s). The content, format and frequency of the clinical data reports will be agreed upon by the PSRT and the CDM(s) in advance of study implementation. In addition to the routine safety data reviews, the PSRT will convene on an ad hoc basis to make decisions regarding the handling of any significant safety concerns. If necessary, experts external

to MATRIX representing expertise in the fields of microbicides, biostatistics, HIV acquisition and medical ethics may be invited to join the PSRT safety review. A recommendation to pause or stop the trial may be made by the PSRT at this time or at any such time that the team agrees that an unacceptable type and/or frequency of AEs has been observed.

The Independent Safety Physician(s) (ISP) will review participant safety data as part of their regular reviews (see Section 10.8.1), since no Data and Safety Monitoring Board (DSMB) oversight is planned for MATRIX-001. These reviews will take place approximately every 3 months, or as needed. The ISP will be an independent investigator(s) with no interest (financial or otherwise) in the outcomes of this study. At the time of these reviews, or at any other time, the ISP or PSRT may convene a panel (composed of the ISP, PSRT and protocol statistician[s]) to review study findings. This panel may recommend that the study proceed as designed, proceed with design modifications, or be discontinued. If at any time a decision is made to discontinue enrollment and/or study product use in all participants, CONRAD will notify USAID, the FDA, Gilead, and any investigators conducting studies under the same IND, and the site IoRs/designees will notify the responsible IRBs/IECs expeditiously per local guidelines.

8.3 Adverse Events Definitions and Reporting Requirements

8.3.1 Adverse Events

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 study participants and is applied beginning at the time of enrollment. The term "investigational products" for this study refers to the TAF/EVG vaginal insert, 20/16 mg. AEs should be assessed for relatedness to study procedure.

Study participants will be provided instructions for contacting the study site to report any untoward medical occurrences they may experience. In cases of potentially life-threatening events, participants will be instructed to seek immediate emergency care. Where feasible and medically appropriate, participants will be encouraged to seek evaluation where a study clinician is based, and to request that the 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 may be obtained and required data elements will be recorded on study CRFs. All participants reporting an untoward medical occurrence will be followed clinically until the occurrence resolves (returns to baseline) or stabilizes.

Study site staff will document in source documents and in the study database all AEs reported by or observed in enrolled study participants regardless of severity and presumed relationship to study product. AE severity will be graded per the DAIDS Table for Grading Adult and Pediatric Adverse Events, Corrected Version 2.1, July 2017 and/or Addenda 1 (Female Genital Grading Tables for Use in Microbicide Studies [Dated November 2007].

For any SAEs that are continuing at a participant's SEV, the IoR/designee must establish a clinically appropriate follow-up plan for the AE. At a minimum, the SAE must be re-assessed by

study staff 30 days after the participant's SEV; additional evaluations also may take place at the discretion of the IoR/designee. The same approach must be taken for any AEs that are found to have increased in severity at the SEV. 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. The PSRT may advise study staff as to whether any additional follow-up may be indicated on a case-by-case basis. For AEs that are re-assessed after study exit, information on the status of the AE at the time of re-assessment will be recorded in source documents only — no updates should be made to AE CRFs based on the re-assessments.

8.3.2 Serious Adverse Events

As per the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use Good Clinical Practice Guidance (ICH E6; https://www.ich.org/page/efficacy-guidelines), SAEs are defined as AEs occurring at any dose that:

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

  NOTE: Per ICH SAE definition, hospitalization itself is not an AE, but is an outcome of the event. Thus, hospitalization in the absence of an AE is not regarded as an AE. The following are examples of hospitalization that are not considered to be AEs:
  - Protocol-specified admission (e.g., for procedure required by study protocol)
  - Admission for treatment of target disease of the study, or for pre-existing condition (unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator)
  - Diagnostic admission (e.g., for a work-up of an existing condition such as persistent pretreatment lab abnormality)
  - Administrative admission (e.g., for annual physical)
  - Social admission (e.g., placement for lack of place to sleep)
  - Elective admission (e.g., for elective surgery)

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

Any SAE including those listed in the protocol, investigator brochure, or package insert must be reported to CONRAD within 24 hours of discovery. If there is any question whether the event meets the criteria for "serious" it should be reported anyway. In addition, a completed SAE form must be emailed to CONRAD as soon as possible.

8.3.3 Adverse Event Relationship to Study Product

Relatedness is an assessment made by a study clinician of whether or not the event is related to the study agent. The PSRT will review and confirm relatedness. The relationship categories that will be used for this study are:

- Related: There is a reasonable possibility that the AE may be related to the study agents
- Not Related: There is not a reasonable possibility that the AE is related to the study agents

8.4 Pregnancy and Infant Outcomes

Pregnant women are excluded from this study.

A participant who is pregnant after enrollment will continue to be followed until the pregnancy outcome is ascertained. A participant who becomes pregnant during the study will have study product discontinued (if initiated) and will be terminated from the study. Please see Section 9.5 for additional details.

8.5 Regulatory Requirements

AEs reported on CRFs will be included in reports to the FDA and other applicable national drug regulatory authorities (DRA). Site IoRs/designees will submit AE information in accordance with local and national regulatory agencies' or other local and national authorities' requirements. Site IoRs/designees also will submit AE information and any other relevant safety information to their IRBs/IECs and national DRA in accordance with IRB/IEC/DRA requirements.

8.6 Social Harms Reporting

Although study sites will make every effort to protect participant privacy and confidentiality, it is possible that participants' involvement in the study could become known to others and that social harms may result. For example, participants could be treated unfairly, or could have problems being accepted by their families, partners and/or communities. Social harms that are judged by the IoR/designee to be serious or unexpected will be reported to the PSRT and responsible site IRBs/IECs according to their individual requirements. In the event that a participant reports social harm, every effort will be made by study staff to provide appropriate care and counseling to the participant, and/or referral to appropriate resources for the safety of the participant as needed. While maintaining participant confidentiality, study sites may engage their community advisory boards (CABs) in exploring the social context surrounding instances of social harm.

9 CLINICAL MANAGEMENT

Guidelines for clinical management and temporary product hold/permanent discontinuation of study product are outlined in this section. In general, the IoR/designee has the discretion to hold study product temporarily at any time if s/he feels that continued product use would be harmful to the participant or interfere with treatment deemed clinically necessary. Unless otherwise specified below, the IoR/designee should immediately consult the PSRT for further guidance on

resuming study product, continuing the hold temporarily, or progressing to permanent discontinuation of study product. The IoR/designee will document all temporary product holds and permanent discontinuations on applicable CRFs.

9.1 Grading System

AE severity grading is described in Section 8.3.

9.2 Dose Modification Instructions

No dose modifications will be undertaken in this study.

9.3 General Criteria for Temporary Hold and Permanent Discontinuation of Study Product

A participant will be permanently discontinued from product use by the IoR/designee for any of the following reasons:

- Failure to follow protocol requirements that is judged severe enough by the investigator to significantly affect study outcomes
- HIV acquisition
- Pregnancy or expresses a desire to become pregnant
- SAEs or other serious medical reasons, as judged by the IoR/designee

A participant will be temporarily held from product use by the IoR/designee for any of the following reasons:

- Grade 2 or higher AE/SAE where short-term resolution is possible
- STI acquisition where short-term resolution is possible

The IoR/designee must consult the PSRT on all temporary product holds instituted at their discretion for further guidance on resuming product use, continuing the temporary hold, or progressing to permanent discontinuation. If product use is temporarily held/permanently discontinued at IoR/designee discretion, but the underlying reason for the temporary hold later resolves, the IoR/designee should consult the PSRT to resume product use at that time. All AEs are defined by the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, as outlined in Section 8.3.

9.4 HIV Infection

Potential participants who test positive for HIV prior to enrollment will be excluded from the study but will be referred to HIV care and management without delay. Participants who test positive for HIV after enrollment must have study product permanently discontinued by the IoR/designee (if initiated). A participant who is confirmed to be HIV positive during the study will have all follow-up visits discontinued and the participant will be considered terminated from the study as per Section 7.4.1. Participants identified as infected with HIV are managed or referred for management according to the local standard of care. Guidance regarding management and referral for participants confirmed to be HIV-positive can be found in Section 13.10. Sites will not be responsible for paying for HIV-related care.

9.5 Pregnancy

Pregnancy testing will be performed at designated study visits and participants will be encouraged to report all signs or symptoms of pregnancy to study staff. The IoR/designee will counsel any participant who becomes pregnant regarding possible risks to the fetus according to site SOPs. The IoR/designee also will refer the participant to all applicable services; however, sites will not be responsible for paying for pregnancy-related care.

Potential participants who test positive for pregnancy prior to enrollment will be excluded from the study but will be referred to all applicable services. Participants who test positive for pregnancy after enrollment will have study product discontinued (if initiated) and will be terminated from the study as per Section 7.4.2. A participant who is pregnant at study termination will continue to be followed until the pregnancy outcome is ascertained (or, in consultation with the PSRT, it is determined that the pregnancy outcome cannot be ascertained). Pregnancy outcomes will be reported on relevant CRFs; outcomes meeting criteria for SAE reporting also will be reported on the Adverse Event CRF. The study site will make every reasonable effort to contact participants and collect infant outcome up to approximately one year after delivery for those pregnancies that result in live birth.

9.6 Criteria for Early Termination of Study Participation

Participants may voluntarily withdraw from the study for any reason at any time. IoRs/designees 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, after consultation with the PSRT. Participants also may be withdrawn if CONRAD, USAID, MATRIX, government or regulatory authorities, including the South African Health Products Regulatory Authority (SAHPRA), Kenya Pharmacy and Poisons Board (KPPB), FDA and Office for Human Research Protections (OHRP), or site IRBs/IECs 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 (see details regarding the Early Termination Visit in Section 7.3.5). Study staff members will record the reason(s) for all withdrawals in participants' study records.

10 STATISTICAL CONSIDERATIONS

10.1 Overview and Summary of Design

MATRIX-001 is a Phase 1, multi-site, randomized, placebo-controlled, double-blinded study of daily vaginal administration of the TAF/EVG insert, 20/16 mg. MATRIX-001 will examine the safety, PK, modeled PD, disintegration, and acceptability of inserts containing the combination of TAF and EVG applied vaginally, daily for 3 days, and QOD for 14 days.

10.2 Study Endpoints

Primary Endpoints

Safety

- Changes after dosing:
  - Any TEAE, Grade 2 or higher

Secondary Endpoints

Pharmacokinetics (PK)

- Concentrations of TFV, TAF and EVG in plasma after dosing
- Concentrations of TFV, TAF and EVG in CVF and RF after dosing
- Concentrations of TFV, TFV-DP, TAF and EVG in CVT after dosing

Modeled *in vitro* PD

- Anti-HIV activity in CVF obtained at baseline and after dosing
- Anti-HSV activity in CVF obtained at baseline and after dosing

Acceptability

- Responses to key questions on acceptability (e.g., satisfaction, comfort with insertion, willingness to use) at enrollment and after dosing

Subclinical Safety and Vaginal Microbiome

- Changes in HIV-1 target immune cell phenotype (e.g., CD45, CD68, CD3, and CD1a) and HIV-1 activation/proliferation markers (e.g., HLA-DR) in CVT
- Changes in soluble markers of innate mucosal immunity and inflammatory response in CVF (e.g., IL-1α, IL-6, IL-10, TNFα, RANTES, MIP-1α, IP-10, GM-CSF, IL-8, IL-1RA, SLPI, and BD2)
- Changes in the Nugent Score, relative abundance of vaginal microbial species and community state type (CST) measured by 16S RNA sequencing

Exploratory Endpoints

Modeled Tissue PD

- p24 antigen production in CVT infected with HIV-1 *ex vivo*, at baseline and after dosing

10.3 Primary Study Hypothesis

It is hypothesized that there will be no clinically significant change in safety endpoints from baseline when the TAF/EVG Insert, 20/16 mg, is administered vaginally once daily for 3 consecutive days and QOD for 14 days by healthy, non-pregnant, HIV-uninfected adult women at low risk for HIV acquisition.

10.4 Sample Size and Power Calculations

The sample size will be calculated assuming a clinically significant difference between the TAF/EVG insert and placebo of 60% or more. Therefore, the sample size needed to detect such

difference at 5% level of significance and 80% power is 20 (10 participants in each arm) per site for a total of 60 participants to complete. Replacement participants will be enrolled in the event of LTFU.

The sample size estimated above will have enough power to conduct a stratified analysis by study site to show the generalizability of the intervention findings.

10.5 Randomization Procedures

Participants will be randomized (1:1) to receive either combination TAF/EVG or placebo vaginal insert. Participants will be randomized 1:1:1 to one of three timepoint groups for tissue sample collection post dosing.

Participants	Randomization Group
30	TAG/EVG insert
30	Placebo insert

Sample Collection Groups	Timepoints after Dosing
Timepoint group 1	24 hours (7a)
Timepoint group 2	48 hours (7b)
Timepoint group 3	72 hours (7c)

10.6 Participant Accrual, Follow-up and Retention

A sufficient number of women may be consented and undergo assessment procedures in order to have at least 60 (20 in each site) female participants complete the study. All participants that undergo baseline sampling will be included in the analysis. Participants who initiate product use but discontinue the study prior to completion may not re-enroll. Participants who discontinue the study early may be replaced to reach the target of having 60 women with complete study data.

10.7 Blinding

This study will be a double-blinded randomized clinical trial. In case of a severe adverse event and the need to unblind, a pharmacist with access to the randomization code will provide appropriate information.

10.8 Data and Safety Monitoring and Analysis

10.8.1 Study Monitoring

DSMB oversight is not planned for this study. The MATRIX Clinical Trials Hub will conduct interim reviews of study progress, including rates of participant accrual, retention, completion of primary and main secondary endpoint assessments, and study or laboratory issues. The ISP will conduct interim review of a closed safety data report. These reviews will take place approximately every

three months, or as needed. At the time of these reviews, or at any other time, the ISP or PSRT may convene a panel (composed of the ISP, PSRT and protocol statistician[s]) to review study findings. This panel may recommend that the study proceed as designed, proceed with design modifications, or be discontinued. Safety monitoring will be done by the PSRT.

10.8.2 Primary Analysis(es)

Product safety will include summaries overall of the incidence of treatment-emergent adverse events, incidence of product-related urogenital adverse events, and incidence of other findings from pelvic examinations (e.g., ulcerations, abrasions, edema, and bleeding). To compare the incidence of TEAEs between the two study arms, Chi-squared test for independence or Fisher's exact test will be used as per the assumptions.

Changes from baseline in clinical laboratory endpoints will be summarized. Shift tables will be produced where relevant.

Evaluation for Study Objectives:
Study objectives will be evaluated by clinical review of descriptive summaries and graphical displays.

Descriptive Statistics:
Categorical variables will be summarized by frequencies and percentages. Continuous variables will be summarized by mean (SD) when found normally distributed. Median (IQR) will be used otherwise. Normality will be assessed graphically by Q-Q plots and statistically by Shapiro-Wilk test for normality. Categorical variables will be summarized by frequency and percentages (e.g. incidence of adverse events).

In addition to descriptive summaries, pharmacokinetic analysis will include time concentration curves for each participant and pooled by sample timepoint group.

To assess for the comparability between the two arms of the study at baseline, t-test or Mann-Whitney test will be used to assess for the differences in continuous variables as appropriate based on the normality assumption. While chi-squared test for independence or Fisher's exact test will be used for categorical variables as appropriate.

10.8.3 Secondary and Exploratory Analyses

Safety:
To assess for the difference in subclinical safety endpoints between TAF/EVG and placebo group, the incidence of different TEAES will be calculated and risk ratio will be estimated. Chi-squared test or fisher exact test will be used as appropriate. A binary variable will be created for each one of the TEAEs of interest, then Multivariable logistic regression will be used to assess for the difference in the occurrence of TEAEs. In this model the risk ratio will be calculated instead of the odds ratio using Stata command (*oddrisk*).

While for quantitative laboratory safety parameters, mixed-effect design will be used to assess for the changes in these parameters over time within the TAF/EVG and placebo group separately

and will assess for the differences in the laboratory parameters between the TAF/EVG and placebo group.

PK and PD:
PK analysis will include descriptive statistics by sample time point of EVG, TFV, and TAF concentrations for all plasma, CVT, and CVF sample types, in addition to TFV-DP concentrations from CVT samples.

PD endpoints will be summarized using descriptive statistics by time point as well as changes from baseline. Estimates and 95% confidence intervals will be provided. Analysis will include summaries of anti-HIV and anti-HSV activity in CVF, and AUC (Area under the curve) and/or SOFT endpoints will be performed to determine if there is a difference between infection of samples collected at baseline and after treatment. Anti-viral activity in CVF (the change in HIV-1 and HSV infections for samples obtained on treatment vs. baseline) will be summarized.

Changes from baseline in clinical laboratory endpoints will be assessed using paired t test or Wilcoxon signed rank test based on the normality of the data. Repeated measures ANOVA or Friedman test for repeated measures will be used to assess the change from baseline though the endpoint.

Mixed effect-design will used to compare within and between participants changes for different PK and PD endpoints from baseline to endpoint.

Disintegration and acceptability:
Disintegration endpoints will be summarized descriptively.

Acceptability endpoints will be summarized by assessment time point using descriptive statistics (e.g., frequencies, means), as appropriate. We will compare ratings of key acceptability measures over time (e.g., after initial insertion and after actual use), assessing whether they changed with increased experience with product insertion using a one-sample t test. In addition, analyses will examine sociodemographic and behavioral correlates of acceptability endpoints using linear regression models with robust standard errors (for rating outcomes) and using multivariable logistic regression for dichotomous outcomes such as willingness to use (e.g., disinterest in future use).

Qualitative Analysis:
Qualitative analyses from the MATRIX 001 study will use a variety of techniques to provide an in-depth characterization of the contextual factors that affected participants' product preferences. The primary source of qualitative data used in the analysis will consist of raw textual data. Qualitative data will be audio-recorded, translated and transcribed in English, and coded for thematic analyses using Dedoose or a similar qualitative software. Data coding will be used as a primary analytical approach for data reduction; that is, to summarize, extract meaning, and condense the data. Whenever possible, we will also compare study sites and explore differences or similarities related to product preferences due to different socioeconomic, cultural and geographical contexts. The findings and interpretations of the data will be critically discussed until there is group consensus on the dominant themes and meanings contained in the data. Whenever possible, site staff will be involved to corroborate findings from the analysis team.

10.8.4 Missing Data

All attempts will be made to avoid missing data. However, missing values will remain as missing, i.e., no attempt will be made to impute. In general, only observed values will be used in data analyses and presentations. The one exception to this is summarizing concentration data; values that are detectable but below the lower limit of quantification (LLOQ) will be set to one-half the value LLOQ; concentrations below the limit of detection will be set to 0. Otherwise, no imputation is anticipated. No transformation will be applied.

11 DATA HANDLING AND RECORDKEEPING

11.1 Data Management Responsibilities

Data collection tools will be developed by the CDM(s) in conjunction with the protocol team. Quality control and data integrity are managed manually and systematically with reports and queries routinely generated and provided by the CDM(s) to the study sites for verification and resolution. As part of the study activation process, each study site will identify all CRFs to be used as source documents. Study CRF data will be entered and cleaned using a data management system compliant with the ICH Good Clinical Practices (GCP) and US CFR guidelines for electronic data capture.

IDI files generated in the field will be electronically transferred to RTI International using a secure File Transfer Protocol (FTP) site, where they will be uploaded and managed using a qualitative software package. RTI International will act as a hub and manage all qualitative data for the study. A convention for file naming will be developed, and all data will be labeled according to this process. Transcripts will be transferred to RTI International as they are completed. RTI International will save all versions of all files on a secure, password-protected server in the US.

11.2 Source Documents and Access to Source Data/Documents

All study sites will maintain source data/documents in accordance with MATRIX's Good Documentation Practice (GDP) guidelines (www.matrix4prevention.org).

Each IoR/designee will maintain, and store securely, complete, accurate and current study records throughout the study. In accordance with U.S. regulations, for the investigational products tested, IoRs/designees will maintain all study documentation for at least two years following the date of marketing approval for the indication in which they were studied. If no marketing application is filed, or if the application is not approved, the records will be retained for two years after the investigation is discontinued, or longer as per local regulatory requirements and the US FDA is notified.

Study records must be maintained on site for the entire period of study implementation. Thereafter, instructions for record storage will be provided by CONRAD. No study records may be moved to an off-site location or destroyed prior to receiving approval from CONRAD.

11.3 Quality Control and Quality Assurance

Study sites will conduct quality control and quality assurance procedures in accordance with MATRIX's Quality Management Plan (www.matrix4prevention.org).

12 CLINICAL SITE MONITORING

Study monitoring will be carried out by ACRO for the African sites and by CRA Resources for the US site. Study monitors will do the following:

- Review informed consent forms, procedures, and documentation
- Assess compliance with the study protocol, ICH GCP guidelines, and applicable regulatory requirements (US and non-US), including US Code of Federal Regulations (CFR) Title 45 Part 46 and Title 2 Parts 200 and 225
- 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 of investigational study products
- Assess implementation and documentation of internal site quality management procedures

Monitoring visits may be conducted on-site or remotely. Remote visits may include remote source document verification using methods specified for this purpose. Remote monitoring visits may be performed in place of or in addition to onsite visits to ensure the safety of study participants and data integrity.

For on-site visits, the IoR/designee will allow study monitors to inspect study facilities and documentation (e.g., informed consent forms, clinic and laboratory records, other source documents, CRFs), as well as observe the performance of study procedures. The IoR/designee also will allow inspection of all study-related documentation by authorized representatives of MATRIX, CONRAD, USAID, FDA, SAHPRA, KPPB, OHRP, IRBs/IECs and other local, US, or international regulatory authorities. A site visit log will be maintained at the study site to document all visits.

13 HUMAN SUBJECTS PROTECTIONS

Site investigators will make efforts to minimize risks to participants. Participants and study staff members will take part in a thorough informed consent process. Before beginning the study, IoRs/designees will have obtained IRB/IEC approval and the protocol will have been submitted to the FDA and other applicable national drug regulatory authorities. IoRs/designees will permit audits by USAID, the FDA, SAHPRA, KPPB, OHRP, MATRIX, IRBs/IECs, and other local, US, or international regulatory authorities or any of their appointed agents.

Changes to this protocol may be implemented by investigators prior to IRB/IEC approval, if those changes are required to eliminate apparent immediate hazards to the study participant; see 45

CFR 46.108(a)(3)(iii) under the 2018 Requirements (https://www.ecfr.gov). These changes must be documented as Protocol Deviations and reported to the Protocol Team and IRBs/IECs as soon as possible; see ICH E6(R2), Good Clinical Practice, Section 4.5.4 (https://www.fda.gov/science-research/clinical-trials-and-human-subject-protection/ich-guidance-documents). In the event of a public health emergency, investigators should adhere to the recommendations of their local institutions, IRBs/IECs and local health departments. When conflicts exist between local directives, MATRIX, Protocol Team and/or USAID policies or guidance, sites should follow the requirement that is most protective of study participants and site staff.

13.1 Institutional Review Boards/Ethics Committees

The participating institution is responsible for assuring that this protocol, the associated site-specific informed consent forms (ICFs), and study-related documents (such as participant education and recruitment materials) are reviewed by an IRB/IEC responsible for oversight of research conducted at each study site and, as required, by applicable national DRA. Any amendments to the protocol must be approved by the responsible IRBs/IECs and (if applicable) national DRA prior to implementation.

Subsequent to the initial review and approval, the responsible IRBs/IECs must review the study at least annually. Each IoR/designee will make safety and progress reports to the IRBs/IECs at least annually and within three months after study termination or completion. These reports will include the total number of participants enrolled in the study, the number of participants who completed the study, all changes in the research activity, and all unanticipated problems involving risks to human subjects or others. In addition, the results of all ISP reviews of the study will be provided to the IRBs/IECs.

13.2 Protocol Implementation

Prior to implementation of this protocol, and any subsequent full version amendments, each site must have the protocol and the protocol consent forms approved, as appropriate, by their local IRBs/IECs and any other applicable regulatory entities. Upon receiving final approval, sites will submit copies of all relevant protocol/amendment documents (i.e., IRB/IEC approval letters with a detailed list of approved documents, approved ICF documents, etc.) to the MATRIX Clinical Trials Hub Regulatory team.

The MATRIX CRM(s) will review the submitted document packet to ensure receipt of all required protocol/amendment documents prior to study activation at the sites. Sites will receive a Study Activation Notification from the MATRIX CRM(s) that indicates successful completion of the protocol readiness process. A copy of the Study Activation Notification should be retained in the site's regulatory files.

Upon receiving final IRB/IEC and any other applicable approval(s) for a protocol/amendment, sites are required to submit copies of all relevant protocol/amendment documents (i.e., IRB/IEC submission letters, IRB/IEC approval letters with a detailed list of approved documents, related correspondence with IRB/IEC, approved ICF documents, etc.) to CONRAD prior to study activation. Upon receiving final IRB/IEC and any other applicable approval(s) for an amendment, activated sites should implement the amendment immediately but are still required to submit copies of all relevant amendment documents to CONRAD.

13.3 Study Coordination

CONRAD holds the Investigational New Drug (IND) application for this study. Delegation of any sponsor responsibilities for this study will be specified in writing between CONRAD and MATRIX

Study implementation will be directed by this protocol, which may not be amended without prior written approval from the Protocol Co-Chairs and Protocol Team representatives from MATRIX and USAID. Study implementation will also be guided by a common SSP Manual that provides further instructions and operational guidance on conducting study visits; data and forms processing; specimen collection, processing, and shipping; AE assessment, management and reporting; dispensing study products and documenting product accountability; and other study operations. Standardized study-specific training will be provided to sites by the MATRIX CRM(s) and other designated members of the Protocol Team.

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. The PSRT will address issues related to study eligibility and AE management and reporting as needed to assure consistent case management, documentation, and information-sharing across sites. Rates of accrual, adherence, follow-up, and AE incidence will be monitored closely by the team as well as the ISP and the MATRIX Clinical Trials Hub.

13.4 Risk Benefit Statement

13.4.1 Risks

General
The study may cause an inconvenience to daily life due to visit scheduling. Participants will need to refrain from vaginal and anal activity, including sex, during study participation at specified timepoints.

Phlebotomy
Phlebotomy may lead to discomfort, feelings of dizziness or faintness, and/or bruising, swelling, having a blood clot, excessive bleeding, and/or infection.

Pelvic Examination and Procedures
The pelvic examination with speculum insertion may cause discomfort, redness, itching, and/or irritation in the external genital and vaginal area.

CVF/CVL Collection
Collection of CVF may cause discomfort or pressure in the vagina or genital area.

RF Collection
There is the risk of mild discomfort in addition to a slight risk of bleeding with the insertion of rectal swabs and sponges for collection of RF.

CVT Biopsy Collection

There is a risk of mild pain and/or sharp pinch when biopsies are taken. Bleeding sometimes occurs after biopsy collection and can usually be stopped with pressure or medication to the biopsy area. If medicine is used, it may cause temporary dark-colored discharge from the vagina. In rare cases, a stitch might need to be placed to stop the bleeding. Soreness and discomfort in the vagina may occur for one to two days after the procedure along with some vaginal spotting. There is a small risk of infection and heavier bleeding. Participants will be instructed to contact the clinic if symptoms are bothersome, if heavy bleeding is noted or if the participant develops any abnormal odor or discharge from the vagina. Participants will be counselled to refrain from NSAIDs or aspirin use 3 days before and after PK sample collection.

Other Risks

Disclosure of HIV and STI status may cause worry, sadness or depression. Disclosure of HIV-positive status has been associated with depression, suicidal ideation, and denial as well as social isolation. Trained study staff will be available to help participants deal with these feelings.

Participation in clinical research includes the risks of loss of confidentiality and discomfort with the personal nature of questions when discussing sexual behaviors.

Sexual partner notification in response to diagnosed STI or HIV infection could cause problems in participants' relationships. Participants also could have problems in their partner relationships associated with study-required abstinence.

Site staff will make every effort to protect participant privacy while in the study. Although study sites will make every effort to protect participant privacy and confidentiality, it is possible that participants' involvement in the study could become known to others, and that social harms may result (i.e., because participants could become known as HIV-positive or at "high risk" for HIV infection). For example, participants could be treated unfairly or discriminated against, or could have problems being accepted by their families and/or communities.

Risks Associated with vaginal inserts

The inserts may cause irritation of the vagina and external genitalia, including pain, itching, irritation, vaginal discharge, or rash. The insert may also cause urinary symptoms including dysuria, frequency, or urgency.

Risks Associated with oral TAF and oral EVG

There is a low probability of adverse events associated with systemic levels of TAF and EVG. Those include: headache, nausea, fatigue, diarrhea, abdominal pain, back pain, cough, arthralgia, and dyspepsia. These side effects may or may not be associated with the use of TAF or EVG when the drugs are administered vaginally.

13.4.2 Benefits

Participants in this study may experience no direct benefit. 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 acquisition and 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 and pelvic exams, and routine laboratory testing related to blood, liver, and kidney function. Participants will be provided STI treatment at no cost, and referrals may be provided if needed. 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 participants may have the opportunity to access expedient treatment and decreased morbidity due to early diagnosis and treatment of abnormalities identified during tests, examinations and referrals.

13.5 Informed Consent Process

Written informed consent will be obtained from each study participant prior to screening. Written informed consent also will be obtained for long-term specimen storage and possible future testing, although consent for specimen storage is not required for study participation. In obtaining and documenting informed consent, the IoR and their designees will comply with applicable local and US regulatory requirements and will adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki. Study staff must document the informed consent process in accordance with MATRIX's GDP guidelines (www.matrix4prevention.org). Participants will be provided with copies of the ICF if they are willing to receive them.

In addition to the ICF, the Protocol Team will work with study staff and community representatives to develop appropriate materials about the study and a standardized approach to the informed consent process to be implemented at study sites, which will be detailed in the MATRIX-001 SSP Manual.

The informed consent process will cover all elements of informed consent required by research regulations. In addition, the process specifically will address the following topics of importance to this study:

- The unknown safety and unproven efficacy of the study product
- The need to practice safer sex behaviors regardless of study treatment group
- The importance of participants in both study groups to the success of the study
- The importance of adherence to the study visit and procedures schedule
- The potential medical risks of study participation (and what to do if such risks are experienced)
- The potential social harms associated with study participation (and what to do if such harms are experienced)
- The real yet limited benefits of study participation
- The distinction between research and clinical care
- The right to withdraw from the study at any time

13.6 Participant Confidentiality

All study procedures will be conducted in private, and every effort will be made to protect participant privacy and confidentiality to the extent possible. Study sites will implement confidentiality protections that reflect the local study implementation plan and the input of study

staff and community representatives to identify potential confidentiality issues and strategies to address them.

All study-related information will be stored securely at the study site. All participant information will be stored in locked areas with access limited to study staff. All laboratory specimens, study data collection, and administrative forms will be identified by coded participant number only to maintain participant confidentiality. All records that contain names or other personal identifiers, such as locator forms and ICFs, will be stored separately from study records identified by code number. All local databases will be secured with password protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link participants' ID numbers to identifying information will be stored in a separate, locked file in an area with limited access. All digital audio files will be stored on password-protected computers. Audio files will be translated and transcribed in English and securely stored. Participants' study information will not be released without their written permission, except as necessary for review, monitoring, and/or auditing by the following:

- Representatives of the FDA, SAHPRA, KPPB, OHRP, USAID, and/or contractors of USAID, and other local, US, or international regulatory authorities
- ACRO and CRA Resources
- CONRAD representatives
- MATRIX designees/ representatives
- Study staff
- Site IRBs/IECs

13.7 Special Populations

13.7.1 Pregnant Women

Women who test positive for pregnancy at the Screening or Enrollment Visit will not be eligible to participate in this study. Should a participant test positive for pregnancy after Enrollment, a product discontinuation will be implemented (if study product use initiated). Follow-up will be completed and data collected per Section 7.4.2. During the informed consent process, participants will be informed that the TAF/EVG vaginal insert is not an effective method of contraception and the effects of TAF/EVG on a developing human fetus are unknown.

13.7.2 Children

The US NIH has mandated that children, defined as younger than 18 years old, be included in research trials when appropriate. This study meets "Justifications for Exclusion" criteria for younger children as set forth by the NIH (specifically, "insufficient data are available in adults to judge potential risk in children" and "children should not be the initial group to be involved in research studies"). As such, this study does not plan to enroll children.

13.8 Compensation

Pending IRB/IEC approval, participants will be compensated for time, inconvenience and travel in this study. Site-specific reimbursement amounts will be determined per local IRB/IEC guidelines and will be specified in the study ICFs of each individual site.

If a participant becomes ill or injured as a result of participation in this trial, medical treatment for the adverse reaction or injury will be provided appropriately. The site staff will refer the participant for ongoing treatment for the injury, if needed. Clinical trial insurance is provided by CONRAD and will be responsible for compensating the study participant for appropriate medical expenses for treatment of any such illness or injury. An HIV infection that occurs during the course of the trial will not be considered an injury or illness caused by trial participation.

13.9 Communicable Disease Reporting

Study staff will comply with local requirements to report communicable diseases including HIV identified among study participants to health authorities. Participants will be made aware of reporting requirements during the informed consent process.

13.10 Access to HIV-related Care

13.10.1 HIV Counseling and Testing

HIV test-related counseling will be provided to all potential study participants who consent to undergo HIV screening to determine their eligibility for this study, and to all enrolled participants at each follow-up HIV testing time point. Testing will be performed in accordance with the algorithm in Appendix II. Counseling will be provided in accordance with standard HIV counseling policies and methods at each site and additionally will emphasize the unknown efficacy of the study products in preventing HIV infection. Participants must receive their HIV test results to take part in this study. Condoms will be available to participants as part of standard risk reduction counselling.

13.10.2 Care for Participants Identified as HIV-Positive

An individual who has been identified as infected with HIV will be referred for management according to the local standard of care. Should a participant test positive for HIV after Enrollment Visit, follow-up procedures will be performed as per Section 7.4.1.

13.11 Study Discontinuation

This study may be discontinued at any time by USAID, MATRIX, CONRAD, the US FDA, SAHPRA, KPPB, the OHRP, site IRBs/IECs, and other local, US or international regulatory authorities.

14 PUBLICATION POLICY

USAID and MATRIX policies and a written agreement between CONRAD and MATRIX will govern publication of the results of this study. Any presentation, abstract, or manuscript will be submitted by the investigator to USAID, MATRIX and CONRAD for review prior to submission.

15 APPENDICES

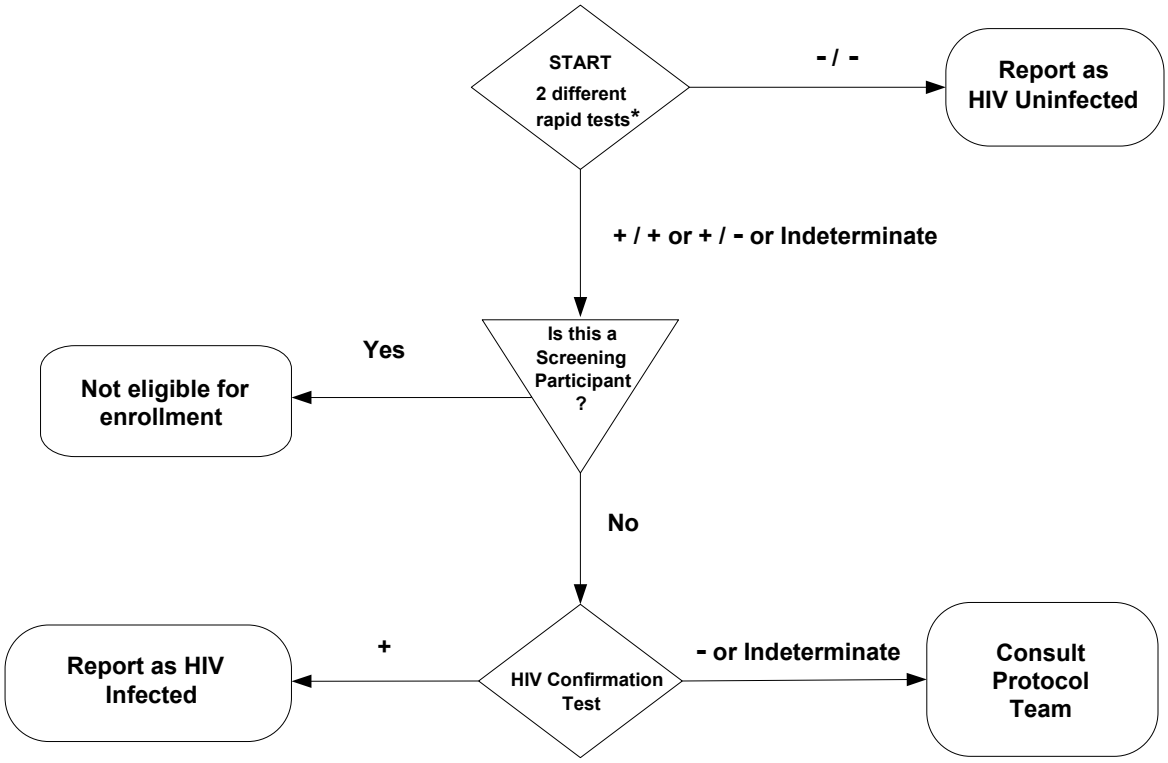
APPENDIX I: SCHEDULE OF STUDY VISITS AND EVALUATIONS

		Screening Visit 1	**Enrollment** Visit 2	**Random-ization** Visit 3	**Post 3rd Dose** Visit 4	**4th & 5th Dose** Visits 5-6	**Post Last Dose** Visits 7a,b,c	**SEV** Visit 8
				10-14 days post V2	*3 days post V3*	*V5 is 10-14 days post V4*	*Participants attend only 1 timepoint 11, 12 or 13 days post V6*	*10 days post V7*
ADMINISTRATIVE AND REGULATORY								
Informed consent process		X						
Assign a unique PTID		X						
Assess/confirm eligibility		X	X					
Collect/review/update locator information		X	X	X	X	X	X	X
Provide reimbursement		X	X	X	X	X	X	X
Schedule next visit/contact		*	*	X	X	X	X	
Randomization (1:1) study product (after HIV and pregnancy tests)				X				
Randomization (1:1:1) PK tail sampling (after HIV and pregnancy tests)				X				
BEHAVIORAL/COUNSELING								
Protocol adherence counseling		X	X	X	X	X	X	
HIV pre and post/ risk reduction counselling		X	X	X			X	X
Demographic information		X						
Behavioral questionnaire			X				X	
Product use questionnaire			X	X	X		X	
IDI (subset)								X
CLINICAL								
Physical exam (directed after Screening)		X	*	*	*	*	*	*
Vital signs and weight			X	X	X	X	X	X
Collect/review/update medical and menstrual history		X	X	X	X	X	X	X
Pelvic exam (bimanual exam at Screening)		X	X	X	X	X	X	X
Assess AEs				X	X	X	X	X
Assess CMs		X	X	X	X	X	X	X
LABORATORY								
URINE	Pregnancy test	X	X	X	*	*	X	X
	Urine dipstick/ urinalysis	X	*	*	*	*	*	*

		Screening Visit 1	**Enrollment** Visit 2	**Random-ization** Visit 3	**Post 3rd Dose** Visit 4	**4th & 5th Dose** Visits 5-6	**Post Last Dose** Visits 7a,b,c	**SEV** Visit 8
				10-14 days post V2	*3 days post V3*	*V5 is 10-14 days post V4*	*Participants attend only 1 timepoint 11, 12 or 13 days post V6*	*10 days post V7*
BLOOD	HIV test	X	X	X			X	X
	HBsAg	X						
	CBC with platelets, Chemistries	X					X	
	HSV-2, Syphilis	X					X	
	Plasma for archive		X					
	PK (Plasma): TAF, TFV, EVG				X	X	X	X
PELVIC	GC, CT, TV testing	X	*	*	*	*	X	*
	Saline/KOH wet mount for candidiasis &/or BV	*	*	*	*	*	*	*
	PSA (semen marker)		X	X	X	X	X	X
	Pap smear	*						
	Gram stain for Nugent score, Vaginal pH		X	X	X	X	X	X
	Vaginal microbiota (16S RNA microbiome)			X	X	X	X	X
	CVF for TAF, TFV, EVG PK levels			X	X	X	X	X
	CVF for Anti-HSV2, Anti-HIV		X	X	X	X	X	X
	CVL (secreted soluble markers)		X	X	X	X	X	X
	BXV for PK – TAF, TFV, EVG, TFV-DP				X		X	
	BXV for IHC		X				X	
	BXC for HIV (EVMS only)		X		X		X	
RECTAL	RF for TAF, TFV, EVG PK levels (if participant agrees)			X	X	X	X	X
STUDY PRODUCT SUPPLY								
Study product self-insertion in clinic				X		X		
Dispense doses for home use				X		X (Visit 6 only)		

X Required
** If indicated and/or per local standard of care*

APPENDIX II: ALGORITHM FOR HIV TESTING – SCREENING/ENROLLING/FOLLOW-UP



*CLIA certified labs may perform 1 rapid test

APPENDIX III: PROHIBITED MEDICATIONS AND PRODUCTS

The following medications and products should not be used during the study:
Daily NSAIDS
Systemic corticosteroids (e.g. dexamethasone)
Endothelin Receptor Antagonists (e.g. bosentan)
Antibiotics
Anticonvulsants (e.g. carbamazepine, oxcarbazepine, phenobarbital, phenytoin)
Antimycobacterials (Rifbutin, Rifampin, Rifapentine)
Anticoagulants or other drugs known to prolong bleeding and/or clotting
Antifungals (e.g. ketoconazole)
Antivirals
Antiretrovirals (e.g. acyclovir, valacyclovir, Viread®, Atripla®, Emtriva®, or Complera®)
St. John's Wort
Drugs that may interact with TAF or EVG as specified in the Vitekta and Vemlidy package inserts
Over-the-counter medications that may alter the genital mucosa/bacteria (e.g., probiotics)

APPENDIX IV: SAMPLE INFORMED CONSENT FORM (SCREENING, ENROLLMENT, LONG-TERM STORAGE AND FUTURE TESTING)

SAMPLE INFORMED CONSENT FORM

MATRIX-001
A Phase I Randomized, Placebo-Controlled, Double-Blind Study to Assess Safety, Pharmacokinetics, and Modeled Pharmacodynamics of a Vaginal Insert Containing Tenofovir Alafenamide and Elvitegravir

USAID

Version 1.0
4 May 2023

PRINCIPAL INVESTIGATOR: *[SITES TO INSERT]*
INSTITUTION: *[SITES TO INSERT]*
AFTER HOURS CONTACT DETAILS: *[SITES TO INSERT]*
STUDY SITE CONTACT DETAILS: *[SITES TO INSERT]*
SHORT TITLE: Safety and PK study of TAF/EVG Vaginal Insert

INFORMED CONSENT
[*SITES TO INSERT APPROPRIATE GREETING*] You are invited to take part in this research study because you are an adult female 18-50 years old. Approximately sixty (60) women will take part in this study across three sites in the United States, South Africa and Kenya. This study is funded by the US Agency for International Development (USAID) and conducted by CONRAD as part of the MATRIX (Microbicide R&D to Advance HIV Prevention Technologies through Responsive Innovation and eXcellence) Collaborative. The study products are supplied by CONRAD. At this site, the person in charge of this study is [*SITES TO INSERT NAME OF CRS PI/IOR*].

KEY INFORMATION
- The study product in this clinical trial is a vaginal insert that contains tenofovir alafenamide (TAF) and elvitegravir (EVG). The vaginal insert is in a solid form that looks like a tablet that is made to dissolve quickly in the vagina. It contains 20 mg TAF and 16 mg EVG. Both drugs are used to treat HIV in oral form. This study will focus on comparing the safety and acceptability of the vaginal insert containing TAF and EVG with the placebo vaginal insert without TAF or EVG.
- The purposes of this study are:
    - To find out if it is safe to apply the TAF/EVG insert in the vagina.
    - To better understand how TAF and EVG enter and exit the body when the insert is used in the vagina.
    - To understand whether you find it acceptable to use the insert when applied to the vagina.
- If you are eligible and choose to participate, you will be randomly assigned to receive 10 doses of the TAF/EVG vaginal insert, 20/16 mg or the placebo vaginal insert. During the study,

you will have laboratory tests for research purposes and to make sure you do not have any side effects.

- You will be asked to attend eight (8) clinic visits at this research clinic and will be followed for approximately 8-12 weeks. The total length of your participation in this study will be about 3 months.
- At some of the clinic visits, the following will occur (other things may happen that are not listed here but are in the detailed descriptions of the study procedures):
    - A physical and/or pelvic exam will be performed.
    - Blood will be obtained to test for HIV and/or other sexually transmitted infections (STI) and for research purposes.
    - Urine will be collected to test for pregnancy and infections (if applicable).
    - Rectal (optional) and vaginal fluids will be collected for research purposes and to test for STIs (if applicable). At 3 of the 8 visits, cervicovaginal tissue will also be collected.
    - You will be asked to complete questionnaires about the vaginal insert, vaginal products, and/or vaginal hygiene practices.
    - We may also ask you to do one in-depth interview with a staff member at your final visit. We may audio-record the interview. It is your choice if you want to do the interview.
- Some common risks from the use of TAF and/or EVG in oral forms include: headache, diarrhea, nausea, fatigue, abdominal pain, cough, back pain, vomiting and rash. Uncommon adverse reactions include depression, insomnia and suicidal thought and attempt in patients with a history of depression or psychiatric illness.
- You may not experience any direct benefit from participation in this study, but information learned from this study may help in the development of ways to prevent the spread of HIV in the future. You will receive HIV/STI risk reduction counseling, HIV and STI testing, physical and pelvic examinations, and routine laboratory testing.
- Taking part in this research study is voluntary. You do not have to participate, and you can stop your participation in the study at any time.

Please take the time to read this entire form and ask questions before deciding to join the study. If you are willing to take part in the study, you will sign this form. A copy of this form will be offered to you. Signing this form does not mean you will be able to join the study. You must first complete the screening tests and clinical examinations to see if you are eligible. It is important to know that your participation in this research study is your decision and taking part in this study is completely voluntary (see Your Rights as a Research Participant/Volunteer for more information).

WHY IS THIS RESEARCH BEING DONE?

This research study is being done to see if an investigational vaginal insert that contains TAF and EVG would be **safe and acceptable** for women to use. Although the product is intended for prevention of HIV, the study is not designed to evaluate how effective the product is for prevention of HIV. This consent form will refer to the vaginal insert as "insert" or "study insert". The word "investigational" means the vaginal insert is experimental and is not yet approved.

EVG is an anti-HIV medication that has been approved by the United Stated Food and Drug Administration (FDA) for oral use in combination with other medications for the treatment of HIV.

EVG works in a specific way to potentially prevent HIV from making copies of itself, thereby stopping the spread of HIV in the human body.

Oral EVG has been tested in combination with other medications in large clinical studies for treatment of HIV infection. These studies have shown that EVG in combination with other drugs is generally safe, with the most frequently reported adverse events being gastrointestinal in nature. EVG in the form of an insert has been tested in animals in the vagina and in the rectum for drug absorption and safety with no findings of concern.

TAF is a medication that is FDA approved for the treatment of chronic hepatitis B or in combination with other medications for the treatment of HIV. TAF also inhibits the replication of HIV virus, therefore stopping the spread of HIV in the human body.

TAF has been tested in clinical studies for safety and efficacy alone and as part of combination therapy that includes EVG. Similarly, study results indicated that TAF taken alone or in combination with other anti-HIV medications is generally safe.

Researchers now would like to learn about the safety of the TAF/EVG insert applied vaginally and how TAF and EVG enter and exit the body. In this study, TAF and EVG are being tested as a vaginally-applied insert for the first time using multiple doses.

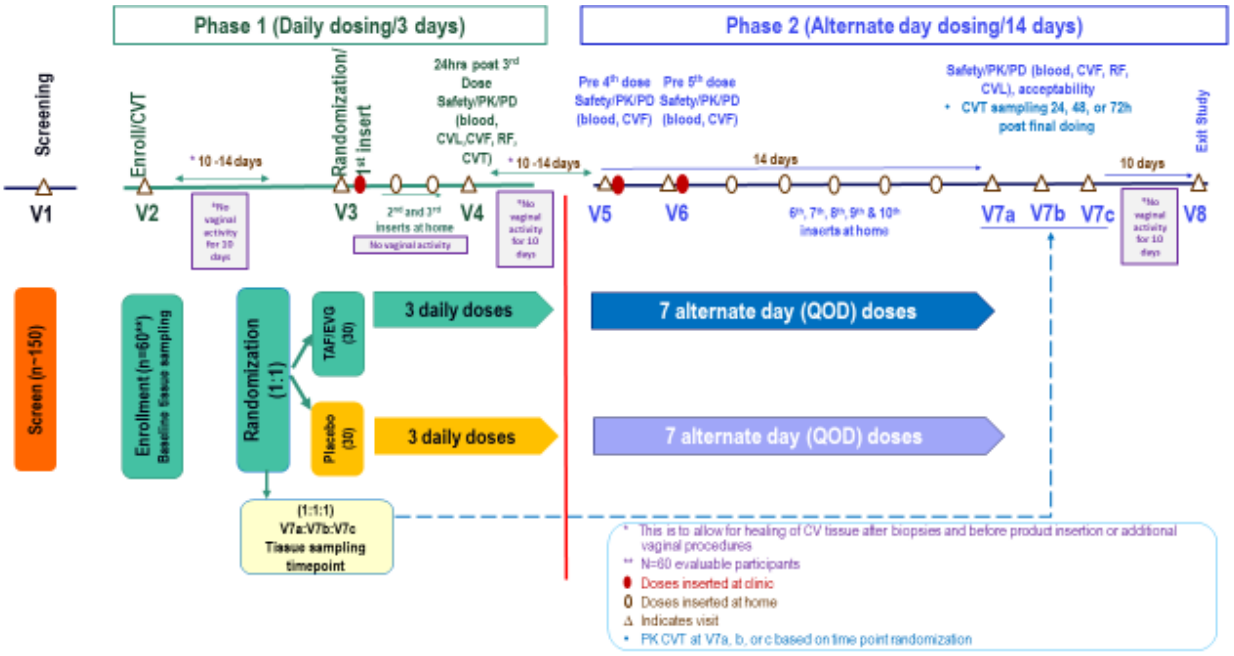
WHO WILL BE IN THIS RESEARCH STUDY?
Approximately sixty (60) women who are 18-50 years old will be enrolled in the study across three sites in the United States, South Africa and Kenya.

DO I HAVE TO BE IN THIS STUDY?
You do not have to be in this study. You can still get the care you need even if you do not join the study. If you decide to join the study, you can change your mind later.

WHAT WILL I BE ASKED TO DO IF I JOIN THIS RESEARCH STUDY?
Following this screening visit the site investigators will assess if you are eligible for the study. If eligible, you will have a total of eight (8) visits over approximately 8-12 weeks. You will be required to use an acceptable method of contraception. You will be randomized to receive the TAF/EVG insert or a placebo insert (does not contain any medication). You will be asked to use the vaginal insert daily for 3 consecutive days and then every other day for 14 days. During this time, you will have blood collected, pelvic exams, vaginal and (if you agree) rectal swabs collected, and cervicovaginal biopsies taken. You are also asked to agree to not use vaginal products or engage in vaginal and/or anal activity for 10-14 days after cervicovaginal biopsies are taken (at Visits 2, 4 and 7) and while using the first 3 vaginal inserts (between Visits 3 and 4).

WHAT WILL HAPPEN DURING THE STUDY VISITS?
The study includes a total of eight (8) clinic visits, including the Screening Visit today. All visits will take place at this research clinic.

If it seems like you can join, you will be asked to come back for an Enrollment visit within 8 weeks from today. At the visit following Enrollment (Visit 3), you will begin using the TAF/EVG vaginal insert or a placebo vaginal insert, depending on which study group you have been assigned to.



Screening Visit Procedures:
The procedures done at this visit will let us know if you can join this study and will take about *[SITES TO INSERT APPROXIMATE DURATION]*.

Study staff will:

- Ask you questions to confirm that you are willing and able to join the study.
- Ask you to provide study staff with your contact information (i.e., where you live and how we can get in touch with you). If you enroll in the study, study staff will review this information with you during future visits.
- Ask you questions about your medical health (including what medications you are taking), menstrual history, and your understanding of the study requirements. If you enroll in the study, study staff will review this information with you during future visits, if needed.
  - Study staff may also ask to view your medical records with your permission.
- Talk with you about the requirements of the study, including the importance of completing clinic visits and study activities and procedures according to the study schedule. If you enroll in the study, study staff will review this information with you during future visits.
  - **It is important that you know if you are not currently using an acceptable method of contraception, or if you do not think you can be sexually abstinent as required by the study, you will not be eligible to participate in this study.**
- Test your urine for pregnancy and for infections.
  - If you are pregnant you cannot join this study.
  - Study staff will talk with you about ways to avoid becoming pregnant.
  - You will answer questions about whether you are using an effective method of contraception and intend to use this method for the entire time that you are in this study. Effective methods include:
    - Sterilization by you or your partner (tubal ligation, vasectomy, etc.).
    - Use of hormonal methods except contraceptive vaginal rings for at least 3 months before enrollment.

        - Intrauterine devices (IUDs) inserted at least 6 months before enrollment.
        - [*US SITE ONLY:* Correct and consistent condom use.]
        - [*US SITE ONLY:* Abstinence from penile-vaginal intercourse.]
- Perform a full physical examination to include general appearance, measurements of height, weight and vital signs, and evaluation of the abdomen, heart and lungs.
- Perform a pelvic examination:
    - The study clinician will use a speculum (a plastic or metal instrument inserted in the vagina). Study staff will ask if you are experiencing symptoms of an infection. They will check your vagina and cervix for signs of infection and other problems.
    - Collect a small amount of vaginal fluid via swab(s), like a Q-tip. These swabs will be used to test for sexually transmitted infections and diseases (commonly known as STIs or STDs) and other problems.
    - Study staff may also collect samples from your cervix for a "Pap test" or "Pap smear". Study staff will inform you of the results of your Pap test when available. It takes about ***[SITES TO INSERT AMOUNT OF TIME]*** before Pap test results are ready. If you have a written report confirming a normal Pap test in the past 3 years, you will not need to have a Pap test taken at this screening visit. The results of your Pap test may affect whether you can join the study.
- Take a blood sample ***[SITES TO INSERT AMOUNT]***:
    - To test the health of your blood, liver and kidneys.
    - To test for infections typically passed through sex, including HIV and Hepatitis B.
        - You will be told your test results as soon as they are available. You will talk with the study staff about the meaning of your results, how you feel about them, and learn about ways to prevent HIV and other STIs. Sometimes HIV tests are not clearly positive, but also not clearly negative. In that case, we will do more tests until we are sure of your status. To participate in the study, you must receive the results of your HIV test. If the test shows you have HIV, you cannot join the study. We will refer you to available sources of medical care and other services you may need. The study staff will tell you about other studies you may be eligible for, if any.
    - For research purposes, including to test for herpes. These tests will be conducted at the end of the study and will not be shared with you.
- If needed, give you treatment or refer you for treatment of STIs or other urinary or reproductive tract infections. If you enroll in the study, study staff will also do this during future visits.
- Inform you about other services, if needed. If you enroll in the study, study staff will also do this during future visits.
- Provide you with the results of your tests, when available. It is expected that all your results will be available by ***[SITES TO SPECIFY TIMEFRAME]***. If you enroll in the study, study staff will also do this during future visits.
- Reimburse you for your visit. If you enroll in the study, study staff will also do this during future visits.
- Schedule your next visit to enroll in the study, if you are willing and eligible.
    - Your menstrual cycle will be considered when scheduling your next visit because, ideally, no bleeding should occur around that visit.

It may be necessary to conduct more than one clinic visit to complete all required screening procedures.

If you enroll in the study, you will be asked to abstain from the following activities for specified periods of time prior to your clinic visits. See stated length of time below:

- Beginning 48 hours before enrollment visit, during daily dosing phase and approximately 48 hours before and 10 days after cervicovaginal tissue (biopsy) collections:
    - Vaginal and/or anal intercourse
    - Finger stimulation
    - Insertion of any objects into the vagina or rectum including
        - Sex toys
        - Female condoms, diaphragms, or other vaginal barrier methods
        - Menstrual cups and tampons (acceptable to use outside of dosing phases)
- 72 hours before and after cervicovaginal tissue collection:
    - Use of aspirin greater than 81 mg
- For the duration of the study
    - Use of any drugs which could prolong bleeding and/or clotting or otherwise interfere with study results per study criteria (i.e., daily NSAID use, St. John's Wort, blood thinners, etc.)
    - Use of vaginal products including
        - Spermicides, lubricants, douches, medications, or moisturizers
        - Contraceptive vaginal rings

If you do not join the study, blood and other samples collected at the Screening visit(s) will not be kept or used for any tests other than those listed above. If you do join the study, test results from samples collected for research purposes throughout the study will not be shared with you.

Enrollment Visit (Visit 2) Procedures:
Your Enrollment Visit (the visit where you enter the study) will take about *[SITES TO INSERT APPROXIMATE DURATION]*.

The following procedures are specific to the Enrollment Visit, which will take place within 8 weeks from your Screening Visit.

Study staff will:

- Ask you questions to confirm that you are willing and able to continue in the study.
- Ask you questions about your thoughts on the study product and about using vaginal products as well as other vaginal hygiene practices.
    - It is important that you know that you will answer these questions in private and your responses will be kept confidential.
- Test your urine for pregnancy and, if needed, for infections.
- Collect vital signs and weight. Perform a directed physical examination if needed.
- Take a blood sample [***SITES TO INSERT AMOUNT***]:
    - To test for HIV.
    - In case there is a question about your HIV test results at a later time.
- Perform a pelvic examination.
    - To check your vagina and cervix for signs of infection and other problems. Study staff will also collect vaginal fluid for research purposes. If needed, they will also collect vaginal fluid to test for infections and these results will be provided to you.

- The study clinician will perform a cervicovaginal lavage (CVL). For the CVL, a clinician rinses your vagina and cervix with a small amount of sterile fluid and collects that fluid in a tube for testing. The CVL fluid collected will be used for research purposes only.
- The study clinician will perform a cervicovaginal biopsy to collect small tissue samples from your vagina [*US SITE ONLY:* and cervix] for research purposes. The study clinician will take approximately one adequate sample from your vagina [*US SITE ONLY:* and two samples from your cervix], each about the size of a grain of rice. It is important that you do not put anything in your vagina for 2 days before the biopsy tissue collections and 10-14 days after, which includes avoiding sexual intercourse, because you may be at higher risk for getting or spreading an infection until the biopsy sites have healed. It is also important that you do not take any aspirin doses higher than 81 mg per day for 3 days before and after the cervical biopsy tissue collections, because you may be at higher risk of bleeding.

- Schedule your next visit, if you enroll in the study. Study staff will also do this during future visits.
  - Your menstrual cycle will be considered when scheduling your next visit (Visit 3) because, ideally, no bleeding should occur around that visit.

Randomization Visit (Visit 3) Procedures:
Visit 3 will take approximately *[SITES TO INSERT APPROXIMATE DURATION]*.

The following procedures are specific to the Randomization Visit, which will take place 10-14 days after your Enrollment Visit.

Study staff will:

- Randomly assign you to active (TAF/EVG) or placebo vaginal insert. Participants in both study groups will have the same study visit schedule.
- Randomly assign you to one of three tissue collection timepoints. Participants in the three study groups will have the same study visit schedule except for the timing of Visit 7, which will take place either 24, 48 or 72 hours after inserting their last (10^{th}) dose of study product.
- Ask you about any health or medical problems you may be currently experiencing or that have occurred since your last visit, including what medications you are taking. Study staff will also do this during future visits.
- Ask you questions about your thoughts on the study product after initial insertion.
- Test your urine for pregnancy and, if needed, for infections.
- Take a blood sample ***[SITES TO INSERT AMOUNT]*** to test for HIV.
- Collect vital signs and weight. Perform a directed physical examination, if needed.
- Perform a pelvic examination
  - To check your vagina and cervix for signs of infection and other problems. Study staff will also collect vaginal fluid for research purposes. If needed, they will also collect vaginal fluid to test for infections and these results will be provided to you.
  - The study clinician will perform a CVL. The CVL fluid collected will be used for research purposes, including to measure the amount of study drug present in your body when using the vaginal insert.
- If you agree, collect a rectal fluid sample using a swab for research purposes. If you choose not to provide a rectal sample, you can still participate in all other study activities.

We will reconfirm the decision you make today at all study visits should you change your mind about rectal fluid collection.

- Give you one vaginal insert to self-insert at the clinic.
  - You will also be given two more vaginal inserts to use at home, with instructions to self-insert one daily for the next two days.
  - Study staff will talk with you about what to do if you have any problems or symptoms while using the vaginal insert.

Post Third Dose Visit (Visit 4) Procedures:
Visit 4 will take approximately *[SITES TO INSERT APPROXIMATE DURATION]*. The following procedures are specific to Visit 4, which will take place approximately 24 hours after the third insert dose.

Study staff will:

- Ask you questions about your thoughts on the vaginal insert after using it daily for three days.
- Test your urine for pregnancy and/or for infections, if needed.
- Take a blood sample ***[SITES TO INSERT AMOUNT]*** for research purposes, including to measure the amount of study drug present in your body when using the vaginal insert.
- Collect vital signs and weight. Perform a directed physical examination, if needed.
- Perform a pelvic examination.
  - To check your vagina and cervix for signs of infection and other problems. Study staff will collect vaginal fluid for research purposes, including to measure the amount of study drug present in your body when using the vaginal insert. If needed, they will also collect vaginal fluid to test for infections and these results will be provided to you.
  - The study clinician will perform a CVL. The CVL fluid collected will be used for research purposes, including to measure the amount of study drug present in your body when using the vaginal insert.
  - The study clinician will perform a cervicovaginal biopsy to collect small tissue samples from your vagina [*US SITE ONLY:* and cervix] for research purposes, including to measure the amount of study drug present in your body when using the vaginal insert. The study clinician will take approximately two adequate samples from your vagina [*US SITE ONLY:* and two samples from your cervix], each about the size of a grain of rice.
- If you agree, collect a rectal fluid sample using a swab for research purposes, including to measure the amount of study drug present in your body when using the vaginal insert. If you choose not to provide a rectal sample, you can still participate in all other study activities.

Post Fourth and Fifth Dose Visits (Visits 5 and 6) Procedures:
Visit 5 will take place approximately 10-14 days after Visit 4. Visit 6 will take place approximately 2 days after Visit 5. Each visit will take between *[SITES TO INSERT APPROXIMATE DURATION]*.

At these visits, study staff will:

- Test your urine for pregnancy and/or for infections, if needed.
- Take a blood sample ***[SITES TO INSERT AMOUNT]*** for research purposes, including to measure the amount of study drug present in your body when using the vaginal insert.

- Collect vital signs and weight. Perform a directed physical examination, if needed.
- Perform a pelvic examination.
  - To check your vagina and cervix for signs of infection and other problems. Study staff will collect vaginal fluid for research purposes, including to measure the amount of study drug present in your body when using the vaginal insert. If needed, they will also collect vaginal fluid to test for infections and these results will be provided to you.
  - The study clinician will perform a CVL. The CVL fluid collected will be used for research purposes, including to measure the amount of study drug present in your body when using the vaginal insert.
- If you agree, collect a rectal fluid sample using a swab for research purposes, including to measure the amount of study drug present in your body when using the vaginal insert. If you choose not to provide a rectal sample, you can still participate in all other study activities.
- Give you one vaginal insert to self-insert at the clinic.
  - At Visit 6, you will also be given five more vaginal inserts to use at home, with instructions to self-insert one every other day for the next ten days.
  - Study staff will talk with you about what to do if you have any problems or symptoms while using the vaginal insert.

Post Last Dose Visit (Visit 7) Procedures:
Visit 7 will take place approximately 11, 12 or 13 days after Visit 6, depending on which of the three biopsy collection timepoints you are assigned. Visit 7 will take between *[SITES TO INSERT APPROXIMATE DURATION]*.

Study staff will:
- Ask you questions about your thoughts on the vaginal insert after using it every other day for two weeks and about using vaginal products as well as other vaginal hygiene practices.
- Test your urine for pregnancy and, if needed, for infections.
- Take a blood sample ***[SITES TO INSERT AMOUNT]***:
  - To test the health of your blood, liver and kidneys.
  - To test for HIV and other infections typically passed through sex.
  - For research purposes, including to measure the amount of study drug present in your body when using the vaginal insert and to test for herpes. These tests will be conducted at the end of the study and will not be shared with you.
- Collect vital signs and weight. Perform a directed physical examination, if needed.
- Perform a pelvic examination.
  - To check your vagina and cervix for signs of infection and other problems. Study staff will collect vaginal fluid to test for infections and for research purposes, including to measure the amount of study drug present in your body when using the vaginal insert.
  - The study clinician will perform a CVL. The CVL fluid collected will be used for research purposes, including to measure the amount of study drug present in your body when using the vaginal insert.
  - The study clinician will perform a cervicovaginal biopsy to collect small tissue samples from your vagina [*US SITE ONLY:* and cervix] for research purposes, including to measure the amount of study drug present in your body when using the vaginal insert. The study clinician will take approximately three adequate samples from your vagina [*US SITE ONLY:* and two samples from your cervix], each about the size of a grain of rice.

- If you agree, collect a rectal fluid sample using a swab for research purposes, including to measure the amount of study drug present in your body when using the vaginal insert. If you choose not to provide a rectal sample, you can still participate in all other study activities.

Study Exit Visit (Visit 8) Procedures:
Your Study Exit Visit will take place approximately 10 days following Visit 7. This visit will take approximately *[SITES TO SPECIFY TIMEFRAME]*.

Study staff will:
- Test your urine for pregnancy and, if needed, for infections.
- Take a blood sample ***[SITES TO INSERT AMOUNT]***:
    - To test for HIV.
    - For research purposes, including to measure the amount of study drug present in your body when using the vaginal insert.
- Collect vital signs and weight. Perform a directed physical examination, if needed.
- Perform a pelvic examination.
    - To check your vagina and cervix for signs of infection and other problems. Study staff will collect vaginal fluid to test for infections and for research purposes, including to measure the amount of study drug present in your body when using the vaginal insert.
    - The study clinician will perform a CVL. The CVL fluid collected will be used for research purposes, including to measure the amount of study drug present in your body when using the vaginal insert.
- If you agree, collect a rectal fluid sample using a swab for research purposes, including to measure the amount of study drug present in your body when using the vaginal insert. If you choose not to provide a rectal sample, you can still participate in all other study activities.
- Ask you to discuss in greater detail your experiences using the vaginal insert, if selected for a longer interview and you agree to participate (see In-Depth Interview Subset section below for details).
- Offer you male condoms, if you need them.

Additional Visits and Procedures
In addition to the procedures listed above, it is possible that study clinicians may need to perform additional tests, if necessary (e.g., if you report having symptoms of a urinary, genital, or other infection and/or other issues). These tests might include the following:
- Physical exam
- Pelvic exam
- Test cervicovaginal samples for STIs
- Test your urine for STIs or other infections
- Test your blood for STIs
- Test your blood to check the health of your blood, liver and kidneys
- Give you treatment or refer you for treatment of STIs or other issues, if needed.

You may be asked to make additional visits so we can do more laboratory tests or have study procedures repeated. We will do this if there are abnormal test results or problems/mistakes during the collection, processing and/or shipping of your samples.

It is important for you to come to every study visit. If you cannot come to the visit, please tell the study staff as soon as possible so that the visit can be rescheduled.

It is important that you remember that at any time during the study, study staff can answer any questions you may have about the procedures mentioned above or any other aspect of this study.

In-depth Interview Subset:
You may be asked to participate in an interview with a trained staff member at your last clinic visit (Visit 8) to discuss your experiences during study participation. A total of approximately 24 participants across three sites will be interviewed. If you are asked to participate in this interview, you will be asked questions about your experiences using the vaginal insert, your preferences and opinions, any problems you may have had using the insert, and whether you used the vaginal insert or not. This interview may take approximately 45-60 minutes and may take place in the clinic or at an alternate location, as schedules permit and as approved by the Institutional Review Board (IRB)/Independent Ethics Committee (IEC). The interviews will be audio-recorded to make sure to record your words exactly how you said them. The audio recording, notes, and analyses from these materials will be kept confidential and will only use study numbers or fake names, and the hardware will be physically protected in a locked area. This means that no one other than the study team will have access to your responses. The information that links you to the research materials will be kept in a secure location that will be accessed only by members of the study team for the purposes of this research. **[*Sites to modify with their site-specific source documentation storage duration requirements if required by their IRBs:*** The audio recordings, notes, and transcripts from these materials will be kept for at least two years after the vaginal insert is approved for marketing or two years after all developmental research on the vaginal insert is stopped.]

WHAT IF I BECOME INFECTED WITH HIV?
We do not know if the vaginal insert will prevent HIV infection, and you may receive the placebo insert with no medication. Persons living with HIV will not be included in this study. Being in this study will not cause HIV infection. But there is always a chance that you can get HIV through unprotected sex or other activities. If you become HIV-positive, you will stop using the study products. The study staff will refer you for medical care and other available services. The study does not pay for this care. If you get HIV, it is possible that the virus is resistant to some drugs. This means that some drugs may not work well to treat your HIV. We will do a blood test to find out if you have drug resistance. These results can help us know which drugs would be best to treat your HIV. [*SITES TO INCLUDE/AMMEND THE FOLLOWING IF APPLICABLE:* If you are interested, study staff will inform you of other available research studies you may be eligible for.]

Depending on local and national health requirements, the study staff may need to report certain diseases, including HIV. The reportable diseases at this site are [*SITES TO INSERT*]. We must inform the following [*SITES TO INSERT MORE DETAILED INFORMATION REGARDING WHO WILL BE INFORMED OF THE REPORTABLE DISEASES*]. [*SITES TO INCLUDE/AMMEND THE FOLLOWING*]: Outreach workers from the [*LOCAL HEALTH AUTHORITY*] may then contact you about informing your partner/s, since they also should be tested. If you do not want to inform your partner/s yourself, the outreach workers will contact them, according to the confidentiality guidelines of the [*LOCAL HEALTH AUTHORITY*].

WHAT IF I BECOME PREGNANT?
The TAF/EVG vaginal inserts are not family planning methods and will not prevent pregnancy. We do not know what effect the study products have on pregnancy, including any effect on the unborn babies. Because of this, pregnant women cannot join this study. Also, you must use an effective family planning method (e.g., birth control pills, hormonal-based methods, intrauterine device [IUD], etc.) other than a vaginal ring, even if you are not currently sexually active.

If you become pregnant during the study, study staff will refer you to available medical care and other services. The study does not pay for this care. You will stop using the study product. We may contact you to find out about the health of your pregnancy and baby. [*SITES TO INCLUDE/AMMEND THE FOLLOWING IF APPLICABLE:* If you are interested, study staff will inform you of other available research studies you may be eligible for.]

RISKS AND/OR DISCOMFORTS

Risks of Blood Draws
You may feel discomfort or pain when your blood is drawn. You may feel dizzy or faint. You may have a bruise, swelling, small clot, or infection where the needle goes into your hand or arm.

Risks of Pelvic Exams
You may feel discomfort or pressure during the pelvic exam and vaginal fluid collection. You may have a small amount of vaginal bleeding or spotting which should stop shortly after the exam.

Risks of Rectal Fluid Collection
You may feel discomfort or pressure in the rectum or anorectal area during rectal fluid collection. There is the risk of mild discomfort in addition to a slight risk of bleeding with the insertion of rectal swabs or sponges.

Risks of Cervicovaginal Tissue Collection Procedures
Cervicovaginal tissue biopsies carry the risk of discomfort or pain during the procedure and for a few hours afterwards. You may have spotting (bleeding) for one or two days. With cervicovaginal biopsies there is also a small risk of infection and heavier bleeding. You may also be at increased risk for STIs and HIV acquisition, if exposed. You will be encouraged to call the clinic to report any problems after the collection, especially if heavy bleeding is noted (soaking through a pad or tampon in an hour or less) or if you develop any abnormal odor or discharge. Note: You are also asked to agree to not use vaginal products or engage in vaginal and/or anal activity for 10-14 days post-biopsy.

Risks of Vaginal Inserts
The vaginal inserts may cause irritation of the vagina and external genitalia, including pain, itching, irritation, vaginal discharge, or rash. The insert may also cause urinary symptoms including dysuria, frequency, or urgency.

Risks of TAF/EVG
The following side effects have been associated with the use of EVG in participants in other studies in which the drug was taken by mouth. These side effects may or may not be associated with the use of EVG when the drug is taken vaginally. The most common adverse reactions (i.e., occurring in 10% or more of participants receiving EVG by mouth) were: headache, diarrhea,

nausea, and tiredness. Other common adverse reactions (i.e., occurring in between 1% and 10% of participants receiving EVG by mouth) were: depression, inability to sleep, pain in abdomen, vomiting, indigestion, and rash. Uncommon adverse reactions (i.e., occurring in between 0.1% and <1% of participants receiving EVG by mouth) were: depression, inability to sleep, and suicidal thoughts and suicide attempt in patients with a history of depression or psychiatric illness.

The following side effects have been associated with the use of TAF in participants in other studies in which the drug was taken by mouth. These side effects may or may not be associated with the use of TAF when the drug is taken vaginally. The most common adverse reactions (i.e., occurring in 5% or more of participants receiving TAF) were: headache, abdominal pain, cough, back pain, fatigue, nausea, joint pain, diarrhea, and dyspepsia. Other common adverse reactions (i.e., occurring in between 1% and 5% of participants receiving TAF) were: vomiting, rash, and flatulence.

There is a theoretical risk of the development of HIV drug resistance to tenofovir or EVG if the participant acquires HIV infection around the time of study drug administration.

It is also possible that you may have an allergic reaction to the study product. Signs of allergic reaction may include: rash, dizziness, itching, muscle aches, nausea, fainting, facial flushing, chest tightness, cough, hives, fever, and shortness of breath.

Risks of HIV and Sexually Transmitted Infection (STI) Testing
HIV and STI testing may make you feel anxious regardless of the test results. Finding out your HIV status may also cause problems with your family, friends, or partner.

Other Possible Risks
You may feel embarrassed and/or worried when talking about sexual activities (if you are currently sexually active), your living situation, ways to protect against HIV and STIs, and your test results. You can choose not to answer questions at any time. Trained study staff will help you with any feelings or questions you have.

We will make every effort to protect your privacy and confidentiality during the study visits. Your visits will take place in private. Reports via computer will be stored in computers that are password-protected and will not include personal information that could identify or link information to you; only your study ID number will be recorded. However, it is possible that others may learn of your participation in this study, and because of this, may treat you unfairly or discriminate against you. If you have any problems, study staff will talk with you and try to help you.

If you are selected for an in-depth interview and you agree, the interview may be performed [*SITES TO SPECIFY MECHANISM:* at the clinic / remotely]. The interview will be audio recorded and questions of a personal nature may be asked. Responding to these questions may make you uncomfortable. The audio files will be put into writing by the person interviewing you or by another person who does not know you and does not have your personal information. You should NOT identify anyone in the interviews and any names that might be mentioned on the recording will only be noted in the transcript using a generic description. The audio files will be stored in computers that are password protected.

BENEFITS
Though you may not experience any direct benefit from participation in this study, information learned from this study may help us learn ways to prevent the spread of HIV in the future. You will receive medical exams and counseling and testing for HIV and STIs. You will also have tests to check your overall health.

This study cannot give you general medical care, but study staff will refer you to another medical provider for care, if needed. Male condoms will be available at no cost, if you need them. If you have an STI diagnosed, you will receive medicine or a referral, if you need it.

NEW INFORMATION
You will be told about any new information learned during this study that may affect your willingness to stay in the study. For example, we will let you know if we learn that the study products may be causing bad side-effects. We will also tell you when study results may be available, and how to learn about them.

WHY YOU MAY STOP TAKING THE STUDY DRUG EARLY OR BE ASKED TO LEAVE THE STUDY
You may need to leave the study early without your permission if:

- The study is cancelled by the US FDA, USAID, CONRAD, the US Office for Human Research Protections (OHRP), MATRIX, the local government or medicines regulatory agency, or the IRB/IEC. An IRB/IEC is a committee that watches over the safety and rights of study participants.
- You are not able to keep appointments.
- Other reasons that may prevent you from completing the study successfully.

The study doctor will ask you to stop using the study products if:

- You are found to be infected with HIV (see "If You Become Infected With HIV" section).
- You become pregnant or are breastfeeding (see "If You Become Pregnant" section).
- You acquire an STI.
- You experience a serious adverse event while on study.
- If you fail to follow study requirements in a manner judged by the study doctor to significantly put you at risk of an adverse reaction or otherwise affect study outcomes. For example, if you report the use of prohibited medications like anticoagulants.
- A study clinician decides that using the study product would be harmful to you, for example, you have a bad reaction to the vaginal insert(s).

If a study doctor asks you to stop using study product, we will ask you to come in for an interim visit during which the procedures scheduled to occur on the Study Exit (Visit 8) will be completed. You will then be exited from the study, unless otherwise informed by study staff.

If you are removed from the study or choose to leave, we will ask you to return any study products you may have and to come back for one final clinic visit. If you do not have the study products with you when you come to the clinic, staff members will make every effort to assist you in returning them as soon as possible. [*SITES TO SPECIFY ALLOWANCES FOR SPECIAL CIRCUMSTANCES*]

ALTERNATIVES TO BEING IN THE STUDY
There are two currently available methods to prevent sexually transmitted HIV: condom use during sex and/or the use of daily oral Truvada® for pre-exposure prophylaxis (PrEP). Oral PrEP is an HIV prevention method where people who do not have HIV take an oral tablet every day to reduce their risk of becoming infected. Study staff can provide you with additional information about PrEP if you are interested in learning more, and where to access oral PrEP.

[*SITES TO INCLUDE/AMEND THE FOLLOWING, IF APPLICABLE*: You may be able to join other studies here or in the community. There may be other places where you can go for HIV counseling and testing and family planning. We will tell you about those studies and those places if you wish.]

COSTS TO YOU
[*SITES TO COMPLETE ACCORDING TO SITE CAPACITY*] There is no cost to you for study visits, study products, physical/clinical exams, laboratory tests or other procedures. We can give you treatments for STIs (other than HIV) at no cost while you are in the study, or we can refer you for available treatment.

REIMBURSEMENT
[*SITES TO INSERT INFORMATION ABOUT LOCAL REIMBURSEMENT:*] You will receive [*SITES TO INSERT AMOUNT $XX*] for your time, inconvenience, and travel to and from the clinic for each scheduled study visit. You may receive [*SITES TO INSERT AMOUNT $XX*] for any extra study visits requested by the study doctor/study team. If you are chosen to take part in the in-depth interview, you will receive [*SITES TO INSERT AMOUNT $XX*].

CONFIDENTIALITY
We will make every effort to keep your information private and confidential. But we cannot guarantee it.

Study visits will take place in private. We will keep the information about your study visits in a secure place that only certain people can access for the purposes of this study. We will only enter your information into computers protected by passwords and will not include information that could identify you. Your identity on these records will be indicated by a number rather than by your name, and the information linking these numbers with your name will be kept separate from the research records. You can choose not to answer questions at any time. If you are selected to do the in-depth interview, you can choose not to answer questions at any time. We will keep the audio recordings and materials from all interviews and discussions confidential and will only use study numbers or fake names. We will store the original records, including the audio recordings, for at least two years after either the study insert is approved for use or research on the insert is stopped. These records will be stored in a secure, locked location.

Your personal information may be disclosed if required by law. For example, if we learn something that would immediately put you or others in danger, the study staff must take steps to keep you and others safe. This means that we have to share any information with the authorities (hospital, police, or social services) that tells us you may be in danger. For example, if you tell us that you plan to hurt or kill yourself, hurt or kill someone else, or if you tell us that someone is abusing or neglecting you.

The study staff may use your personal information to verify that you are not in any other research studies. This study will not use your name or identify you personally in any publication.

[*SITES TO INSERT INFORMATION ABOUT SYSTEMS CURRENTLY IN PLACE TO ENSURE PARTICIPANTS ARE NOT PART OF OTHER CONFLICTING STUDIES, INCLUDING BIOMETRIC IDENTIFICATION SYSTEMS; SEE EXAMPLE BELOW FOR SOUTH AFRICA SITE:*

In clinical studies where study products or other medical devices are being assessed, it is important that volunteers are enrolled in only one clinical study at a time. Using more than one study product may lead to drug interactions and side effects that could potentially be harmful to your health. In addition to compromising the health of the study participant, this can affect the outcome of the study.

The Biometric Co-Enrollment Prevention System (BCEPS) is a web-based system developed by the South African Medical Research Council IT (Information Technology) department, for the prevention of co-enrolment (being enrolled in more than one study at a time). It is a secure system that is used to ensure participant safety and study integrity, in studies where co-enrolment could impact both.

Authorized study staff enters the South African Identify number (SA ID) or SA/foreign Passport Number of the participant into the system to check if they are enrolled in any study within any of the organizations using BCEPS in South Africa. During screening for a study at an Aurum Institute clinic/site, the SA ID number or SA/foreign Passport Number and all fingerprints are captured onto the system. At every study follow-up visit thereafter, their SA ID number or SA/foreign Passport Number and fingerprints will be checked.

In the long term, after study completion, it is important that study staff can verify what studies a participant has volunteered in, in order to ensure that the participant's safety is not compromised in future study participation. The SA ID number or SA/foreign Passport Number and fingerprints will remain in the database on an ongoing basis for a period of up to 15 years after the end of a study. If the participant has not participated in any additional studies for the 15-year period, their SA ID number or SA/foreign Passport Number and fingerprints will be removed from the system.

If a participant refuses for the system to be used at the initial visit or requests to be removed from the system while using a study product, the study investigator will decide if it is safe for the participant to be enrolled into the study or if it is safe to continue using study product without regularly checking for co-enrolment. The study that the potential participant is screening for may also prohibit co-enrolment in which case we will not be able to screen you.]

Your records may be reviewed by:

- Representatives of the US Federal Government, including the US FDA, US OHRP, USAID and/or USAID contractors, and other local, national, or international regulatory authorities
- [*SITES TO INSERT APPLICABLE LOCAL AND NATIONAL AUTHORITIES*]
- CONRAD representatives
- MATRIX representatives
- Study monitors
- Site IRBs/IECs
- Study staff

[*US SITE TO INCLUDE/AMEND THE FOLLOWING:* Federal and state laws and the federal medical Privacy Rule also protect your privacy. By signing this form, you provide your authorization for the use and disclosure of information protected by the Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule. This includes things learned from the procedures described in this consent form. Study staff may also collect other information including your name, address, date of birth, and information from your medical records.]

[*NON-US SITES TO INCLUDE/AMEND THE FOLLOWING:* As part of your participation in this research study, your personal information may be sent to the United States for analysis or storage. This includes things learned from the procedures described in this consent form. Study staff may also collect other information including your name, address, date of birth, and information from your medical records. There are laws in the U.S. to protect your personal information when in that country.]

[*SOUTH AFRICA SITE TO INCLUDE/AMEND THE FOLLOWING:* The Protection of Personal Information Act (POPIA) ensures that all South African institutions collect, process, store, and share your personal information in a responsible manner and that they will be held accountable should they abuse or compromise your personal information.]

People outside the study team may need to see or receive your information for this study, such as those listed above. We cannot do this study without your authorization to use and give out your information to them. You do not have to give us this authorization. If you do not, then you may not join this study.

The use and disclosure of your information has no time limit. You may cancel your authorization to use and disclose your information at any time by notifying the Principal Investigator of this study in writing. 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 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.

RESEARCH-RELATED INJURY

[*SITES TO SPECIFY INSTITUTIONAL POLICY:* It is unlikely that you will be injured by being in this study. If you are injured or get sick from being in this study, please tell study staff immediately.

If you believe that the research procedures have resulted in an injury to you, immediately contact the Principal Investigator who is listed on the first page of this form. If you become ill or injured as a result of participation in this study, medical treatment for the adverse reaction or injury will be provided appropriately. The site staff will refer you for ongoing treatment for the injury, if needed. Clinical trial insurance is provided by CONRAD and will be responsible for compensating you for appropriate medical expenses for treatment of any such illness or injury. An HIV infection that occurs during the course of the trial will not be considered an injury or illness caused by trial participation. The research center or sponsor is not responsible for any loss, injuries and/or damages that are caused by any of the following things:

- Any injury that happens because you used other medicine during the study that you did not tell us about.

- Any injury that happens because you did not follow instructions given by the study doctor or nurse.
- Any injury that happens because of negligence on your part.]

[*SITES TO SPECIFY ANY ADDITIONAL POLICY RELATED TO EMERGENCY MEDICAL ATTENTION*]

[*US SITE TO INCLUDE/AMEND THE FOLLOWING:* To pay these medical expenses, the sponsor will need to know some information about you, like your name, date of birth, and social security number or Medicare Health Insurance Claim Number. This is because the sponsor has to check to see if you receive Medicare and if you do, report the payment it makes to Medicare.]

You are not giving up any legal rights by signing this form.

CLINICALTRIALS.GOV
A description of this research study will be available on https://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.

[*SOUTH AFRICA SITE TO INCLUDE LANGUAGE RELATED TO SOUTH AFRICAN NATIONAL CLINICAL TRIALS REGISTER*]

YOUR RIGHTS AS A RESEARCH PARTICIPANT/VOLUNTEER
[*SITES TO SPECIFY INSTITUTIONAL POLICY:*] Being in this study is completely voluntary. You may choose not to join this study or leave this study at any time. If you choose not to join or to leave the study, you can still join other studies and you can still access non-study services you would normally get at this or another clinic. If you want the results of the study after it is over, let the study staff members know.

PROBLEMS OR QUESTIONS
If you ever have any questions about the study, or if you have a research-related injury, you should contact [*SITES TO INSERT NAME OF THE INVESTIGATOR OR OTHER STUDY STAFF*] at [*SITES TO INSERT TELEPHONE NUMBER AND/OR PHYSICAL ADDRESS*].

If you have questions about your rights as a research participant, you should contact [*SITES TO INSERT NAME OR TITLE OF PERSON ON THE IRB/EC OR OTHER ORGANIZATION APPROPRIATE FOR THE SITE*] at [*SITES TO INSERT PHYSICAL ADDRESS AND TELEPHONE NUMBER*].

[SITES TO OMIT THE FOLLOWING IF A SEPARATE CONSENT FOR STORAGE AND FUTURE TESTING OF SPECIMENS IS REQUIRED]

CONSENT FOR LONG-TERM STORAGE AND FUTURE TESTING OF SPECIMENS and RELATED HEALTH INFORMATION

There might be a small amount of blood, vaginal fluid, cervicovaginal tissue, and rectal fluid left over after we have done all the study-related testing. We would like to ask your permission to store these leftover samples and related health information for use in future studies, such as future research to fight HIV and other related diseases. This health information may include personal facts about you such as your race, ethnicity, sex, medical conditions, and your age range. This health information will not include your name or any personal identifying information. The samples will be stored by your participant number only.

If you enroll in the study and agree, your samples and related health data will be stored safely and securely at facilities that are designed so that only approved researchers will have access to the samples. Some employees of the facilities will need to have access to your samples to store them and keep track of where they are, but these people will not have information that directly identifies you.

There is no time limit on how long your samples will be stored. Your samples may be shipped and/or stored outside of the country. The type of testing planned for your leftover specimens is not yet known. However, samples may be used by CONRAD to complete additional quality assurance testing, ensuring that the tests work correctly and supply accurate data. No genetic testing on either a limited set or the full set of genes is planned for leftover samples that are stored for the purposes of future research. It is important that you know that any future testing or studies planned for these specimens must be approved by an Institutional Review Board before they can be done. You will not receive the results from any future testing of these specimens.

You can still enroll in this study if you decide not to have leftover samples stored for future studies. If you do not want the leftover samples stored, we will destroy them when all protocol-specified testing has been completed. You can withdraw your consent for the storage and future testing of specimens at any time by providing your request in writing to the person in charge of this study. However, researchers will not be able to destroy samples or information from research that is already underway.

_____ Initials and Date — I DO agree to allow my biological specimens and health data to be stored and used in future research studies. I understand my biological specimens may be shipped and stored outside of the country.

_____ Initials and Date — I DO NOT agree to allow my biological specimens and health data to be stored and used in future research studies.

CONSENT FOR RECTAL FLUID SAMPLE COLLECTION

We would like to ask your permission to collect a rectal fluid sample at each of your clinic visits starting with Visit 3 to see if the study drugs are present in your rectum. If you agree, study staff will insert a swab in your rectum to take the sample. We will reconfirm the decision you make today at applicable study visits should you change your mind about rectal fluid collection.

You can still enroll in this study if you decide not to have rectal fluid samples taken. You can withdraw your consent for the swabbing of rectal fluid samples at any time by providing your request in writing to the person in charge of this study.

_____ Initials and Date — I DO agree to allow study staff to collect my rectal fluid samples for this study.

_____ Initials and Date — I DO NOT agree to allow study staff to collect my rectal fluid samples for this study.

[SITES TO OMIT THE FOLLOWING IF A SEPARATE CONSENT IS REQUIRED FOR IDI]

CONSENT TO PARTICIPATE IN AN IN-DEPTH INTERVIEW

We would like to ask your permission to participate in an in-depth interview (IDI) at the end of the study to gather more feedback about the vaginal insert. If you agree and are selected to participate in the IDI, trained study staff will ask you questions about your experiences using the product, about product design, packaging and delivery, and other topics related to product use. The IDI may be conducted at the study site, over a secure digital platform, or an agreed upon location.

The IDI is anticipated to last approximately 45-60 minutes. Study staff will take notes and record the interview. You can choose not to answer questions at any time. We will keep the audio recordings and materials from all interviews and discussions confidential and will only use study numbers or fake names to identify them. These materials will be stored in a secure, locked location for at least two years after either the study insert is approved for use or research on the insert is stopped.

We will reconfirm the decision you make today at later study visits should you change your mind about participating in the IDI.

You can still enroll in this study if you decide not to participate in the IDI. You can withdraw your consent to participate in the IDI at any time.

_____ Initials and Date — I DO agree to participate in an in-depth interview. I understand the interview will be recorded and notes will be taken.

_____ Initials and Date — I DO NOT agree to participate in an in-depth interview.

[SITES TO OMIT THE FOLLOWING IF NOT APPLICABLE]

CONSENT FOR OFF-SITE VISITS

If the site determines that an off-site visit is appropriate and with your permission, members of the research team at this clinic may be able to schedule off-site visits with you at your home or at another location as part of the study. Some of the scheduled study visits and some of the study procedures may take place at your home or other location outside of the research clinic. For example, if you need to receive vaginal inserts or to have a urine or blood sample collected but you are unable to come into the clinic. The study personnel will explain in greater detail the requirements of these visits (like the conditions of the place, the type of visit and the time it will take) and the procedures in-place to maintain your information in a confidential manner. However, it is important that you know that off-site visits may eventually affect your confidentiality even if the study staff take precautions not to disclose the purpose of the visits.

We will only conduct visits outside of the clinic if you give us permission to do so. Please read carefully the following statement and initial and date one option. Choosing not to have study visit procedures outside of the study clinic will not affect your participation in this study. Even if you agree today, you can withdraw your consent for off-site visits at any time by providing your request in writing to the person in charge of this study. In addition, before each off-site visit, we will confirm with you that you still agree and remember today's discussion.

PARTICIPANT INITIALS	
_____ Initials Date	I DO agree to have study visit procedures at a location other than the study clinic by clinic staff, when necessary.
_____ Initials Date	I DO NOT agree to have study visit procedures at a location other than the study clinic by clinic staff, when necessary.

SIGNATURE PAGE

[SITES TO INSERT SIGNATURE BLOCKS AS REQUIRED BY THE LOCAL IRB/IEC:]
All of the above has been explained to me and all of my current questions have been answered. I understand that I can ask questions about any aspect of this research study during the course of this study, and that future questions will be answered by the researchers listed on the first page of this form or their representatives.

Any questions I have about my rights as a research participant will be answered by *[SITES TO INSERT LOCAL IRB/IEC INFORMATION].*

I voluntarily agree to be in this research study. A copy of this permission form will be given to me.

_____
Participant's Name (Print)

_____ _____
Participant's Signature Date

_____
Study Staff's Name Conducting
Consent Discussion (Print)

_____ _____
Study Staff Conducting Date
Consent Discussion (Signature)

_____
Witness Name (Print), if required

_____ _____
Witness Signature, if required Date

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