Phase 1 Safety and Pharmacokinetic Study of a Polyurethane Tenofovir Disoproxil Fumarate Vaginal Ring in Sexually Active Women (TDF IVR-002)

Funded by:
Division of AIDS, US National Institute of Allergy and Infectious Diseases
US National Institutes of Health

DAIDS-ES# 30088

Grant #
U19 AI076980

IND Holder:
Albert Einstein College of Medicine

IND# 116945

Project Leader:
Marla Keller, MD

Version 2.0, August 30, 2016

Phase 1 Safety and Pharmacokinetic Study of a Tenofovir Disoproxil Fumarate Vaginal Ring in Sexually Active Women

Table of Contents

Phase 1 Safety and Pharmacokinetic Study of a Polyurethane Tenofovir Disoproxil Fumarate Vaginal Ring in Sexually Active Women

LIST OF ABBREVIATIONS AND ACRONYMS

AE	Adverse event
ALT	Alanine Transaminase
ARV	Antiretroviral
AST	Alanine Aminotransferase
AUC	Area Under the Curve
BLQ	Below the Limit of Quantification
BV	Bacterial Vaginosis
CAPRISA	Centre for the AIDS Programme of Research in South Africa
$C_{average}$	Average Concentration
CBC	Complete Blood Count
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
C_{max}	Maximum Concentration
CRF	Case Report Form
CVF	Cervicovaginal Fluid
CVL	Cervicovaginal Lavage
DAIDS	Division of AIDS
DBS	Dried Blood Spots
DMPA	Depot Medroxyprogesterone Acetate
EAE	Expedited Adverse Event
EC	Ethics Committee
ECX	Ectocervix
Einstein	Albert Einstein College of Medicine
FACTS	Follow on Africa Consortium for Tenofovir Studies
FDA	Food and Drug Administration (US)
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
hCG	Human Chorionic Gonadotropin
HEC	Hydroxyethylcellulose
HIV	Human Immunodeficiency Virus
HPEU	Hydrophilic Polyether Urethane
HPTN	HIV Prevention Trials Network
HSV	Herpes Simplex Virus
IATA	International Air Transport Association
IgG	Immunoglobulin G
IND	Investigational New Drug
INT	Introitus
IPCP-HTM	Integrated Preclinical/Clinical Program for HIV Topical Microbicides
IPM	International Partnership for Microbicides
IQR	Interquartile Range
IRB	Institutional Review Board
IUD	Intrauterine Device
IVR	Intravaginal Ring
mg	Milligram
MMC	Montefiore Medical Center

MO	Medical Officer
MTN	Microbicide Trials Network
N	Number
NAAT	Nucleic Acid Amplification Test
NaCl	Sodium Chloride
ng/mL	Nanogram per milliliter
NHP	Non-Human Primate
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OAT	Organic Anion Transporter
OHRP	Office of Human Research Protections
PD	Pharmacodynamics
PK	Pharmacokinetics
PL	Project Leader
PoR	Pharmacist of Record
PPB	Pharmacy and Poisons Board (national drug regulatory authority in Kenya)
PrEP	Pre-exposure Prophylaxis
PSI	Particle Sciences Incorporated
PSRT	Protocol Safety Review Team
PTM	Pigtailed Macaque
qPCR	Quantitative Polymerase Chain Reaction
REDCap	Research Electronic Data Capture
RSID	Rapid Semen Identification Test
RTI	Reproductive Tract Infection
SAE	Serious Adverse Event
SD	Standard Deviation
SHIV	Simian-Human Immunodeficiency Virus
SOP	Standard Operating Procedures
STI	Sexually Transmitted Infection
TDF	Tenofovir Disoproxil Fumarate
TFV	Tenofovir
TFV-DP	Tenofovir Diphosphate
T_{max}	Time to Maximum Concentration
UA	Urinalysis
US	United States
USP	United States Pharmacopeia
UTI	Urinary Tract Infection
VAG	Vagina
VVC	Vulvovaginal Candidiasis

PROTOCOL TEAM ROSTER

Marla Keller, MD
Project Leader
Professor
Albert Einstein College of Medicine
1300 Morris Park Avenue
Block Building, Room 512
Bronx, NY 10461
Tel: 718-430-3240
Fax: 718-430-8879
E-mail: marla.keller@einstein.yu.edu

Betsy Herold, MD
Co-Investigator
Professor
Albert Einstein College of Medicine
1225 Morris Park Avenue
Van Etten Building, Room 6A-04
Bronx, NY 10461
Tel: 718-839-7460
Fax: 718-862-1833
E-mail:
betsy.herold@einstein.yu.edu

Jeanne Marrazzo, MD, MPH
Co-Investigator
Professor
University of Alabama
Tinsley Harrison Tower 215
1900 University Blvd.
Birmingham, AL 35294-0006
Tel: 205-975-5500
Fax: 205-996-2416
E-mail: jmm2@uab.edu

Nelly Mugo, MBChB, MMed Ob/Gyn, MPH
Co-Investigator
Kenya Medical Research Institute
University of Nairobi Institute of Infections and Tropical Diseases (UNITID)
College of Health Sciences 3rd Floor
Kenyatta National Hospital, Ngong Road
P.O. Box 19865-00202, Nairobi
Tel: +254-733 629665
E-mail: rwamba@uw.edu, nmugo@kemri.org

Kenneth Ngure, MPH, PhD
School of Public Health
College of Health Sciences
Jomo Kenyatta University of Agriculture & Technology
62000
Nairobi, 00200
Tel: +254-722 362219
E-mail: k_ngure@hotmail.com

Craig Hendrix, MD
Co-Investigator
Pharmacology
Professor
Johns Hopkins University
600 North Wolfe Street, Harvey 502
Baltimore, MD 21287
Tel: 410-955-9707
Fax: 410-955-9708
E-mail: cwhendrix@jhmi.edu

Yungtai Lo, PhD
Biostatistician
Associate Professor
Albert Einstein College of Medicine
1300 Morris Park Avenue
Block Building, Room 206
Bronx, NY 10461
Tel: 718-430-8921
E-mail: yungtai.lo@einstein.yu.edu

George A. Mugendi, MPharm
Clinical Pharmacist
Department of Pharmaceutics and Pharmacy Practice
School of Pharmacy
University of Nairobi
Tel: +254-721 480411
E-mail: gmugendi@pipsthika.org

Yvonne Gayle, Pharm D
Pharmacist
Director of Pharmacy Operations
Montefiore Medical Center Weiler Division
1825 Eastchester Road
Bronx, NY 10461
Tel: 718-904-2825 or 718-904-2838
Fax: 718-904-2158
E-mail: ygayle@montefiore.org

Jenny Stanwix
Research Coordinator
Albert Einstein College of Medicine
1300 Morris Park Avenue
Block Building, Room 514
Bronx, NY 10461
Tel: 718-430-3253
Fax: 718-430-8879
E-mail: jenny.stanwix@einstein.yu.edu

NIH
Jim A. Turpin, PhD
Branch Chief
Preclinical Microbicides and Prevention Research Branch (PMPRB)
NIAID/DAIDS
Room 8B31/MSC 9831
5601 Fishers Lane

Rockville, MD 20852-9831
Tel: 301-451-2732
Fax: 240-627-3465
E-mail: jturpin@niaid.nih.gov

Hans M.L. Spiegel, MD, DrMedSc, DTMH, FAAP
Sr. Medical Officer, PMPRB/Prevention Sciences Program,
Contractor, Kelly Government Solutions
DAIDS, NIAID, NIH
5601 Fishers Lane
Room 8B51A
Rockville, MD 20852
Tel: 240-292-4633
Fax: 240-627-3465
MSC 9831
E-mail: hans.spiegel@nih.gov

Jeanna Piper, MD
Sr. Medical Officer
Clinical Microbicide Research Branch
PSP/DAIDS/NIAID/NIH
5601 Fishers Lane
Room 8B68
Rockville, MD 20852
Tel: 240-292-4798
E-mail: piperj@niaid.nih.gov

Cherlynn Mathias, RN, BSN
Clinical Operations, Office of Director
NIAID, NIH, DHHS
Program Officer/Nurse Consultant
Prevention Science Program
Division of AIDS
5601 Fishers Lane
Room 8B51B
Rockville, MD 20852
Tel: 240-292-4791
E-mail: cmathias@niaid.nih.gov

Phase 1 Safety and Pharmacokinetic Study of a Polyurethane Tenofovir Disoproxil Fumarate Vaginal Ring in Sexually Active Women (TDF IVR-002)

INVESTIGATOR SIGNATURE FORM

Version 2.0

Date: August 30, 2016

Funded by:
Division of AIDS, US National Institute of Allergy and Infectious Diseases
US National Institutes of Health

IND Holder:
Albert Einstein College of Medicine

I, the Investigator of Record, agree to conduct this study in full accordance with the provisions of this protocol. I will comply with all requirements regarding the obligations of investigators as outlined in the Statement of Investigator (Form FDA 1572), which I have also signed. I agree to maintain all study documentation for at least two years following the date of marketing approval for the study product for the indication in which it was 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 and the US Food and Drug Administration is notified.

I have read and understand the information 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.

The signature below constitutes the approval of this protocol, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US Federal regulations and ICH guidelines.

_____
Name of Investigator of Record

_____ _____
Signature of Investigator of Record Date

Phase 1 Safety and Pharmacokinetic Study of a Polyurethane Tenofovir Disoproxil Fumarate Vaginal Ring in Sexually Active Women

PROTOCOL SUMMARY

Short Title:	Safety and PK of a TDF ring in sexually active women
Clinical Phase:	Phase 1
IND Holder:	Albert Einstein College of Medicine
Project Leader:	Marla Keller, MD
Sample Size:	80 women (40 US and 40 African); 20 US women in run-in phase are a sub-group of the 40 US women
Study Population:	Healthy, sexually active, human immunodeficiency virus (HIV) uninfected females between the ages of 18 and 45 who are using contraception
Study Sites:	Albert Einstein College of Medicine (Einstein), Bronx, USA; Partners in Health Research and Development, Thika, Kenya
Study Design:	Two-site, two-arm, randomized single-blind placebo-controlled trial
Study Duration:	Accrual will require approximately 12 months. Each participant will engage in the screening process for up to 28 days prior to enrollment. Twenty US women will participate in a 30-day run-in phase to assess safety prior to initiation of enrollment in Kenya.
Study Products:	Reservoir-type tenofovir disoproxil fumarate (TDF) and placebo polyurethane intravaginal ring (IVR)
Study Regimen:	Participants will be stratified by site and will be randomized in a 3:1 ratio to receive either a TDF or a placebo IVR once every 4 weeks over the investigational product use period
Primary Objectives:	Assess safety of TDF IVR when used continuously for 84 days by healthy, HIV-uninfected, sexually active women, as compared with the placebo IVR
Primary Endpoints:	The safety endpoints are: The proportion of women in each arm experiencing adverse events (AEs): o Genitourinary events Grade 2 or higher as defined by the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events judged to be related to study product o Adverse events Grade 2 or higher as defined by the Division

	of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events
Secondary Objectives:	Examine systemic and genital tract pharmacokinetics (PK) of TDF and its metabolites during and after 84 days of continuous IVR use in sexually active women Evaluate the acceptability of the study IVR in HIV-uninfected, sexually active women over 84 days of use
Secondary Endpoints:	The pharmacokinetic endpoints are: Assessment of TDF and tenofovir (TFV) levels in cervicovaginal fluid (CVF), and TFV in plasma on days 28 (visit 4), 42 (visit 5), 56 (visit 6), 70 (visit 7), and 84 (visit 8) after TDF ring insertion, TFV-DP in dried blood spots (DBS) on days 28 (visit 4), 56 (visit 6), and 84 (visit 8) after TDF ring insertion in all participants, and TFV and TFV-DP in cervical tissue on days 28 (visit 4) and 84 (visit 8) in all participants and on day 56 (visit 6) in US participants only Participant report and partner report of acceptability including genitourinary and emotional (dis)comfort, awareness/feeling during daily activities and during sex, willingness to use during menses and in the future
Exploratory Objectives:	Examine systemic and genital tract PK of TDF and/or its metabolites (TFV, TFV-DP) soon after TDF ring insertion and removal to assess rapidity with which drug concentrations rise post-insertion and fall after ring removal Examine the impact of TDF and placebo IVRs on the vaginal microbiome and the relationship between the vaginal microbiome and PK of TDF and/or its metabolites Evaluate the pharmacodynamics (PD) of luminal drug during and after 84 days of continuous use of a reservoir-type TDF IVR Examine the impact of study IVR on genital tract mucosal immunity
Exploratory Endpoints:	Assessment of TDF and TFV in CVF, TFV and TFV-DP in tissue at 1 hour (Visit 2a), 4 hours (Visit 2b) and 1 day (Visit 2c) after TDF ring insertion, TFV in plasma, TDF and TFV in CVF, and TFV and TFV-DP in tissue at 7 (Visit 2d), 14 (Visit 3a), and 21 (Visit 3b) days after TDF ring insertion, TFV-DP in tissue 5-7 days (Visit 9a) after ring removal, TDF and TFV in CVF and TFV in plasma 10-12 (Visit 10) days after ring removal in US participants only Assess composition of vaginal microflora using broad-range 16S rRNA gene PCR with Illumina sequencing and species-specific quantitative polymerase chain reaction (qPCR) assays before, during and after continuous TDF ring use and correlate changes in bacteria with concentrations of TDF and/or its metabolites

Measure of anti-HIV activity in CVF

Assessment of select innate immune and host defense mediators in vaginal swabs before and after IVR use

Study Schema for All Participants

	Visit 1: Scr	Visit 2: Enr IVR Insertion	Visit 3: Day 14	Visit 4: Day 28 IVR Change	Visit 5: Day 42	Visit 6: Day 56 IVR Change	Visit 7: Day 70	Visit 8: Day 84 IVR Removal	Visit 9: Day 89 Final Visit
Pelvic Exam to assess AEs			X	X		X		X	X
Blood PK		X[1]	X	X	X	X	X	X	X
CVF PK		X[1]	X	X	X	X	X	X	X
Tissue PK				X		X[2]		X	
Behavior studies[3]				X				X	

[1]Blood and CVF will be collected at Enrollment (Visit 2) prior to IVR insertion
[2]Only US participants will have tissue collected at Day 56
[3]Quantitative instrument in all women and qualitative interviews in 20 women and up to 20 male partners at the Thika site at Days 28 and 84 and focus groups in female participants at the Thika site after study completion

Exploratory PK for US Participants Only[1]

	Visit 2a: 1 hour	Visit 2b: 4 hours	Visit 2c: Day 1	Visit 2d: Day 7	Visit 3a: Day 14	Visit 3b: Day 21	Visit 9a: Day 89	Visit 10 Day 94 Final Visit
Pelvic Exam	X	X	X	X	X	X	X	X
Blood PK						X		X
CVF PK	X	X	X	X		X		X
Tissue PK	X	X	X	X	X	X	X	

[1]Only US participants will be randomized to additional sampling at only 1 of 7 time-points: 1 hour (Visit 2a), 4 hours (Visit 2b), 1 (Visit 2c), 7 (Visit 2d), 14 (Visit 3a) or 21 days (Visit 3b) after IVR insertion or 5-7 days after IVR removal (Visit 9a). There will be 5 or 6 US participants per time-point.

Number of Cervical Biopsies Obtained per Time-point

	Visit 4: Day 28 IVR Change	Visit 6: Day 56 IVR Change	Visit 8: Day 84 IVR Removal	Additional time point[1]	Total number of cervical biopsies per participant
US cohort	2	2	2	2	8
Kenya cohort	2		2		4

[1]Only US participants will be randomized to additional tissue sampling at only 1 of 7 time-points: 1 hour (Visit 2a), 4 hours (Visit 2b), 1 (Visit 2c), 7 (Visit 2d), 14 (Visit 3a) or 21 days (Visit 3b) after IVR insertion or 5-7 days after IVR removal (Visit 9a). There will be 5 or 6 US participants per additional time-point.

1 KEY ROLES

1.1 Protocol Identification

Protocol Title: Phase 1 Safety and Pharmacokinetic Study of a Polyurethane Tenofovir Disoproxil Fumarate Vaginal Ring in Sexually Active Women

Short Title: Safety and PK of a TDF Ring in Sexually Active Women

Date:

Project Leader:
Marla Keller, MD
Professor
Albert Einstein College of Medicine
1300 Morris Park Avenue
Block Building, Room 512
Bronx, NY 10461
Tel: 718-430-3240
Fax: 718-430-8879
E-mail: marla.keller@einstein.yu.edu

Co-Investigators:
Betsy Herold, MD
Professor
Albert Einstein College of Medicine
1225 Morris Park Avenue
Van Etten Building, Room 6A-04
Bronx, NY 10461
Tel: 718-839-7460
Fax: 718-862-1833
E-mail: betsy.herold@einstein.yu.edu

Jeanne Marrazzo, MD, MPH
Professor
University of Alabama
Tinsley Harrison Tower 215
1900 University Blvd.
Birmingham, AL 35294-0006
Tel: 205-975-5500
Fax: 205-996-2416
E-mail: jmm2@uab.edu

Nelly Mugo, MBChB, MPH
Kenya Medical Research Institute
University of Nairobi Institute of Infections and Tropical Diseases (UNITID)
College of Health Sciences 3rd Floor
Kenyatta National Hospital, Ngong Road
P.O. Box 19865-00202, Nairobi
Tel: +254-733 629665
E-mail: rwamba@uw.edu, nmugo@kemri.org

Biostatistician:
Yungtai Lo, PhD
Associate Professor
Albert Einstein College of Medicine
1300 Morris Park Avenue
Block Building, Room 206
Bronx, NY 10461
Tel: 718-430-8921
E-mail: yungtai.lo@einstein.yu.edu

Monitor Identification:
Division of AIDS (DAIDS)
National Institute of Allergy and Infectious Diseases (NIAID)
National Institutes of Health (NIH)
6700 B Rockledge Drive
Bethesda, MD 20892 USA

Medical Officers:
Hans M.L. Spiegel, MD, DrMedSc, DTMH, FAAP
Sr. Medical Officer, PMPRB/Prevention Sciences Program,
Contractor, Kelly Government Solutions
DAIDS, NIAID, NIH
5601 Fishers Lane
Room 8B51A
Rockville, MD 20852
Tel: 240-292-4633
Fax: 240-627-3465
MSC 9831
E-mail: hans.spiegel@nih.gov

Jeanna Piper, MD
Sr. Medical Officer
Clinical Microbicide Research Branch
PSP/DAIDS/NIAID/NIH
5601 Fishers Lane
Room 8B68
Rockville, MD 20852
Tel: 240-292-4798
E-mail: piperj@niaid.nih.gov

James F. Rooney, MD
Vice President Medical Affairs
Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, CA 94404
Tel: 650-522-5708
Fax: 650-522-5854
E-mail: jim.rooney@gilead.com

Clinical Laboratories:
Montefiore Medical Center
Department of Pathology
111 East 210th Street
Bronx, NY 10467

Thika Partners in Prevention House
Located next to Thika Nursing Home, Section 9
P.O. Box 19865 - 00202, Nairobi, Kenya
Thika Town

Data Management:
Albert Einstein College of Medicine
1300 Morris Park Avenue
Bronx, NY 10461

Study Operations:
Cherlynn Mathias, RN, BSN
Clinical Operations, Office of Director
NIAID, NIH, DHHS
Program Officer/Nurse Consultant
Prevention Science Program
Division of AIDS
5601 Fishers Lane
Room 8B51B
Rockville, MD 20852
Tel: 240-292-4791
E-mail: cmathias@niaid.nih.gov

Rebecca Guzmán
Clinical Trials Manager
Gilead Sciences, Inc.
333 Lakeside Drive, Building 300
Foster City, CA 94404
Tel: 650-524-3953
Fax: 650-524-9387
E-mail: rebecca.guzman@gilead.com

2 INTRODUCTION

2.1 Background

The UNAIDS Report on the Global AIDS Epidemic 2013 indicates that 35.3 million people were living with human immunodeficiency virus (HIV) and 2.3 million individuals acquired HIV in 2012 [1]. The majority of new infections are transmitted through heterosexual contact, with women and children bearing a disproportionate burden. Women account for 23% of new HIV infections in the United States (US) and 50% globally. The Centre for the AIDS Programme of Research in South Africa (CAPRISA) 004 trial demonstrated that tenofovir (TFV) vaginal gel reduced HIV acquisition by an estimated 39% overall, and by 54% in women with high gel adherence when applied before and after sexual intercourse (BAT24 dosing)[2]. However, subsequent studies with pericoital TFV gel dosing (Follow on Africa Consortium for Tenofovir Studies [FACTS] 001) and daily TFV gel use (Microbicide Trials Network (MTN) 003) failed to show protection [3, 4].

Previous findings highlight the difficulties with adherence to daily or pericoitally dosed intravaginal gels. The development of delivery systems that overcome difficulties associated with adherence, such as intravaginal rings (IVRs), may result in greater reductions in the rates of HIV-1 infection [5]. IVRs are used by women for hormone replacement therapy and contraception [6]. Previous studies have shown that many women have a strong preference for rings compared to gel formulations for vaginal drug administration, citing discretion and ease of use as major advantages [7, 8].

2.2 Rationale

Tenofovir disoproxil fumarate (TDF) is a prodrug of TFV, an adenosine nucleoside monophosphonate (nucleotide) derivative with potent antiretroviral (ARV) activity. TDF is marketed by Gilead Sciences, Inc. (Foster City, CA) under the trade name Viread® and is licensed for the treatment of HIV-1 infection in the US, the European Union, Middle East, and Africa. TDF permeates cells more rapidly than TFV resulting in increased intracellular accumulation of TFV-diphosphate (TFV-DP), the active metabolite [9]. TDF is more potent than TFV and inhibits HIV-1 and herpes simplex virus type 2 (HSV-2) infection in cell and tissue culture models at approximately 100-fold lower concentrations than TFV, suggesting that it may be an excellent candidate for prevention of HIV [10].

2.3 Integrated Preclinical/Clinical Program

The National Institutes of Health (NIH) Integrated Preclinical/Clinical Program for HIV Topical Microbicides (IPCP-HTM) supports microbicide development linked with research conducted as a multi-project, multidisciplinary cooperative agreement. The IPCP-HTM seeks to increase the variety of approaches and availability of possible candidate microbicides appropriate for advancement into clinical trials.

The IPCP program at the Albert Einstein College of Medicine (Einstein) brings together an inter-disciplinary group of research scientists from 5 academic institutions: Einstein, Johns Hopkins University, Northwestern University, University of Washington, University of Nairobi, as well as scientists from the NIH and the Centers for Diseases Control (CDC). The goal of the program is to develop and evaluate a TDF polyurethane reservoir IVR using an iterative, interactive approach between the laboratory components, formulation development efforts, animal model studies, and early Investigational New Drug (IND) clinical studies.

The study outlined here is the second clinical study of the polyurethane TDF vaginal ring (TDF IVR-002) following on the successful completion of a first-in-human study (TDF IVR-001). The initial safety and pharmacokinetic (PK) study was conducted among 30 healthy, sexually abstinent US women 18-45 years of age who used a TDF or placebo IVR continuously for 14 days. The TDF and placebo IVRs were safe and well tolerated. There were 8 product-related adverse events (AEs), which were mild [11]. Median cervicovaginal fluid (CVF) TFV concentrations 14 days after TDF ring use were in excess of the concentration associated with protection in CAPRISA 004 (10^3 ng/mL) [12]. Median CVF TFV concentrations following ring use were also above the median TFV concentration ($5x10^4$ ng/mL) measured after 6 weeks of daily oral TDF in MTN 001 [13], a regimen proven to be 100% effective in the Partners Pre-Exposure Prophylaxis (PrEP) study if taken with high adherence [14]. CVF TFV concentrations were also similar to those achieved with macaque-sized rings in which the animals were completely protected from repeated simian-human immunodeficiency virus (SHIV) vaginal challenges [15].

Importantly, the objectives of this study are congruent with the National Institute of Allergy and Infectious Diseases (NIAID) Topical Microbicide Strategic Plan to support early clinical trials of selected, highly promising candidate topical HIV microbicides. A TDF IVR safety and PK study is proposed in sexually active women at 2 sites (US and Kenya) and is supported by data demonstrating more potent *in vitro* antiviral activity of TDF compared to TFV against both HIV-1 and HSV-2 in cell cultures and murine models [10, 16, 17], pigtailed macaque (PTM) studies demonstrating an excellent safety and PK profile and complete protection against 16 weekly challenges with SHIV [15], and a first-in-human TDF IVR trial which resulted in few product-related AEs and a favorable PK profile [11]. Additional support for advancing a TDF IVR comes from consideration of these findings in the context of data from previously completed oral and topical PrEP studies. Plasma concentrations after 2 weeks of TDF IVR use were similar to serum concentrations found after 6 weeks of daily vaginal TFV gel dosing (C_{max} = 3.9 ng/mL) but were significantly lower than those observed with oral daily TDF dosing (332 ng/mL). Importantly, median tissue TFV-DP concentrations after TDF IVR dosing (120 fmol/mg) were between concentrations achieved in MTN 001 with oral TDF (10 fmol/mg) and TFV gel dosing (1807 fmol/mg) with CVF TFV levels that exceeded those with oral TDF dosing [13].

2.4 Tenofovir Disoproxil Fumarate

TDF (tenofovir DF, or Viread®) marketed by Gilead Sciences, Inc. (Foster City, CA), received marketing approval for the treatment of HIV-1 infection in the US in 2001 and in the European Union, Middle East, and African regions in 2002. In 2012, US Food and Drug Administration (FDA) granted approval for oral use of TDF in combination with emtricitabine (combination marketed as Truvada® by Gilead Sciences, Inc.) to reduce the risk of HIV infection in uninfected individuals who are at high risk and who may engage in sexual activity with HIV-infected partners.

2.5 Tenofovir Disoproxil Fumarate Polyurethane IVR

A new class of IVR was engineered capable of achieving pharmacologically significant fluxes of hydrophilic ARVs like TDF using hollow hydrophilic polyether urethane (HPEU) elastomeric tubes that are sealed and formed into IVRs [18]. The drug product, TDF IVR, developed within the NIH IPCP-HTM (U19 AI076980), is a white (with clear segment), flexible torus-shaped device (Figure 1, left). The corresponding placebo is a clear, flexible torus-shaped device (Figure 1, right). The TDF and placebo rings are formulated using preformed flexible tubing, comprised of biomedical grade hydrophilic, aliphatic polyether urethane, and an inner core compartment comprised of 365 ± 15 milligrams (mg) (86 wt% of formulation) TDF and 55 ± 5 mg (14 wt% of formulation) sodium chloride (NaCl). The terms polyether urethane and polyurethane are similar.

The polyurethane class of biomedical elastomers has long been one of the materials of choice for medical applications. Common polyurethane medical products include urinary catheters, urinary stents, sutures, cardiovascular, orthopedic and dialysis devices. The polyurethane used to formulate the TDF and placebo rings is from the HydroThane™ class of medical grade polymers; polyurethane is also being used in vaginal rings that release TFV alone or TFV and levonorgestrel, which are currently being evaluated in a Phase 1 safety and PK study sponsored by CONRAD (clinicaltrials.gov identifier: NCT02235662). The specific polymer used in the TDF and placebo ring is HydroThane AL 25 93A. The ring dimensions are comparable to commercially available rings. TDF IVR is designed to provide sustained delivery of an average of 5.5 mg/day of TDF for at least 30 days. This HPEU polymer was selected because the polarity of TDF is too high to deliver protective doses of the drug from more common elastomers used in IVR technology such as silicone and poly(ethylene-co-vinyl acetate)[10].

Figure 1. Representative images of TDF (left) and placebo (right) IVRs. Scale = 1 cm.

The reservoir IVR design is composed of HPEU tubing with a drug-bearing core sealed by induction-melt welding into a torus [18]. The tubing wall acts as rate controlling membrane for drug release while providing the necessary mechanical support for ring retention in the vaginal canal. Mechanical properties were adjusted so that the ring has compression stiffness similar to NuvaRing®, a hormonal contraceptive vaginal ring. We included an osmotic agent to attract vaginal fluid into the core to solubilize TDF and establish a reservoir of soluble and diffusible drug to drive release, as simply filling the device with TDF resulted in a long lag time of over 20 days. The final design, which contains NaCl (14 wt%) as an osmotic excipient, was selected by scanning a series of osmoattractants *in vitro*. Figure 1 shows a schematic of the TDF IVR. We were able to achieve highly reproducible release rates *in vitro* from these devices. Results from prototypic TDF IVRs are shown in Figure 2. The daily TDF release rate from TDF IVRs was 0.14% to 2.7% over the 30-day duration. The average TDF release rate for 30-day duration was 5.5 ± 1.5 mg/day (mean ± standard deviation (SD); N=3). Because TDF is hydrolyzed to the monoester (monoPOCTFV) and subsequently to TFV, we also measured these components. Only a small amount of the monoester was detected but there was no detectable hydrolysis *in vitro* to TFV.

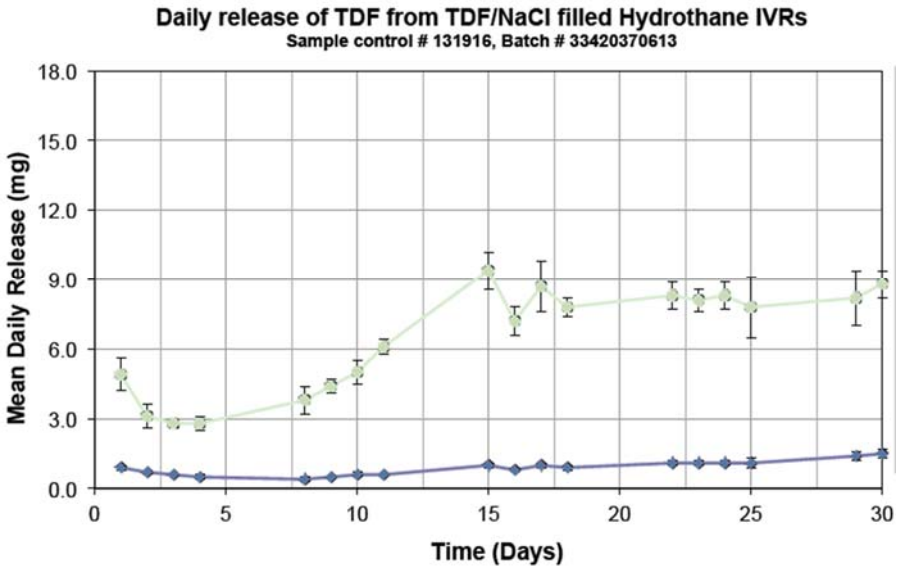


Figure 2. Representative daily TDF (green) and the monoester (blue) *in vitro* release from IVR.

2.6 Non-Human Primate (NHP) PK, Safety, and Efficacy Studies

2.6.1 Non-Human Primate PK Studies

We conducted non-Good Laboratory Practice (GLP) studies in NHPs at the CDC according to Institutional Animal Care and Use Committee (IACUC) protocols and laboratory standard operating procedures (SOP). The CDC does not conduct GLP macaque studies, but the PTM is the most analogous model for topical toxicity in humans and investigators at the CDC are considered experts in this model.

The prototype TDF IVR used in macaque studies had an identical composition to the human TDF IVR, except the overall dimensions of the IVR were reduced to fit a macaque vagina [15]. The PK of the TDF reservoir IVR was investigated in PTM (n=6) in a 28+2 study (28 days of IVR exposure and 2 days after removal), for drug distribution and concentration in vaginal fluid and tissue. Vaginal tissue biopsies were collected proximal and distal to the TDF IVR on day 7, 21, and 30 and analyzed for drug. The IVR design provided TFV fluid and tissue concentrations of $8.1 \pm 6.9 \times 10^4$ ng/mL and $2.7 \pm 9.8 \times 10^4$ ng/mL (mean ± SD), respectively. These concentrations significantly exceeded the TFV concentration of 10^3 ng/mL recovered in cervicovaginal lavage (CVL) that correlated with protection in women receiving 1% TFV gel [12]. Since TDF hydrolytically converts into TFV, more variable levels of TDF were observed [15]. Notably, TFV levels in swabs were $1.4 \pm 2.2 \times 10^4$ ng/mL and in tissue were $7.1 \pm 8.6 \times 10^3$ ng/g; (mean ± SD) two days after ring removal. In vaginal tissue, the TFV concentration exceeded the *in vitro* IC_{50} by approximately 200 times. TFV and TDF were both below the limit of quantification (BLQ) in the plasma (5 ng/mL) at all time-points examined indicating minimal systemic absorption, an important safety parameter. In PK studies using oral TDF (300 mg of Viread®) the plasma C_{max} values of TFV exceeded 300 ng/mL following oral daily administration [13]. The TDF IVR average dose over 30 days was approximately 2.3 mg/day and resulted in plasma levels <5 ng/mL (BLQ).

2.6.2 NHP Effectiveness Study

We conducted a weekly challenge study in sexually mature, normal cycling PTM (Figure 3a) [15]. Six TDF IVR-treated macaques received weekly low dose 50 $TCID_{50}$ SHIV162p3 inoculations starting six days after IVR insertion. Control animals (n=6 real time and n=6 historical controls)

were challenged similarly and 11 of 12 became infected with peak viral RNA levels of 3.4 x 10^6 ± 1.9 x 10^7copies/mL (median ± SD) with a median of 4 exposures to infection, assuming a 7 day eclipse period from time of infection to detection of viral RNA in plasma. In contrast, all TDF IVR-treated animals (6 of 6) remained SHIV viral RNA negative and seronegative after 16 weekly exposures spanning over 4 months and involving 4 IVR changes (Figure 3b) (p=0.0004, Fisher's exact test). All TDF treated macaques remained uninfected after 4 weeks of follow up with a TDF IVR in place. These NHP were further monitored for a year with no evidence of viral infection. Vaginal secretion and tissue levels of TFV remained consistent for the duration of the study [15].

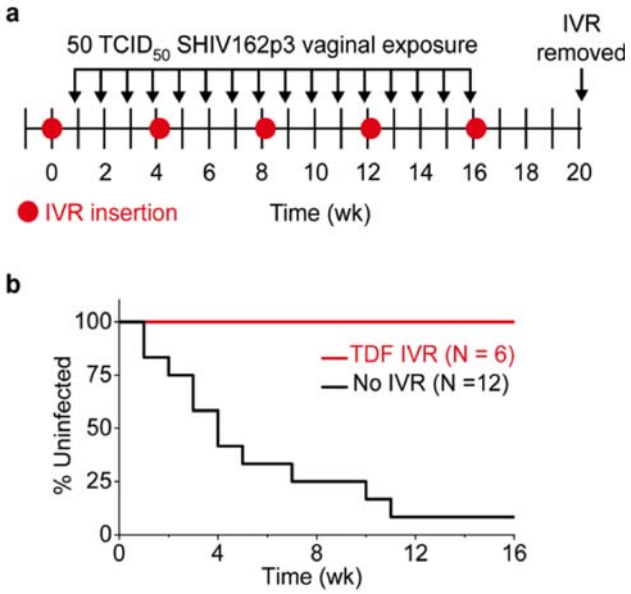


Figure 3. a) Six TDF IVR-treated cycling female macaques received weekly low dose 50 $TCID_{50}$ SHIV162p3 inoculations starting 6 days after the first IVR insertion. Control animals (N=6 real time and N=6 historical controls) were challenged similarly. The ring was replaced as shown in red (every 28 days starting 2 days after the fourth virus exposure). Animals were monitored weekly (until week 20) for presence of SHIV by RT-PCR and confirmed by Western blot and were defined as infected and exposures discontinued if vRNA was detected in plasma for 2 consecutive weeks. b) Kaplan-Meier plot showing time to infection for TDF IVR (N=6; red) and control (N=6 real-time and 6 historical naïve; black) groups (Fisher's Exact test; p=0.0004).

A second efficacy study was conducted in a more stringent model combining repeated 30 mg injections of depot medroxyprogesterone acetate (DMPA) every 6 weeks with vaginal viral challenges weekly for 12 weeks. Twelve macaques were randomized to TDF or placebo rings. All placebo macaques became infected after a median of 2 exposures, whereas only 1 TDF macaque became infected at the eighth exposure (P = 0.0012) [19].

2.6.3 NHP Safety

All of the animals in the 14 and 28-day studies tolerated the ring well without any AEs observed. A pediatric speculum was inserted weekly to assess for any signs of inflammation and to ascertain for retention of the ring (n=4 observations per animal in the 28 day PK study and 20 observations per animal in the 5 month efficacy study). There were no signs of erythema or tissue disruption in any of the animals as assessed by colposcopy. Two animals in the 5-month study had mild vaginal discharge at a single time-point; one at week 12.5 and the other at week 15. There was no discharge observed at any other visit or in any other animal. Transient white discharge is observed occasionally in animals not in PrEP studies and if symptoms persist the animals are tested and treated for vaginal infection. The observed discharge in the 2 animals resolved without intervention. In addition, there were no systemic signs of illness including changes in food intake or changes in behavior observed with daily visual observations in any of the macaques. None of the animals had a weight gain or loss of more than 10% throughout the study period.

The induction of mucosal inflammation due to IVR presence continuously for 6 months was monitored by measurement of 18 vaginal cytokines and chemokines. Although there were some

differences in cytokines before ring compared to after ring placement, when using false discovery rate (FDR)-adjusted p-values, the differences reflected variability between time-points, suggesting that the effects were not due to IVR use [15, 20].

Safety was also evaluated using the rabbit vaginal irritation model and a murine model. As detailed in the IND, no safety concerns were noted. In the murine study, TDF safety was assessed in female Balb/c mice following twice daily vaginal application of 0.3 wt% TDF gel compared to hydroxyethylcellulose (HEC) placebo (3 wt% HEC in water) treated mice over 7 days by examining whether exposure increased the susceptibility of the mice to genital herpes. Increased susceptibility to genital herpes infection has been observed in this model with 7 single daily doses of nonoxynol-9 and 6 wt% cellulose sulfate gel, products that may have increased the risk of HIV acquisition in clinical trials [21-24]. The dosing for this study was designed to deliver in the mouse vagina 10 times the estimated maximum dose released from the TDF IVR in humans scaled to relative surface areas. Approximately 12 hours after the last vaginal dose, the mice were intravaginally challenged with concentrations of virus needed to cause severe disease in 30 and 90% of mice (LD_{30} and LD_{90}), respectively (10 mice per each of the four groups) of control mice and the mice were monitored for signs of disease daily and sacrificed if mice developed substantial genital tract or neurological disease as previously described [23-25]. In parallel studies, additional mice received twice daily gel applications for 3 and 7 days (n=3 for HEC and n=6 per TDF group) or no gel product (n=2) and were sacrificed 12 hours after the last dose. The genital tissue from these mice was excised and RNA extracted from half the tissue for evaluation of cytokines, chemokines, junctional proteins, and mitochondrial protein expression by RT-PCR. The other half of the tissue was embedded in OCT and subsequently evaluated by H&E staining. There was no increase in susceptibility to HSV-2 in mice treated with twice daily dosing of TDF gel. Rather, TDF treatment protected the mice from HSV-2 disease compared to HEC gel (p=0.02). In addition, there were no significant changes in cytokine, chemokine, mitochondrial or junctional protein gene expression and no changes in tissue architecture were observed by histology.

2.7 Rationale for TDF IVR and Preclinical Data

Vaginal formulations of TDF and TFV have not been directly compared in prior NHP or clinical studies. However, *in vitro* studies consistently demonstrate that ~100-fold less TDF compared to TFV is needed to protect cells in culture from HIV or HSV. Consistent with this observation, a 0.3% TDF gel (formulated in HEC and maintained at -80°C until use to prevent hydrolysis to monoPOC or to TFV) was significantly more protective than 1% TFV gel following intravaginal HSV-2 challenge in a murine model [16] and against both HIV and HSV-2 in a transgenic mouse model [17]. Differential mechanisms of genital tract cellular uptake and metabolism may result in a more favorable PK profile for TDF compared to TFV, and hence more potent antiviral activity. We performed membrane permeability and drug transport assays to test this hypothesis in vaginal epithelium (VK2) and Jurkat T cells. The average permeability of TDF through lipid bilayers by parallel artificial

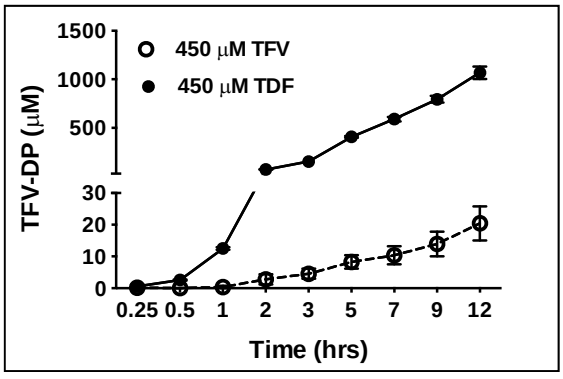


Figure 4. Cells were exposed to 450μM TDF or TFV in RPMI medium for the indicated time (15 minutes-12 h) and at each time point, the intracellular concentrations of drugs and metabolites were measured by HPLC MS/MS. Results for the active intracellular metabolite, TFV-DP, are shown as mean (SEM) of duplicates and are representative of two independent experiments.

membrane permeability assay (PAMPA) was at least 4-5 orders of magnitude greater than that observed for TFV permeability, suggesting that only TDF enters cells by passive diffusion [26]. Renal tubular transport of TFV is thought to occur via organic anion transporters (OAT) [27], but we did not detect OAT1 or OAT3 expression by qRT-PCR in vaginal or cervical epithelial cells, Jurkat T cells, HaCaT cells, primary keratinocytes, or peripheral blood mononuclear cells. This is consistent with several other studies that have also failed to identify OAT1 or OAT3 in human tissue [28, 29]. Instead, studies with pharmacological inhibitors of endocytic pathways indicated that TFV uptake occurs via an energy-dependent endocytic-like pathway. Exposure of vaginal epithelial cells to equimolar concentrations of TDF and TFV resulted in approximately 40-fold higher intracellular concentrations of TFV-DP by HPLC-MS/MS (Figure 4) [26].

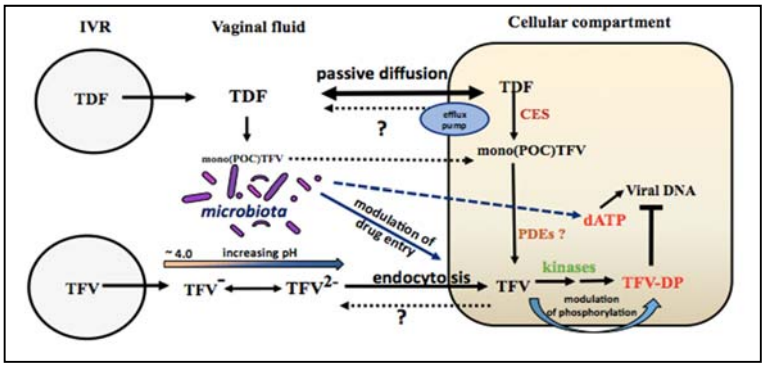


Figure 5. Cellular uptake of TDF vs. TFV

TDF is rapidly taken up by cells by passive diffusion and then converted by carboxylesterases first to the monoester (monoPOCTFV) and then to TFV as illustrated in Figure 5. TFV is subsequently phosphorylated by cellular enzymes to the active metabolite, TFV-DP, which competes with intracellular pools of deoxy-adenosine triphosphate (dATP) for HIV reverse transcription. Genital tract tissue and cells are therefore exposed primarily to TFV and thus the safety of TFV gel and of oral formulations containing TDF (Viread® or Truvada®) is relevant to the safety of the TDF IVR.

2.7.1 Clinical Studies of TFV Gel

There have been several studies with TFV 1% vaginal gel applied either daily or before and after sex (2 doses within 24 h period). In HIV Prevention Trials Network (HPTN) 050, a Phase 1 safety and acceptability study, 1% TFV gel formulation was well tolerated in both HIV uninfected and infected females [30]. The majority of AEs were mild (87%) and limited to the genitourinary tract (77%). Four severe AEs were reported, but only one (lower abdominal pain) was thought to be product-related. HPTN 059 was a Phase 2 expanded safety and acceptability study of 1% TFV gel. No statistically significant differences were seen between those receiving TFV and placebo gels in complete blood count (CBC), liver or renal function tests. Among those using a study gel daily, no participants had pelvic exam findings involving generalized erythema or severe edema or deep epithelial disruption at any follow-up visit during the study. At the Week 24 Visit, no participants had exam findings suggestive of vaginitis, cervicitis, superficial disruption, disrupted blood vessels, or intermenstrual bleeding.

MTN-001 was a Phase 2 study of adherence to and PK of oral and vaginal preparations of TFV among 144 sexually active HIV-negative females at sites in Uganda, South Africa and the US. Participants followed each study regimen (oral, vaginal and a combination of oral and vaginal) for six weeks. All regimens (oral 300 mg TDF, 1% TFV gel (40 mg) or both) were well tolerated. Transient and mild nausea was more frequent in the oral (15%) and combination periods (14%) when compared to the vaginal period (3%) ($p<0.001$). Headache was also more frequent during the combination dosing period (8%) compared to vaginal dosing periods (2%) ($p<0.01$) with intermediate frequency with oral only dosing (5%). Hypophosphatemia was the most commonly reported AE, but did not differ in frequency among regimens: 11% vaginal, 15% oral, 15% combination ($p>0.05$). Serum concentrations after vaginal dosing were 56-fold lower than after oral

dosing (p<0.001) whereas vaginal tissue TFV-DP was ≥130-fold higher with vaginal compared to oral dosing (p<0.001) [13]. In a CONRAD study among 45 female participants, TFV exposure was low in blood plasma following single and multiple dose gel exposure [31]; median C_{max} was 4.0 and 3.4 nanogram/milliliter (ng/mL), respectively (C≤29 ng/mL). TFV concentrations were high in CV aspirates and vaginal tissue after single and multiple dosing, ranging from 1.2 x 10^4 to 9.9 x 10^6 ng/mL and 2.1 x 10^2 to 1.4 x 10^6 ng/mL, respectively, and did not noticeably differ between proximal and distal tissue. TFV-DP was high in endocervical cells, ranging from 7.1 x 10^3 to 8.8 x 10^6 ng/mL. TFV-DP was detectable in approximately 40% of the vaginal tissue samples, ranging from 1.8 x 10^2 to 3.5 x 10^4 ng/mL. These findings demonstrate that multiple dose TFV gel exposure resulted in high genital tract concentrations with minimal systemic absorption [13, 31].

MTN-003 was a Phase 2B safety and effectiveness study of 1% TFV vaginal gel, oral TDF tablet and oral TDF/emtricitabine tablet conducted in 5,029 women in Sub-Saharan Africa. While the three products did not prove to be effective in the intent-to-treat analysis, and most participants did not use them daily as recommended (~30% had TFV detected in plasma) no safety concerns were identified for any of the products. Of note, a secondary analysis of TFV gel's efficacy that compared women in the 1% TFV gel arm with PK evidence of consistent adherence to non-adherers reported a 48% reduction in HIV acquisition (p=0.03)[4]. These results suggest that delivery of products that requires minimal daily adherence may be more suitable and provide rationale for advancing a TDF IVR.

MTN-011 was a Phase 1 PK and pharmacodynamics (PD) study of 1% TFV vaginal gel used pericoitally by 24 women in the US. TFV PK was determined following single applications of gel 1 h before, 24 h before, or 1 h before and after (BAT24 dosing) penile-vaginal sexual intercourse without a condom. Importantly, TFV levels decreased significantly after sex in both vaginal and cervical tissue. Vaginal tissue levels saw a 75% decrease after 1 h application and a 71% decrease after 24 h application, while cervical tissue levels saw a 75% decrease after 1 h application and a 55% decrease after 24 h application. Levels of TFV were restored to those observed in the absence of sex when BAT dosing was used [32]. These results suggest that sex is associated with a significant decrease in luminal and tissue TFV concentrations and provide strong rationale for sustained drug delivery.

FACTS 001 was a Phase 3 safety and effectiveness study of pericoital 1% TFV gel using BAT24 dosing in 2,059 women at high risk for HIV in South Africa. Seventy percent of women were under 25 years of age, and the use of TFV gel pericoitally failed to reduce HIV acquisition in women randomized to TFV versus placebo gel. The HIV incidence rate was 4% in both groups. A high level of TFV measured in CVL was significantly associated with a reduction in HIV acquisition (HR: 0.52; 95% CI: 0.27-0.99; p=0.04) [3].

2.8 First Clinical Study of TDF IVR

2.8.1 Safety and acceptability

TDF IVR-001 was a first-in-human single blind, randomized placebo-controlled trial to evaluate the safety and PK during 14 days of TDF IVR use in 30 healthy, sexually abstinent US women ages 18-45 [11]. The objectives were to assess the safety of a TDF and placebo IVR and to measure PK in plasma, dried blood spots (DBS), CVF, and cervical tissue over 14 days of continuous ring use. There were a total of 43 AEs. Twenty-nine AEs occurred in 12 women in the TDF arm and 14 were reported in 7 placebo recipients. Twenty-three AEs involved the reproductive tract, 8 of which were judged to be product-related in 6 participants who received TDF versus 1 who received placebo. All product-related AEs were due to vaginal or cervical discharge and were mild (Grade 1). An

additional 8 reproductive tract AEs were related to study procedures and 7 were due to intermenstrual bleeding. There were 2 non-product-related Grade 2 AEs (angioedema of eye, friable cervix due to study procedures). There were no Grade 3-4 AEs, and no serious AEs. There were no discontinuations due to AEs. There were no colposcopic changes due to product use. There were 10 colposcopic findings; 6 were present prior to ring placement and 4 observed at the final study visit were due to speculum trauma. There were no changes in Nugent scores over the dosing period. There were a total of 9 laboratory abnormalities in 7 participants. Six occurred in 5 participants in the TDF arm and 3 occurred in 2 in the placebo arm. All lab abnormalities occurred 14 days after ring insertion, were Grade 1 (mild), and were not related to study product. Participants completed a computer-assisted survey. None reported ring removal or expulsions and 2 reported physical discomfort. Twelve women (9 TDF and 3 placebo) reported that the vagina was wetter after ring use. All strongly liked or somewhat liked the ring, all reported that it was very easy or somewhat easy to wear, and all definitely or probably would recommend it to others. Although the TDF and placebo IVRs are not identical in appearance we learned from this first trial that it may be difficult for participants to distinguish between the TDF and placebo IVRs because of mucus and secretions adherent to the ring after insertion and use.

2.8.2 PK and PD

The TDF IVR provided high tenofovir disoproxil (TD) (the fumarate disassociates from TDF in solution) and CVF TFV concentrations (Table 1). The median average vaginal TD and TFV concentrations during ring use were 1.1 x 10^5 and 7 x 10^4 ng/mL, respectively (Table 1) and concentrations did not differ significantly between proximal (vaginal, ectocervix) and distal (introitus) sites or change significantly from day 1 to day 14, indicating that the drug reached steady-state conditions in the vagina one day after ring insertion (Figure 6a). Median TFV-DP levels 14 days after TDF IVR placement were 120 fmol/mg tissue (interquartile range 90-550). Plasma TFV concentrations were quantifiable in 53% (8/15) women at Day 7 (0.34 ng/mL [BLQ, 0.56]) and 73% (11/15) women at Day 14 (1.5 ng/mL [BLQ, 2.1])(Figure 6b). TFV-DP concentrations in DBS were measured to explore the possibility that DBS may provide a marker of recent and cumulative adherence in future studies. TFV-DP was detected in 78% (11/14) women at Day 14 (117 fmol/punch [63, 260]). Anti-HIV activity of CVF in women who received the TDF IVR increased significantly from a median of 29% inhibition to 96% (p=0.005) and 94% (p=0.025) on Days 7 and 14, respectively, in studies using Jurkat CCR5+ T cells (Figure 7).

Table 1: Summary of drug concentrations in CVF, plasma, and tissue

Matrix & Analyte	N	C_{ave} (ng/mL) median (IQR)	N	C_{max} (ng/mL) median (IQR)	N	T_{max} (days) median (IQR)	N	AUC0-14 (ng/mL) median (IQR)
CVF VAG TDF	14	$1.1x10^5$ ($5.6x10^4$-$1.5x10^5$)	14	$2.4x10^5$ ($1.4x10^5$-$3.6x10^5$)	14	6 (3-7)	14	$2.0x10^6$ ($6.9x10^5$-$3.2x10^6$)
CVF ECX TDF	14	$6.6x10^4$ ($2.6x10^4$-$1.3x10^5$)	14	$2.1x10^5$ ($8.1x10^4$-$3.2x10^5$)	14	5 (4-7)	13	$1.3x10^6$ ($3.4x10^5$-$2.1x10^6$)
CVF INT TDF	14	$4.1x10^4$ ($1.6x10^4$-$7.5x10^4$)	14	$9.6x10^4$ ($3.7x10^4$-$1.7x10^5$)	14	4 (1-6)	13	$6.1x10^5$ ($2.4x10^5$-$1.2x10^6$)
CVF VAG TFV	15	$7.0x10^4$ ($4.4x10^4$-$1.3x10^5$)	15	$9.1x10^4$ ($7.3x10^4$-$1.9x10^5$)	15	14 (1-14)	15	$1.1x10^6$ ($6.7x10^5$-$2.1x10^6$)
CVF ECX TFV	15	$5.8x10^4$ ($3.5x10^4$-$8.4x10^4$)	15	$8.5x10^4$ ($5.2x10^4$-$1.7x10^5$)	15	7 (3-14)	13	$9.7x10^4$ ($5.7x10^5$-$1.3x10^6$)
CVF INT TFV	15	$6.3x10^4$ ($1.6x10^4$-$1.1x10^5$)	15	$9.2x10^4$ ($3.6x10^4$-$2.0x10^5$)	15	4 (1-14)	14	$9.4x10^5$ ($2.5x10^5$-$1.3x10^6$)
Tissue TFV	15	5.4 (2.8-8.8) ng/mg						
Tissue TFV-DP	15	120 (90-550) fmol/mg						
Plasma TFV			15	1.9 (1.2-2.4)	12	14 (14-14)		

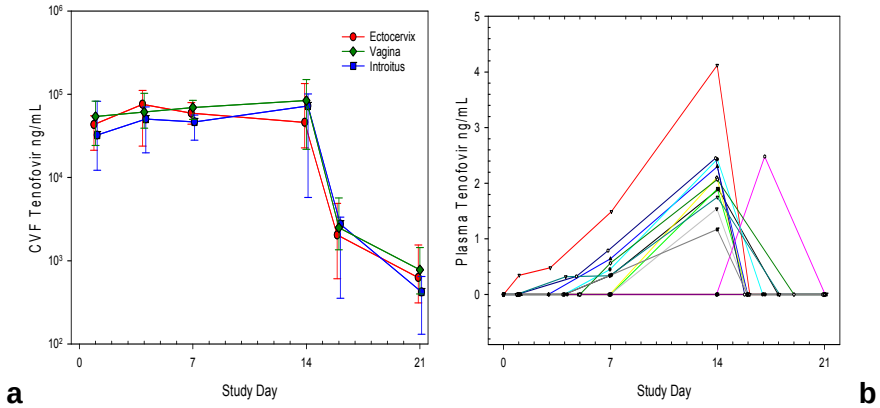


Figure 6. CVF TFV concentrations versus time plots are shown for the 14-day dosing period and the week after ring removal (a). Median with upper and lower quartiles is shown. Plots are shown of plasma TFV concentrations in relation to time for the 15 women who received a TDF IVR (b).

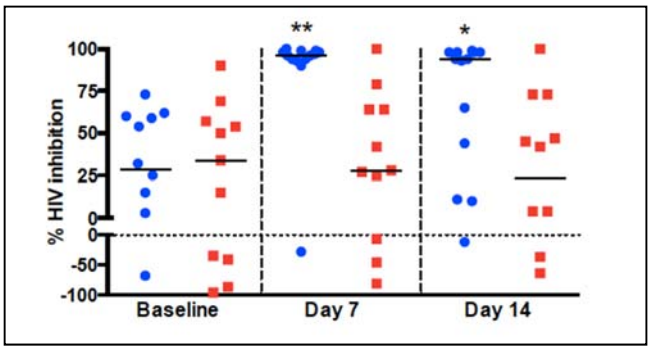


Figure 7. *Ex vivo* challenge of CVF. Blue circles represent the anti-HIV activity of CVF from participants who received TDF, red squares indicate the activity of those who received placebo, and the black bars correspond to the median percent HIV inhibition for each time-point. Compared to enrollment, there was a significant increase in percent HIV inhibition at one week and two weeks in the TDF group (* $p<0.05$, ** $p<0.01$).

2.8.3 Characterization of IVRs and vaginal microbiota after use

TDF recovered from 15 TDF IVRs after use was 260 ± 20 mg, which is consistent with an average *in vivo* release rate of 6.2 ± 1.4 mg/day based on product content of 365 ± 15 mg TDF (mean ± SD). This *in vivo* release rate is similar to the 14-day average *in vitro* release rate of 5.0 ± 0.16 mg/day in acetate buffer at pH 4.2 and 37°C. The mean concentration of monoPOCTFV recovered from rings was 13 ± 5 mg, which represents ~8.0 ± 2.7 mol% of total drug and is comparable to the 6-10% measured in rings after 30 days of *in vitro* release testing. Assessment of the vaginal microbiota by broad-range PCR and pyrosequencing of amplified 16S rRNA genes indicated that in 5 TDF recipients and 1 placebo recipient, the community transitioned from a *L. crispatus/jensenii* dominant environment to a *L. iners*-dominant environment. Semiquantitative cultures of the ring surface showed colonization with common vaginal flora and no bacteria were isolated from the core of 26 rings [11].

2.8.4 Clinical Study of TDF IVR in Sexually Active Women

A TDF IVR clinical study in sexually active women in the US and Kenya meets the objective of this IPCP-HTM to advance sustained delivery of an ARV to reduce HIV acquisition in women and is supported by data demonstrating more potent *in vitro* antiviral activity of TDF compared to TFV against both HIV-1 and HSV-2 [10], *in vitro* and animal studies demonstrating an excellent safety and PK profile, complete protection against SHIV in a PTM model [15], extensive safety and PK

data of oral TDF in healthy and HIV-infected adults [33-35], vast data from clinical trials of 1% TFV vaginal gel, and a first-in-human trial of TDF IVR use in 30 US women which resulted in few product-related AEs and a favorable PK profile [11].

2.9 Study Hypothesis, Rationale for Study Design and Inclusion of a Site in Thika, Kenya

We hypothesize that the TDF IVR will be safe and well tolerated among healthy, sexually active women in the US and Kenya. We also hypothesize that there will be a favorable PK profile in sexually active women. The null hypothesis is that there will be no difference in the safety profile between the active product and the placebo.

2.9.1 Rationale for Study Design

Building on the results of the first TDF IVR study in sexually abstinent women, we propose a longer Phase 1 study to assess the safety and PK of a TDF IVR in sexually active women. Vaginal rings have been tested in several trials targeting women 18 years of age and older and were found to be safe. A Phase 1 safety and PK study of a silicone elastomer matrix dapivirine and placebo vaginal ring (International Partnership for Microbicides [IPM] study 024) found the rings to be safe and well tolerated in 16 women following 28 days of continuous use. No differences were observed between the groups in the incidence of AEs, genital symptoms, pelvic/colposcopy findings, or safety laboratory findings. There were no product-related AEs [36]. An additional Phase 1 study (MTN-013/IPM 026) found the dapivirine vaginal ring to be safe in women who wore it for 28 days. There was no difference in genitourinary AEs between the dapivirine and placebo arms. In general, women found the ring acceptable, though 17% reported that they preferred not to wear a ring during menses [37]. Two Phase 3 efficacy trials of a dapivirine vaginal ring in more than 4,500 women at sites across southern and eastern Africa demonstrated an overall reduction in the risk of HIV infection by ~30% [38, 39]. An acceptability trial of NuvaRing® is ongoing in Western Kenya in 220 healthy women age 18-34 who are using the ring for 6 months. Preliminary review of qualitative data indicates that most women liked the ring and experienced fewer side effects than with their previous contraceptive method. A sub-set of women indicated that experiencing monthly menses was an important benefit of the ring (personal communication from the CDC).

Given the drug release kinetics observed *in vitro*, in NHP studies, and in the first Phase 1 study, TDF and TFV (formed following hydrolysis of the prodrug TDF) concentrations in CVF are expected to reach steady state within one day and within one month for tissue and maintain steady state concentrations for the duration of the planned 84 days of ring use. Sampling at multiple time points will provide information on drug kinetics, and, by sampling after ring removal how long drug persists. The latter may be important as behavior studies suggest that some women may remove a ring at time of menses or around sex [8]. Tissue sampling in US women in the hours and days immediately following ring insertion will provide valuable information about early tissue levels and the time required to achieve drug concentrations needed for protection. To capture systemic absorption, blood will be sampled to determine drug concentrations in plasma and DBS. To capture the concentrations of drug within the genital tract, CVF will be collected from the posterior fornix using Dacron swabs. Cervical tissue sampling will be highly informative given that it is the likely site of drug action. However, biopsies must be taken sparingly as they are uncomfortable to some and alter the healing process. Accordingly, 2 biopsies for PK studies will be obtained in US women at Days 28 and 56 when the ring is changed, and at Day 84 when the final ring is removed. US women will also have an additional 2 biopsies collected at only 1 of 7 time-points (5 or 6 women per time-point at either 1 hour, 4 hours, 1, 7, 14, or 21 days after ring insertion, or 5-7 days after ring removal). Participants in Kenya will have 2 cervical biopsies collected at each of 2 study visits (Days 28 and 84) (Table 2). The study will be single-blinded because the TDF and placebo rings are not identical in appearance. All participants will be informed that 2 biopsies will be taken

at study visits in which biopsies will be collected. Cervical biopsies will be obtained without local anesthetic. Participants may take acetaminophen before and/or after the biopsies are collected if desired. Participants will be instructed to abstain from sex for 7 days after biopsy collection to allow adequate time to heal.

Table 2: Number of Cervical Biopsies Obtained per Time-point

	Visit 4: Day 28 IVR Change	Visit 6: Day 56 IVR Change	Visit 8: Day 84 IVR Removal	Additional time point[1]	Total number of cervical biopsies per participant
US cohort	2	2	2	2	8
Kenya cohort	2		2		4

[1]Only US participants will be randomized to additional tissue sampling at only 1 of 7 time-points: 1 hour (Visit 2a), 4 hours (Visit 2b), 1 (Visit 2c), 7 (Visit 2d), 14 (Visit 3a) or 21 days (Visit 3b) after IVR insertion or 5-7 days after IVR removal (Visit 9a). There will be 5 or 6 US participants per additional time-point.

2.9.2 Rationale for Inclusion of a Site in Thika, Kenya

The site in Kenya at which the study will be conducted is a highly experienced clinical research site, that has for the last 10 years, conducted multiple HIV prevention studies. Dr. Nelly Mugo, an experienced obstetrician, gynecologist, and research scientist at the Kenya Medical Research Institute (KEMRI) in Nairobi, has a strong publication record in women's health. She was the site PI for the University of Washington Partners in Prevention HSV-2/HIV-1 Transmission Study and the Partners PrEP Study, which demonstrated that oral TDF and TDF-emtricitabine protected against HIV in heterosexual men and women. Dr. Mugo successfully led the Thika site in the enrollment and retention of several hundred HIV discordant couples.

The Thika site is located 25 miles northeast of Nairobi in Kiambu County Kenya. The rates of sexually transmitted infection (STI) in previous studies have been fairly low. The most common infections are Trichomonas and candidiasis. The prevalence of HIV is higher among women (5.6%) in Kiambu County than in men (2%). The flow of participants through the Thika clinic includes a counseling visit that is focused on the prevention of STI, including HIV. The counselors are skilled and experienced in HIV and STI risk reduction. This experience is demonstrated through the lowered HIV-1 incidence among participants at high risk for HIV randomized to the placebo arm to less than 2% (HSV/HIV Transmission Study and Partners PrEP Study) compared to incidence rates of 10-14% reported elsewhere.

The fact that there is a high rate of HIV and STI is known in the Thika community, and many women want to take an active part in designing potential solutions, and their families support such involvement. The Thika team has worked with the local community conducting research for the past 10 years. The site also has extensive experience collecting biopsies in research studies. Two studies were recently completed as part of this NIH grant in which 56 participants had 2 cervical and 2 vaginal biopsies collected. For 39 women, biopsy sampling was repeated at a second visit. Cervical, vaginal and vulvar biopsies are being done currently in Thika as part of 2 other NIH studies: "Ancillary study of genital mucosal sampling among female participants in the Partners PrEP study," which is being led by Dr. Jared Baeten (NIH R01 AI096968) and "Incident HSV-2 and genital health in Kenyan adolescent girls; an inception cohort," which is being led by Dr. Anna Wald (NIH P01 AI030731). In the Partners PrEP study, 237 women had 2 cervical and 2 vaginal biopsies performed. For 36 women, biopsies were collected at 2 visits. In the HSV study, 87 vulvar biopsies have been collected. Both the acceptability and tolerance of biopsies has been high. Thus far, the

Thika site has performed 736 cervical, 736 vaginal, and 87 vulvar biopsies. There have been no adverse events related to biopsy procedures in any of these 3 studies. Most women reported no challenges with abstinence after counseling after biopsy collection. In addition, coerced or forced sex has not been a concern in this region. Women who agree to participate are fully informed regarding the risks of the procedures and are monitored very closely. The study team has experience building rapport with young women, it is expected that some women will decline participation, and the site staff will not enroll any participant believed to be at risk of harm. Women will be strongly encouraged to involve their male partner, which will likely improve retention and adherence to IVR use and study visits.

3 STUDY OBJECTIVES

3.1 Primary Objectives

- Assess the safety of TDF IVR when used continuously for 84 days by healthy, HIV-uninfected, sexually active women, as compared with the placebo IVR

3.2 Secondary Objectives

- Examine systemic and genital tract PK of TDF release during and after 84 days of continuous IVR use in sexually active women
- Evaluate the acceptability of the study IVR in HIV-uninfected, sexually active women over 84 days of use

3.3 Exploratory Objectives

- Examine systemic and genital tract PK of TDF and/or its metabolites (TFV, TFV-DP) soon after TDF ring insertion and removal to assess rapidity with which drug concentrations rise post-insertion and fall after ring removal
- Examine the impact of TDF and placebo IVRs on the vaginal microbiome and the relationship between the vaginal microbiome and PK of TDF and/or its metabolites
- Evaluate the PD during and after 84 days of continuous use of a reservoir-type TDF IVR
- Examine the impact of study IVR on genital tract mucosal immunity

4 STUDY DESIGN

4.1 Identification of Study Design

This is a two-site, two-arm, randomized single blind placebo-controlled trial.

4.2 Summary of Major Endpoints

4.2.1 Primary Endpoints

Primary endpoints are the proportion of women in each arm with:

- Genitourinary events Grade 2 or higher as defined by the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric AEs, Version 1.0, Dec 2004 (Clarification dated August 2009), Addendum 1, (Female Genital Grading Table for Use in Microbicide Studies) judged to be related to study product

- Adverse events Grade 2 or higher as defined by the DAIDS Table for Grading the Severity of Adult and Pediatric AEs, Version 2.0, Nov 2014

4.2.2 Secondary Endpoints

The secondary endpoints will be:

- Assessment of TDF and TFV levels in CVF, and TFV in plasma on days 28 (visit 4), 42 (visit 5), 56 (visit 6), 70 (visit 7), and 84 (visit 8) after TDF ring insertion, TFV-DP in DBS on days 28 (visit 4), 56 (visit 6), and 84 (visit 8) after TDF ring insertion in all participants, and TFV and TFV-DP in cervical tissue on days 28 (visit 4) and 84 (visit 8) in all participants and on day 56 (visit 6) in US participants only

- Participant report and partner report of acceptability including genitourinary and emotional (dis)comfort, awareness/feeling during daily activities and during sex, willingness to use during menses and in the future

4.3 Description of Study Population

The study population will include approximately 80 healthy 18-45 year old females who are HIV-uninfected, non-pregnant, non-breastfeeding, sexually active and using contraception, as described in Sections 5.2 and 5.3. Forty US women and 40 Kenyan women will be stratified by site and will be randomized 3:1 (30 TDF:10 placebo at each site).

The Kenya site will not initiate enrollment until an interim analysis is performed on the first 20 US women who have completed 30 days of ring use. Enrollment at both sites will include women using a copper intrauterine device (IUD) or a hormonal contraceptive method other than an IVR. To ensure participants are protected from pregnancy and STI during study participation, condoms will be distributed at each study visit. As this is a Phase 1 trial of a TDF IVR, only healthy women with a low risk profile for HIV will be recruited and will be asked to wear a TDF or placebo IVR for 84 days. Participants will be strongly encouraged to include their male partner in the study. If participants are willing, education and counseling will be provided to male partners at all study visits and up to 20 males at the Thika site will be invited to participate in qualitative interviews if able and willing to provide written informed consent.

4.4 Time to Complete Enrollment

The approximate time to complete study enrollment is expected to be 12 months. Given the need to carefully screen and exclude women who are not able to comply to study procedures, we anticipate that the Thika site will not enroll more than 1 participant per week.

4.5 Study Groups

Two study groups are planned at each site. All groups will be assigned to complete a total of 9 study visits (Screening [visit 1], Enrollment [visit 2], Day 14 [visit 3], 28 [visit 4], 42 [visit 5], 56 [visit 6], 70 [visit 7], 84 [visit 8], 89 [visit 9]). Each US participant will be asked to undergo additional PK sampling at one of the following time-points: either 1 hour (visit 2a), 4 hours (visit 2b), 1 (visit 2c), 7

(visit 2d), 14 (visit 3a), or 21 days (visit 3b) after ring insertion, or 5-7 (visit 9a) and 10-12 days (visit 10) after ring removal.

The study groups are as follows:
1) Reservoir-type polyurethane TDF IVR group
2) Reservoir-type polyurethane Placebo IVR group

4.6 Expected Duration of Participation

The expected duration for participants is approximately 120 days (including a screening visit, an enrollment visit, 84 days of continuous IVR use, and 5-7 days following IVR removal). Participants will undergo Visit 2 within 28 days of screening. No study data will be collected after the Final Study/Early Termination Visit unless the participant is pregnant at the Final Study/Early Termination Visit. Participants who have AEs at the Final Study/Early Termination Visit that have not resolved or stabilized will be followed beyond the Final Study/Early Termination Visit until a clinically acceptable resolution of the AE(s) is confirmed and documented. Clinical acceptability of resolution will be determined by the Project Leader (PL) in consultation with the Protocol Safety Review Team (PSRT). Participants identified as infected with HIV during the study will be managed as per Section 9.6, HIV-1 Infection. Participants who are pregnant at the Final Study/Early Termination Visit may be followed as per Section 9.7, Pregnancy.

4.7 Clinical Study Sites

The study will take place at the Albert Einstein College of Medicine in Bronx, USA and the Partners in Health Research and Development site in Thika, Kenya. The Clinical Research Center (CRC) at Einstein is a component of the Block Institute for Clinical and Translational Research (ICTR) at Einstein and Montefiore Medical Center (MMC). The CRC provides infrastructure for studies of normal and abnormal body function and for the investigation of the cause, progression, prevention, and treatment of human disease. Resources include specialized nursing staff, 2 out-patient facilities, and support for intensive PK studies. This study will also be conducted at the Thika Partners Study Clinic (Partners in Health Research and Development) affiliated with the University of Washington International Clinical Research Center (ICRC), Kenyatta National Hospital, and KEMRI, located one hour from Nairobi. This site has led many initiatives and analyses on the relationship of hormonal contraception and HIV transmission risk. The site has demonstrated excellent capacity to recruit and retain women and has implemented single-site intensive studies with outstanding quality [40].

5 STUDY POPULATION

5.1 Selection of the Study Population

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

5.1.1 Recruitment

Participants at Einstein will be recruited from the gynecology, medicine and adolescent practices at Montefiore Medical Center (MMC), the local community, as well as community-based locations. The Institutional Review Board (IRB) will approve recruitment materials prior to use.

Participants in Kenya will be recruited through contacting participants exiting from current on-going studies at exit, participants who were previously screened and expressed interest in future

research, and community mobilization using community health workers. Other target areas for recruitment will be local colleges and family planning clinics. The recruitment materials will be submitted to the KEMRI Scientific Ethics Review Unit for approval.

5.1.2 Retention

Once a participant is enrolled in the study, study staff will make every effort to retain the participants in follow-up to minimize possible bias associated with loss-to-follow-up. This sample size was chosen with the goal of achieving 32 participants with complete data per site (24 TDF and 8 placebo) and includes additional women to account for those who may not complete the study or are lost to follow-up. SOP will be established and followed for participant retention.

5.2 Inclusion Criteria

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

- Age 18-45 years (inclusive) at screening.
- General good health (by volunteer history and per investigator discretion) without any clinically significant systemic disease (including, but not limited to significant liver disease/hepatitis, gastrointestinal disease, kidney disease, thyroid disease, osteoporosis or bone disease, and diabetes).
- Able and willing to provide written informed consent to be screened for and take part in the study.
- Able and willing to provide adequate locator information.
- Able and willing to avoid receptive vaginal and anal intercourse for 1 week after each biopsy.
- HIV-uninfected based on testing performed by study staff during screening procedures (per applicable algorithm in Appendix II).
- Using a copper IUD or any hormonal contraceptive method, other than an IVR, for a minimum of 2 months and intending to use the same method for the duration of study participation.
- Per participant report, sexually active, defined as having vaginal intercourse at least once in the month prior to screening.
- Have a regular sex partner and willing to have at least 4 sex acts per month for the duration of the study. *Sex act is defined as penile-vaginal penetrative intercourse. Study staff will provide condoms to all study participants. Participants will not be restricted from engaging in oral sex.*
- Has not used pre- or post-exposure prophylaxis for HIV exposure in the 3 months prior to Screening.
- Per participant report at Screening and Enrollment, agrees not to participate in other research studies involving drugs, medical devices, or vaginal products for the duration of study participation.
- At Screening, participant states she is able and willing to refrain from taking traditional herbs or medicines and is willing to refrain from inserting any non-study vaginal products or objects into the vagina, including but not limited to, spermicides, diaphragms, contraceptive vaginal rings, vaginal medications, vaginal probiotics/pre-biotics, menstrual cups, cervical caps (or any other vaginal barrier method), douches, lubricants, vaginal drying agents and sex toys (vibrators, dildos, etc.). *Tampons may be used, but for menses only.*
- Vaginal and cervical anatomy that, in the opinion of the investigator, lends itself to easy genital tract sample collection.

5.3 Exclusion Criteria

Women must meet none of the following criteria prior to genital sampling at Enrollment:

- Participant report of any of the following at Screening:
  - Sex in the past 3 months or any possibility of sex during study participation with a partner who is HIV+ or with a partner of unknown HIV status.
  - Known adverse reaction to polyurethane or to any components of the study product or allergy to both silver nitrate and Monsel's solution.
  - Active hepatitis B infection.
  - Chronic, recurrent, and/or acute vulvar or vaginal symptoms (pain, irritation, spotting, etc.).
  - Known bleeding disorder that could lead to prolonged or continuous bleeding with biopsy.
  - Intending to become pregnant during the period of study participation.
  - Currently breastfeeding or planning to breastfeed during the course of the study.
  - Menopause.
  - History of unexplained or unresolved intermenstrual bleeding in the 3 months prior to screening.
  - History of gynecological procedures (including genital piercing) on the external genitalia, vagina or cervix in the last 14 days.
  - Hysterectomy.
  - Women using contraceptive IVRs because the study product is an IVR.
  - Systemic use in the last 2 weeks or anticipated use during the study period of any of the following: corticosteroids, anticoagulants or ARVs.
  - Plans to relocate away from the study site area during the period of study participation.

- Grade 1 or higher laboratory abnormality, as defined by the DAIDS Table for Grading the Severity of Adult and Pediatric AEs, Version 2.0, Nov 2014.
- At Screening or Enrollment, is pregnant (based on urine pregnancy test).
- In the last three months, diagnosed with or treated for any STI.
  Note: Women with a history of condylomata or *genital herpes may be considered for eligibility as long as there are no lesions on exam. HSV 1 and 2 serologies will be obtained. Participants will be included regardless of the results of these serologies*.
- Reproductive tract infection (RTI) or pelvic inflammatory disease (PID) requiring treatment per local guidelines in Kenya and CDC guidelines in the US at Screening or Enrollment.
  Note: Otherwise eligible women diagnosed with symptomatic vulvovaginal candidiasis (VVC), *symptomatic BV or urinary tract infection (UTI) will be eligible if Visit 2 (Enrollment) is scheduled after all symptoms have resolved and at least two weeks after completing treatment. Women with recurrent VVC and symptomatic BV despite treatment will be offered another course of treatment and will not be eligible.*
- Positive test for *Trichomonas vaginalis*, *Neisseria gonorrhea* or *Chlamydia trachomatis* at screening.
- Positive test for hepatitis B (defined as positive for hepatitis B surface antigen).
- Reactive serologic test for syphilis at screening (per local guidelines).
  Note: Women with a history of syphilis that was not acquired and/or treated within the past 3 months and that has been appropriately treated may be considered for eligibility.
- At Screening or Enrollment, has a clinically apparent Grade 1 or higher pelvic exam finding (observed by study clinician or designee) per the DAIDS Table for Grading the Severity of

Adult and Pediatric AEs, Addendum 1, Female Genital Grading Table for Use in Microbicide Studies.
Note: Cervical friability bleeding associated with speculum insertion and/or specimen collection judged to be within the range of normal according to the clinical judgment of the PL/designee is considered expected non-menstrual bleeding and is not exclusionary.

- Pap result Grade 2 or higher according to the Female Genital Grading Table for Use in Microbicide Studies Addendum 1 to the DAIDS Table for Grading Adult and Pediatric AEs, Version 1.0, December 2004 (Clarification dated August 2009).
  Note: Women 21 years of age or older with a documented normal result within the 12 months prior to enrollment need not have Pap smear during the screening period. Women with a Grade 1 abnormal Pap smear can be enrolled upon completion of the initial phase of evaluation if no current treatment is indicated (based on local standard of care for management of abnormal cervical cytology). Need for a repeat Pap within 6 months does not preclude enrollment prior to that result becoming available.
- At Screening, has severe pelvic relaxation such that either the vaginal walls or the uterine cervix descend beyond the vaginal introitus with Valsalva maneuver.
- Has any condition that, in the investigator's opinion, would preclude informed consent, make study participation unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the study objectives.

5.4 Co-enrollment Guidelines

As indicated in Section 5.2, participants should not take part in other research studies involving drugs, medical devices, or vaginal products after the Screening Visit and while taking part in this study. Should any participant report concurrent participation in contraindicated studies after enrolling in this study, the PL will consult with the PSRT regarding ongoing ring use and other potential safety considerations associated with co-enrollment.

5.5 Withdrawal Criteria

Participants who sign the informed consent and agree to participate in the study, but do not meet eligibility criteria will not undergo baseline genital sampling procedures at Visit 2 and will not continue in the study. Once a participant undergoes baseline genital sampling procedures, she may be withdrawn from the study for the following reasons:

- Failure to follow protocol requirements that is judged severe enough by the investigator to significantly affect study outcomes.
- Pregnancy or desire to become pregnant.
- HIV seroconversion.
- Sexual activity with a partner of unknown or positive HIV status.
- Medical reasons, including diagnosis of an STI prior to IVR insertion.
- Use of prohibited medications or practices.
- Personal reasons (participant request).
- Discontinuation of treatment arm or of entire study.

6 STUDY PRODUCT

6.1 Study Product(s) Description

Each participant will receive an IVR containing TDF or a placebo ring. The vaginal ring will be worn continuously for 28 days and will be replaced with new rings twice, which will each be worn for 28 days, for a total of 84 days. A ring will be inserted into the vagina by the study clinician at Visit 2, changed by the study clinician at Visits 4 and 6 and removed by the study clinician at Visit 8. All participants will be seen 5-7 days after final ring removal.

6.2 Administration

IVRs will be inserted by the study clinician at Visit 2 (Day 0), Visit 4 (Day 28), and Visit 6 (Day 56). Study participants will be given a 24-hour telephone number to call if the ring is removed, expelled, and/or lost; or if they have concerns/problems wearing the ring. Study participants will be shown how to insert and remove the ring and a handout with detailed instructions will be provided.

6.3 Study Product Formulation

6.3.1 Study IVRs

To manufacture rings, hydrophilic elastomer HydroThane AL 25-93A (AdvanSource Biomaterials Corp., Wilmington, MA) tubing (wall thickness=0.7 mm) is extruded, tubing is cut to 17.1 ± 0.1 cm in length, and one end is sealed as described previously [15, 18]. The open tube is filled with a mixture of TDF and NaCl. The formulation of TDF and NaCl (86:14) is filled to achieve a final concentration of 365 ± 15 mg of TDF and 55 ± 5 mg of NaCl per TDF IVR. Table 3 indicates the composition of the TDF IVR. For the placebo formulation, one-end sealed tubes are filled with 55 ± 5 mg of NaCl per IVR. The open end of the filled tubes is sealed to form a rod and the ends of each rod are butt-welded together to form a ring. To set the shape, the rings are placed in a heated circular-shaped mold and then cooled. The rings are packaged in heat-sealed pouches and placed at 65°C for 5 days to load the wall of the IVR with TDF. The cross-sectional and outer diameters of the IVR are 5.5 mm ± 0.2 mm and 55 mm, respectively (Figure 8).

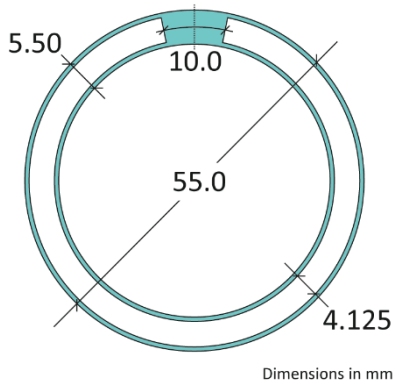


Figure 8. Schematic drawing of the TDF IVR. Measurements are in mm.

Table 3. Composition of the TDF IVR.		
Material	**Mass per Ring (grams)**	**% of Total Ring Mass**
TDF	0.36 ± 0.15	17%
NaCl (US Pharmacopeia) [USP]	0.05 ± 0.05	2.8%
HydroThane AL 25 93A	1.7	80.2%
Total	2.12	100%

TDF and placebo IVRs used in the first human trial required 0.78 ± .05 N to compress 10% of the IVR outer diameter. Mechanical failures occurred in 13 of 18 IVRs, which were tested upon extension at high tensile forces (an average force of 96.8 ± 24.8 N) but not at lower forces, indicating that IVRs used in human studies are sufficiently robust to withstand large tensile forces unlikely to be experienced in expected use without failure. Fourier Transform Infrared (FTIR) spectra of the polymer was measured before and after ring use in the first human study in order to investigate the presence of 4,4' methylene bis(cyclohexyl isocyanate). This compound did not form during ring use.

As detailed in Section 10.7, the study will be single-blinded because the TDF and placebo rings are not identical in appearance (Figure 1). In performing safety assessments, the study clinicians may be able to distinguish TDF from placebo IVRs, which is a potential weakness of the study. However, all study participants and laboratory staff will be blinded to the treatment assignments. Study participants will not be shown the actual rings at insertion or removal. At the randomization and monthly visits, rings will be inserted and removed by a study clinician. In order to objectively perform safety assessments, every effort will be made to have the clinician performing safety assessments be different from the clinician who inserts and changes rings. Prior to randomization, participants will be shown how to insert and remove a ring and participants will receive a handout with detailed instructions. An empty polyurethane ring will be used to demonstrate and practice insertion and removal. Participants will be told that each TDF and placebo IVR contains powder but participants will not be told that the amount of powder differs in the TDF compared to the placebo ring. We learned from the first human trial that after ring insertion and use, it is difficult to distinguish between TDF and placebo IVRs because of mucus and secretions adhering to the ring. Participants will be instructed to re-insert a ring if it is removed or expelled and all participants will be informed that they can call anytime and return to the study site for re-insertion, if needed.

6.4 Supply and Accountability

6.4.1 Supply

Particle Sciences, Inc. (PSI) will manufacture all the study vaginal rings under good manufacturing practice (GMP). Each ring will be individually packaged in a sealed foil pouch. PSI will label and ship all study IVRs directly to the Pharmacist of Record (PoR) at the study sites. PSI will use room temperature shipper systems with phase change gel packs and temp tales and packaging material will be included to cushion the rings during shipping and handling. Prior to shipping study product, the PoR in Thika will apply for an import license from the Pharmacy and Poisons Board (PPB) Trade Affairs Department (KENTrade). PSI will ship study product using a reputable shipping company, ensuring that temperature conditions are met and chain of custody is maintained. A detailed Pharmacy Plan is included in Appendix III.

6.4.2 Storage and Dispensing

The vaginal rings will be stored at 20°C to 25°C (68° to 77°Fahrenheit) with excursions permitted between 15° and 30°C (between 59° and 86°F). Stability data to date includes 12 month stability

studies conducted on TDF IVRs at 25°C/60% relative humidity and at 40°C/75% relative humidity. Study IVRs will only be dispensed by a licensed pharmacist upon receipt of a written prescription signed by an authorized prescriber. Dispensing will take place on the day of Enrollment and at monthly follow-up visits until the final study visit. Dispensing may also take place at interim (unscheduled) visits, as needed.

6.4.3 Accountability

The pharmacist is required to maintain complete records of all study vaginal rings received and subsequently dispensed. Procedures to be followed are provided in Appendix III. Used IVRs will be collected upon removal or expulsion to assess for residual drug levels. Used rings will be flash frozen and stored in the laboratory at each site at -80°C until shipment to the US. Each IVR given to a participant must be documented by the clinic staff when it is returned. This includes a ring that is brought back to the clinic by the participant and any ring removed at the clinic visit. Any study products not returned must also be documented by the clinic.

6.5 Adherence Counseling

Prior to randomization, participants will receive instruction on how to insert and remove an empty IVR. Participants will also receive study IVR adherence counseling at the Ring Insertion Visits and at additional follow-up visits. Site staff will counsel participants to refrain from removing the ring (except as directed) and from using prohibited medications and practices as described in Section 6.7. Site staff will also provide counseling and a 24-hour telephone number to call in case of ring removal/expulsion. Participants will be instructed to rinse with water and re-insert a ring if it has been removed or expelled. Instructions will be provided regarding how long a ring can be out and still be re-inserted versus replaced with a new study ring. Bottled water will be provided to participants in Thika. Participants will also be counseled not to use any products to ease ring removal or re-insertion (e.g. lubricants, creams and lotions).

The site staff will counsel participants to remove the vaginal ring immediately and contact study site staff if they experience a rash, itching, or other skin trouble, joint pain, or difficulty breathing, as these may be signs of an allergic reaction.

6.6 Concomitant Medications

Enrolled study participants may use concomitant medications during study participation. All concomitant medications as well as illicit substances reported throughout the course of the study will be recorded on case report forms designated for that purpose. All prescription medications, over-the-counter preparations, vitamins, nutritional supplements, and herbal preparations will be recorded on forms for concomitant medications.

6.7 Prohibited Medications and Practices

Several concomitant medication/practices will not be permitted. Participants will be asked about concomitant use of prohibited non-study vaginal products or other devices including but not limited to spermicides, diaphragms, contraceptive IVRs, vaginal medications, vaginal probiotics/pre-biotics, menstrual cups, cervical caps, tampons (except for menses only), douches, lubricants, non-vaginal cleansing or moisturizing products, and sex toys (e.g., vibrators, dildos, etc.). These medications and practices are restricted in order to protect the integrity of the lower genital tract and reduce the possibility of AEs due to agents other than the study ring and product. Participants will also be instructed to not use any products on an expelled or removed vaginal ring. Participants will be provided or referred to family planning services for the provision of alternative methods, as

applicable. If participants are unable to restrict the use of prohibited medications and practices, their participation in the study will be discontinued.

Concomitant use of prohibited medications during the two weeks prior to Visit 2 until the end of the study of any of the following systemically administered drugs: traditional herbs or medicines, corticosteroids, anticoagulants or ARVs, will be assessed.

Participants will be permitted to use acetaminophen before and/or after the cervical biopsy procedure.

7 STUDY PROCEDURES

An overview of the study visit and evaluations schedule is presented in Appendix I. Presented in this section is additional information on visit-specific procedures. Detailed instructions to guide and standardize procedures are provided in the Study SOP.

7.1 Pre-Screening

As part of participant outreach and recruitment strategies, study staff may pre-screen potential study participants at either on-site or off-site locations. During these interactions, study staff may explain the study to potential participants and ascertain elements of presumptive eligibility to be confirmed at an on-site screening visit. Process information (e.g., number of potential participants contacted, number presumptively eligible) may be recorded and stored at the study site in the absence of written informed consent from potential participants, provided the information is collected in such a manner that it cannot be linked to participant identifiers. Procedures and documentation will comply with local IRB requirements.

7.2 Screening Visit

Screening may take place up to 28 days prior to Enrollment. Multiple visits may be conducted to complete all required procedures, if necessary. Written informed consent for screening will be obtained before any screening procedures are initiated. For participants who do not meet the eligibility criteria, screening will be discontinued once ineligibility is determined. The screening visit components and procedures are outlined in Table 4.

NOTE: Otherwise eligible participants diagnosed with symptomatic VVC, symptomatic BV or UTI at screening will be offered treatment with oral medication, and Visit 2 may be scheduled at least two weeks after completing treatment if all symptoms have resolved.

Visit 1 should be scheduled at a time when the volunteer does not expect to be menstruating.

Table 4: Screening Visit (Visit 1).

Screening Visit	
Component	**Procedures**
Administrative and Regulatory	• Assign participant ID • Obtain written informed consent for screening • Collect demographic information • Collect locator information • Assess eligibility (behavioral and clinical) • Provide reimbursement for study visit

		• Schedule next study visit
Behavioral		• Provide counseling o HIV testing process o Contraceptive adherence o Protocol adherence o HIV/STI risk reduction o Dispense condoms
Clinical		• Collect obstetric history • Collect medical/menstrual/sexual history • Collect concomitant medications • Perform physical examination (see section 7.11) • Perform pelvic exam • Provide available test results • Treat for UTI/RTIs/STIs or refer for other findings[1] (Participants diagnosed with an STI at Screening will be excluded from study participation)
Laboratory	**Urine**	• Collect urine sample o Qualitative Human Chorionic Gonadotropin (hCG) o Dipstick urinary analysis (UA)[1] o Urine culture[1]
	Blood	• Collect blood samples o CBC o Serum chemistries listed in section 7.12.1 o Syphilis serology o Herpes serology (HSV-1 and 2 IgG) o HIV-1 test (per local guidelines) o Hepatitis B surface antigen o Liver function tests listed section 7.12.1
	Pelvic Samples	• Collect pelvic samples o Vaginal swab for Nucleic Acid Amplification Test (NAAT) for GC/CT/Trichomonas o Pap smear[1] o Swab for gram stain/Nugent score and pH o Vaginal fluid for wet mount microscopy[1]

[1]If clinically indicated

7.3 Enrollment and Ring Insertion Visit (Visit 2)

The Enrollment and Ring Insertion Visit (Visit 2) will be scheduled within 28 days of the Screening Visit. The components and procedures are shown in Table 5.

NOTE: Otherwise eligible participants diagnosed with symptomatic VVC, symptomatic BV or UTI prior to ring insertion at Visit 2 will be offered treatment with oral medication, and Visit 2 may be rescheduled after completing treatment and all symptoms have resolved.

Table 5: Ring Insertion Visit (Visits 2, 2a, 2b).

Ring Insertion Visit (Visits 2, 2a, 2b)	
Component	**Procedures**
Administrative and Regulatory	• Review/update locator information • Confirm eligibility (behavioral and clinical) • Schedule next study visit • Provide reimbursement for visit

Behavioral		• Provide counseling o Contraceptive adherence o Protocol adherence o Product use/adherence o HIV testing process o HIV/STI risk reduction
Clinical		• Update medical/menstrual/sexual history • Review/update concomitant medications • Perform physical examination[1] • Perform pelvic exam • Provide available test results • Treat for UTI, VVC, symptomatic BV[1] (Participants diagnosed with an STI prior to ring insertion at Visit 2 will be discontinued from the study)
Laboratory	**Urine**	• Collect urine sample o Qualitative hCG
	Blood	• Collect blood samples o HIV test (per local guidelines) o PK prior to IVR insertion
	Pelvic Samples	• Collect pelvic samples prior to IVR insertion o Swabs for CVF, gram stain, pH and rapid semen identification (RSID) test, o Swab of lateral vaginal wall for qPCR for vaginal bacteria and endocervical swab for mediators/antimicrobial activity • Swabs for CVF 1 hour and 4 hours post IVR insertion (Visits 2a and 2b)[2] • Swab of lateral vaginal wall for qPCR for vaginal bacteria 4 hours post IVR insertion (Visit 2b)[2] • Collection of 2 ectocervical biopsies for drug levels 1 hour and 4 hours post-insertion (Visit 2a and 2b)[2]
Study Product Supply		• The study IVR will be inserted by the clinician and time of insertion will be recorded. • Provision of condoms • Instruct participants to not have sex for 1 week to allow healing of biopsy sites[2]

[1]If clinically indicated, [2]In 5 participants at the US site 1 hour after ring insertion (Visit 2a) and in 6 participants at the US site 4 hours after ring insertion (Visit 2b)

7.4 Follow-up Visits after IVR insertion

The following procedures will occur at Visit 3 (after 1st ring is inserted), Visit 5 (after 2nd ring is inserted), and Visit 7 (after 3rd ring is inserted). Visits 2c, 2d, 3a and 3b will be scheduled in US participants only 1, 7, 14, and 21 days after ring insertion, respectively. Visits 5 and 7 will be scheduled 14 days after the 2nd and 3rd ring insertion. The components and procedures are shown in Table 6.

Table 6: Follow-up Visits After IVR Insertion (Visits 2c, 2d, 3, 3a, 3b, 5, 7)

Follow-up Visits After IVR Insertion (Visits 2c, 2d, 3, 3a, 3b, 5, 7)	
Component	**Procedures**
Administrative and Regulatory	• Review/update locator information • Record/update AEs • Schedule next study visit

		• Provide reimbursement for visit
Behavioral		• Provide counseling o Contraceptive adherence o Protocol adherence o Product use/adherence; management of ring expulsion and/or removal o HIV/STI risk reduction
Clinical		• Update medical/menstrual history • Review/update concomitant medications • Perform pelvic exam at Visits 2c, 2d, 3, 3a, 3b, and only at Visits 5 and 7 if clinically indicated • Treat for UTIs/RTIs/STIs or refer for other findings[1] (If a symptomatic infection is diagnosed, study procedures will continue per investigator discretion; the participant will be treated or referred as appropriate, and, if indicated, IVR will be removed. See Section 9.5 for specific management)
Laboratory	**Urine**	• Collect urine sample for qualitative hCG[2]
	Blood	• Collect blood for PK[3]
	Pelvic Samples	• Perform appropriate clinical and laboratory work-up to evaluate abnormal pelvic exam findings and participant-reported symptoms[1] • Collect pelvic samples o Swabs for CVF, gram stain, pH, and RSID test[4] o Collection of ectocervical biopsies for drug levels[5] o Swab of lateral vaginal wall for qPCR for vaginal bacteria[6]
Study Product Supply		• Insert study IVR in case of ring removal/expulsion[1] • Collect used study IVR in case of ring removal/expulsion[1] and assess residual drug levels • Provision of condoms • Instruct participants to not have sex for 1 week to allow healing of biopsy sites[5]

[1]If clinically indicated, [2]Visits 2d, 3a and 3b, [3]Visits 3, 3b, 5 and 7, [4]Visits 2c, 2d and 3b and in all participants at Visit 3, [5]Biopsies will be collected at Visits 2c, 2d, 3a (n=6 US participants per Visit), and 3b (n=5 US participants per Visit), [6]Visit 2c only

7.5 IVR Removal Visits

The following procedures shown in Table 7 will occur when the ring is changed (Visits 4 and 6) and at the final IVR removal visit (Visit 8), which will take place 28-30 days after each ring is inserted.

Table 7: IVR Removal Visits (Visits 4, 6, and 8).

IVR Removal Visits (Visits 4, 6, and 8)	
Component	**Procedures**
Administrative and Regulatory	• Review/update locator information • Record/update AEs • Schedule next study visit • Provide reimbursement for visit
Behavioral	• Administer acceptability questionnaire[1] • In-depth qualitative interview[2]

Clinical		• Update medical/menstrual history • Review/update concomitant medications • Perform physical examination[3] • Perform pelvic exam • Treat for UTI/RTIs/STIs or refer for other findings[3] (If a symptomatic infection is diagnosed, study procedures will continue per investigator discretion; the participant will be treated or referred as appropriate. See Section 9.5 for specific management)
Laboratory	**Urine**	• Collect urine sample o Qualitative hCG
	Blood	o Collect blood samples o CBC o Serum chemistries listed in section 7.12.1 o Liver function tests listed in section 7.12.1 o HIV-1 test (per local guidelines) o HSV-1 and HSV-2 IgG at Visit 8 only o Collect blood for PK including DBS
	Pelvic Samples	• Perform appropriate clinical and laboratory work-up to evaluate abnormal pelvic exam findings and participant-reported symptoms[3] • Collect pelvic samples o Swabs for CVF, gram stain, pH and RSID test o Swab of lateral vaginal wall for qPCR of vaginal bacteria o Endocervical swab for mediators and antimicrobial activity o Collection of an ectocervical biopsy for drug levels from two quadrants (two cervical biopsies in total)[4] o Collection of used IVR
Study Product Supply		• Study clinician to remove used study IVR at Visits 4, 6, and 8 and record time of ring removal. The second and third study IVRs will be inserted by the clinician at Visits 4 and 6, respectively, and time of insertion will be recorded. • Collect used study IVR in case of ring removal/expulsion and assess residual drug levels • Provision of condoms • Instruct participants to not have sex for 1 week to allow healing of biopsy sites[4]

[1]Visits 4 and 8, [2]Will be conducted in 20 Kenyan women at Visits 4 and 8, [3]If clinically indicated, [4]All participants will undergo biopsy collection at Visits 4 and 8. Only US women will have cervical biopsies collected at Visit 6.

7.6 Follow-up Visit after IVR Removal

The following procedures shown in Table 8 will occur at Visit 9. Visit 9 will be scheduled for all participants 5-7 days after final ring removal. Four US participants will have 2 cervical biopsies collected (Visit 9a) and will have a final study visit 5-7 days later (Visit 10) for blood and CVF PK sampling.

Table 8: Follow-up Visit after IVR Removal (Visits 9, 9a and 10).

Follow-up Visit After IVR Removal (Visit 9, 9a and 10)		
Component		**Procedures**
Administrative and Regulatory		• Review/update locator information • Record/update AEs[1] • Provide reimbursement for visit
Behavioral		• Provide counseling o Contraceptive adherence o HIV/STI risk reduction
Clinical		• Update medical/menstrual history • Review/update concomitant medications • Perform physical examination[2] • Perform pelvic exam • Treat for UTI/RTIs/STIs or refer for other findings[2] (If a symptomatic infection is diagnosed, study procedures will continue per investigator discretion; the participant will be treated or referred as appropriate.)
Laboratory	**Urine**	• Collect urine sample for qualitative hCG[3]
	Blood	• Collect blood for PK
	Pelvic Samples	• Collect pelvic samples o Swabs for CVF, gram stain, pH and RSID test o Swab of lateral vaginal wall for qPCR for vaginal bacteria (Visit 9 only) o Collection of 2 ectocervical biopsies for drug levels (Visit 9a)[4, 5]
Study Product Supply		• Provision of condoms • Instruct participants to not have sex for 1 week to allow healing of biopsy sites[4,5]

[1]Participants who have AEs at Final Visit (Visit 9 or Visit 10) that have not resolved or stabilized will be followed beyond the Final Visit until a clinically acceptable resolution of AE(s) is confirmed and documented. Clinical acceptability of resolution will be determined by the PL and assessed by the study clinician. [2]If clinically indicated, [3]Visit 9a, [4]Biopsies will be collected in 6 US participants (Visit 9a), [5]Participants who undergo biopsy collection at Visit 9a will be asked to return for a final study visit 5-7 days later (Visit 10) for blood and CVF PK sampling.

7.7 Follow-up Procedures for Participants Who Temporarily or Permanently Discontinue Study Product Use (Participant Volunteer or PL Discretion)

Participants who temporarily or permanently discontinue IVR use will be instructed to return the study IVR and will continue to complete study visits and procedures as originally scheduled (except that study IVRs will no longer be provided during the time the participant will not be using the study IVR). All protocol-specified study procedures will continue except the following:

- Provision of study product
- Pelvic exams*
- PK specimen collection**
- Provision of adherence counseling
- Acceptability and adherence assessments**

*Unless required for AE follow-up. See section 9.5 for specific management of events.

**PK specimen collection and acceptability and adherence assessments may be continued per investigator discretion.

7.8 Interim Visits

Interim visits may be performed at any time during the study and are shown in Table 9. All interim contacts and visits will be documented in participants study records.

Table 9: Interim Visit(s) if clinically indicated.

Interim Visit(s)		
Component		**Procedures**
Administrative and Regulatory		• Review/update locator information • Record/update AEs • Provide reimbursement for study visit • Schedule next study visit
Behavioral		• Provide counseling o Contraceptive adherence o Protocol adherence o Product use/adherence o HIV/STI risk reduction
Clinical		• Update medical/menstrual history • Review/update concomitant medications • Perform physical examination[1] • Perform pelvic exam[1] • Treat for UTI/RTIs/STIs or refer for other findings[1] (If a symptomatic infection is diagnosed, study procedures will continue per investigator discretion; the participant will be treated or referred as appropriate, and, if indicated, IVR will be removed. See Section 9.5 for specific management)
Laboratory	**Urine**	• Collect urine sample[1] o Urine hCG[1] o Dipstick UA[1] o Urine culture[1]
	Blood	• Collect blood samples[1] o Syphilis serology[1] o HIV-1 test[1] (per local guidelines)
	Pelvic Samples	• Perform appropriate clinical and laboratory work-up to evaluate abnormal pelvic exam findings and participant-reported symptoms, including but not limited to:[1] o NAAT for GC/CT/Trichomonas o Viral swab for HSV culture o Vaginal fluid for wet mount microscopy and vaginal pH
Study Protocol		• Collect IVR[1]

[1]if clinically indicated

7.9 Pharmacokinetics

All enrolled participants will undergo PK specimen collection procedures as shown in Table 10. These PK specimens will occur at study visits as described in the table below. Plasma and CVF will be collected to assay for TDF and TFV levels, and DBS and cervical biopsies will be collected to measure TFV-DP levels. PK studies will be performed under the direction of Dr. Mark Marzinke, Director, Clinical Pharmacology Analytical Laboratory, Johns Hopkins University.

Blood and pelvic PK specimens should be collected within approximately one hour of each other and within the acceptable sample windows. Blood will be collected prior to genital sampling. Cervical biopsies will be obtained after all other genital specimens are collected as bleeding from the biopsy sites may interfere with specimen collection. Participants will report ring adherence, including the reason for ring expulsion or removal as well as the date and length of time that the ring was out of the vagina. Study staff will record this information. In addition, women will be asked questions regarding vaginal and other practices (e.g., sex, tampon use, etc.) in an effort to further inform the PK analysis. These data may be collected at study visits in which PK assessments are scheduled to occur. Staff will also record all PK specimen collection times.

Table 10: PK Specimen Collection Schedule.

Study Visit	**PK Specimen Collection**
Visit 1 Screening	
Visit 2 Enrollment (Initial IVR insertion)	Plasma, CVF (pre-IVR insertion)
Visit 2a (5 US women 1 hour after IVR insertion)	CVF, Ectocervical tissue
Visit 2b (6 US women 4 hours after IVR insertion)	CVF, Ectocervical tissue
Visit 2c (6 US women 1 day after IVR insertion)	CVF, Ectocervical tissue
Visit 2d (6 US women 7 days after IVR insertion)	CVF, Ectocervical tissue
Visit 3	Plasma, CVF
Visit 3a (6 US women 14 days after IVR insertion)	Ectocervical tissue
Visit 3b (5 US women 21 days after IVR insertion)	Plasma, CVF, Ectocervical tissue
Visit 4 (IVR removal and IVR insertion)	Plasma, DBS, CVF, Ectocervical tissue in all women
Visit 5	Plasma, CVF
Visit 6 (IVR removal and IVR insertion)	Plasma, DBS, CVF, Ectocervical tissue in US women only
Visit 7	Plasma, CVF
Visit 8 (IVR removal)	Plasma, DBS, CVF, Ectocervical tissue in all women
Visit 9	Plasma, CVF
Visit 9a (6 US women 5-7 days after IVR removal)	Ectocervical tissue
Visit 10 (6 US women 10-12 days after IVR removal)	Plasma, CVF

The study site will record the date and time of all sample collections. The acceptable study windows for PK specimen collection are listed in Table 11. For visits with blood and genital sampling, blood will be collected prior to genital sampling.

Table 11: Study Windows.

Sampling Time point	**Acceptable Range Post-IVR Insertion**
1 hour	1-2 hours
4 hours	4-6 hours
Day 1	20-28 hours
Day 7 (Days 7-9)	164-220 hours
Day 14 (Days 14-16)	332-388 hours
Day 21 (Days 21-23)	500-556 hours
Day 28 (Days 28-30)	668-724 hours
First IVR removed and second IVR inserted	
Day 42 (Days 42-44)	1004-1060 hours
Day 56 (Days 56-58)	1344-1396 hours
Second IVR removed and third IVR inserted	

Day 70 (Days 70-72)	1676-1732 hours
Day 84 (Days 84-86)	2012-2068 hours
Third (final) IVR removed	**Acceptable Range Post-IVR Removal**
Day 89 (Days 89-91)	2132-2188 hours
Day 94 (Days 94-96)	2252-2304 hours

7.10 Behavioral Measures

Dr. Kenneth Ngure, a behavioral scientist in Thika, will oversee the primary behavioral study aim which will be addressed using (1) a brief quantitative instrument in all participants conducted at Visit 4 (day 28) and Visit 8 (Day 84); (2) in-depth qualitative interviews conducted at Visit 4 (Day 28) and at Visit 8 (Day 84) in 20 female participants and up to 20 male partners at the Thika site; and (3) focus groups which will be offered to female participants in Thika who do not have qualitative interviews and will be conducted after completion of the study. Male participants will be 18 years of age or older.

(1) The quantitative instrument will be structured around the following topics:

- Product acceptability during sex and menses
- Partner/relationship power items

(2) We will recruit 20 female participants and up to 20 male partners in Kenya to participate in in-depth qualitative interviews that address use of study product during the trial. Female participants and their partners will be interviewed separately at 2 time-points in the study. Interviews will be conducted by a trained qualitative researcher using an interview guide. Participants will be asked about their experience with the ring, report of frequency of study IVR removal/expulsions and duration without IVR inserted in vagina, change in feelings about the ring, concerns about wearing the ring continuously for 84 days, wearing a ring during sex and menses, partner-related concerns about the ring, explorations of the context around ring removal/expulsion, and desire for a ring that is changed monthly versus every 3, 6, or 12 months. Each interview will last approximately 60 minutes in duration and will be conducted early at Visit 4 (28 days after ring use) and exit, Visit 8 (84 days after ring use) to capture change in acceptability and ring experiences. Interviews will be audiotaped, transcribed and entered into Dedoose online qualitative tool for coding and analysis. We will also obtain informed consent to conduct two qualitative interviews at Visit 4 (28 days after ring use) and Visit 8 (84 days after ring use) in up to 20 male partners of female study participants at the Thika site. Female participants who disclose ring use to their partner will be asked if their partner is willing to participate in in-depth interviews. Couples who agree will be randomly selected.

(3) A sub-set of female participants in Thika will be invited to participate in a focus group discussion to ascertain their experiences using the TDF or placebo IVR. We expect to implement 2 focus groups at the Thika site of 5-8 women each; an experienced focus group moderator will lead each discussion. The focus group guide will address acceptability and adherence, interest in using IVR in the future for HIV prevention, as well as emergent themes from previous qualitative interviews. The focus group(s) will be audiotaped and transcribed, and the moderators will prepare a report of themes and findings.

7.10.1 Acceptability

Acceptability will be measured via questions about product use and user-related attributes, such as genitourinary discomfort, awareness/feeling the study IVR during daily activities, emotional comfort wearing the ring continuously for 84 days, wearing the ring during sex and menses, and partner-related concerns regarding ring use.

7.10.2 Adherence

Adherence will be assessed by biological markers of product use, including drug concentrations from dried blood spots, CVF, tissue and measurement of residual drug in IVRs upon removal. If feasible, specific vaginal bioanalytes will be quantified on the surface and within the removed IVR that are deposited over time as a cumulative measure of adherence. Bioanalytes include amine products penetrating the wall of the ring and carbohydrates accumulating on its surface.

7.11 Clinical Evaluations and Procedures

Physical exams will include the following assessments to be conducted at the Screening Visit:

- General appearance
- Weight
- Vital signs
- Temperature
- Height

A directed physical exam may be performed at the discretion of the examining clinician in response to symptoms or illnesses present at the time of the exam.

The required sequence of procedures and specimen collection performed during pelvic exams will be specified in the study SOP.

7.12 Laboratory Evaluations

The schedule of study visit and laboratory evaluations is presented in Appendix I.

7.12.1 Local Laboratory Testing (Montefiore Medical Center or Kenyatta National Hospital)

Blood

HIV-1 (see Appendix II)
Syphilis serology (per local guidelines)
HSV-1 IgG and HSV-2 IgG
Hepatitis B surface antigen
CBC with differential
- White blood cell count
- Hemoglobin
- Platelets
- Absolute Lymphocyte Count
- Absolute Neutrophil Count

Serum chemistries
- Random glucose
- Creatinine
- Sodium
- Potassium
- Calcium
- Alkaline Phosphatase
- Alanine aminotransferase (AST)
- Alanine transaminase (ALT)
- Total Bilirubin

Urine

Qualitative hCG

Urine dipstick
- Blood
- Leukocytes
- Nitrite
- Ketones
- Protein
- Glucose

Urine microscopy
Urine culture

Pelvic Specimens
Vaginal pH
Vaginal swab for NAAT for GC/CT/Trichomonas (will be done at U. of Washington HIV/STD Research Lab in Mombasa, Kenya)
Wet mount microscopy of vaginal fluid for BV and candidiasis
Pap test

7.12.2 Laboratory Testing (Albert Einstein College of Medicine, Johns Hopkins University, Particle Sciences and University of Washington)

Blood Specimens
Plasma and DBS for drug levels

Pelvic Specimens
Gram stain for Nugent score
Vaginal swab for semen testing by RSID
Swabs to measure drug levels
Endocervical swab for quantification of soluble immune mediators
Ectocervical tissue to assess PK
Used study IVR to assess residual drug levels and bioanalytes
Swabs for vaginal flora assessment by PCR

7.13 Specimen Collection, Preparation, Handling and Shipping

The study sites will adhere to the standards of Clinical Laboratory Improvement Amendments (CLIA), GLP, Good Clinical Practice (GCP) and the site Manual of Operations for proper collection, processing, labeling, transport, and storage of specimens.

7.13.1 Instructions for Specimen Preparation, Handling, and Storage

Vaginal and endocervical swabs and plasma will be immediately transported on ice to the local laboratory and stored at -80°C. Slides will be gram stained on site, stored at room temperature, and shipped to the US for Nugent scoring. Used rings will be flash frozen and stored at -80°C until shipment to the US. DBS processing will occur on site and will be stored until shipment to the US for analysis.

Ectocervical biopsies will be snap-frozen and shipped in liquid nitrogen to the US for drug levels. We have previously exported tissue samples from Kenya to the US using a liquid nitrogen carrier.

7.13.2 Specimen Shipment

Vaginal swabs for microbiota will be stored at the local lab at -80°C and shipped to Dr. David Fredricks' lab at the University of Washington at the completion of the study. We have previously exported clinical samples (plasma, DBS cards, swabs and used rings) on dry ice from Kenya to the US.

7.14 Biohazard Containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study as recommended by the CDC and NIH. All biological specimens 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

The PL is responsible for continuous close safety monitoring of all study participants, and for alerting the PSRT if unexpected concerns arise. The PSRT will include the PL, Co-investigators from the University of Washington and Thika, DAIDS Medical Officer (MO), and two independent investigators with no interest (financial or otherwise) in the outcomes of this study. The research coordinators will prepare routine AE and clinical data reports for review by the PSRT, which will meet via conference call approximately once per month or 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

The PL is responsible for the initial evaluation and reporting of safety information at the participant level, and for alerting the PSRT if unexpected concerns arise. The PL will review incoming safety data on an ongoing basis. Events identified as questionable, inconsistent, or unexplained will be queried for verification. AE reports submitted in an expedited manner to the DAIDS Safety Office will be forwarded to the DAIDS MO.

The PSRT will meet approximately every month via conference call to review clinical data reports. The content, format and frequency of the clinical data reports will be agreed upon by the PSRT 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. 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.

If the protocol team has serious safety concerns they will request a review of data by the PSRT. PSRT 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, the PL will notify the FDA, the PPB and the responsible IRBs expeditiously.

8.3 Adverse Events Definitions and Reporting Requirements

8.3.1 Adverse Events

An adverse event 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 the study groups, and is applied to all groups beginning from the time of randomization.

The term “investigational product” for this study refers to the following:
1) Reservoir-type polyurethane TDF IVR group
2) Reservoir-type polyurethane Placebo IVR group

Study participants will be instructed to contact the study site staff to report any AEs they may experience at any time between enrollment and completion of their participation. In the case of a life-threatening event, they will be instructed to seek immediate emergency care. Where feasible and medically appropriate, participants will be encouraged to seek evaluation where the study clinician is based, and to request that the clinician be contacted upon their arrival. Sites will obtain written permission from the participant to obtain and use records from non-study medical providers to complete any missing data element on a CRF related to an AE. All participants reporting an untoward medical occurrence will be followed clinically, until the occurrence resolves (returns to baseline) or stabilizes.

The PL will determine AE resolution or stabilization in their best clinical judgment, but may seek DAIDS MO and/or PSRT medical consultation regarding follow-up or additional evaluations of an AE. Study site staff will document in source documents all AEs reported by or observed in enrolled study participants regardless of severity and presumed relationship to study product. Study staff also will record all AEs on case report forms. The DAIDS AE Grading Table Version 1.0, December 2004 (Clarification dated August 2009), Addenda 1 and 3 (Female Genital and Rectal Grading Tables for Use in Microbicide Studies) will be the primary tools for grading AEs for this protocol. AEs not included in that table will be graded by the DAIDS AE Grading Table, Version 2.0 November 2014. In cases where an AE is covered in multiple tables, Addendum 1 (Female Genital Grading Table for Use in Microbicide Studies) will be the grading scale utilized.

8.3.2 Serious Adverse Events

Serious adverse events (SAEs) will be defined by the Manual for Expedited Reporting of Adverse Events to DAIDS (Version 2.0, dated January 2010) 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, and is not subject to expedited reporting.

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 patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

8.3.3 Adverse Event Relationship to Study Product

The relationship of all AEs to study product will be assessed per the Manual for Expedited Reporting of Adverse Events to DAIDS (Version 2.0, dated January 2010) and clinical judgment. Per the Manual for Expedited Reporting of Adverse Events to DAIDS (Version 2.0, dated January 2010), the relationship categories that will be used for this study are:

Related: There is a reasonable possibility that the AE may be related to the study agent(s)

Not related: There is not a reasonable possibility that the AE is related to the study agent(s)

8.4 Expedited Adverse Event (EAE) Reporting Requirements

Expedited Adverse Event Reporting

Requirements, definitions and methods for expedited reporting of AEs are outlined in Version 2.0 of the DAIDS EAE Manual, which is available on the RSC website at http://rsc.tech-res.com/safetyandpharmacovigilance/. The DAIDS Adverse Experience Reporting System (DAERS), an internet-based reporting system, must be used for expedited AE reporting to DAIDS. In the event of system outages or technical difficulties, expedited AEs may be submitted via the DAIDS EAE Form. For questions about DAERS, please contact NIAID CRMS Support at CRMSSupport@niaid.nih.gov. Please note that site queries may also be sent from within the DAERS application itself.

Where DAERS has not been implemented, sites will submit expedited AEs by documenting the information on the current DAIDS EAE Form. This form is available on the RSC website: http://rsc.tech-res.com/safetyandpharmacovigilance/. For questions about EAE reporting, please contact the RSC (DAIDSRSCSafetyOffice@tech-res.com).

EAE reporting procedures specific to this protocol are that once the sites have submitted EAEs via DAERS (as above), the RSC Safety Office will also prepare the draft safety reports and send them to the PL and DAIDS medical officers for review.

Study sites will be contacted by the DAIDS MO if any further information or clarification is needed after the report is evaluated by the PL and DAIDS MOs. The RSC Safety Office will then prepare the final report, which will go to the PL for signature and submission to the FDA. Copies of this final

report will be filed with the PL and RSC. Additionally, the RSC Safety Office will distribute safety reports to all DAIDS sites that use products under investigation in this study.

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

EAE Reporting Requirements for this Study

The SAE EAE Reporting Category, as defined in Version 2.0 of the DAIDS EAE Manual, will be used for this study. The study agents for which expedited reporting are required are: TDF and placebo IVR.

Grading Severity of Events

The Division of AIDS Table for Grading the Severity of Adult and Pediatric AEs, Version 1.0, December 2004 (Clarification dated August 2009), Addenda 1 and 3 (Female Genital and Rectal Grading Tables for Use in Microbicide Studies) will be the primary tools for grading AEs for this protocol. AEs not included in those tables will be graded by the DAIDS AE Grading Table Version 2.0, November 2014. In cases where an AE is covered in all tables, the DAIDS AE Grading Table, Version 1.0, December 2004 (Clarification dated August 2009), Addendum 1 (Vaginal Grading Table for Use in Microbicide Studies) will be the grading scale utilized [and/or as applicable Addendum 3 (Rectal Grading Table for Use in Microbicide Studies) will be the grading scale utilized].

The DAIDS AE Grading Table, Version 1.0, December 2004 (Clarification dated August 2009), Addenda 1 and 3 and DAIDS AE Grading Table, Version 2.0, November 2014 are available on the RSC website at http://rsc.techres.com/safetyandpharmacovigilance/.

EAE Reporting Period

The expedited AE reporting period for this study is defined as the entire study duration for an individual participant (from study enrollment until the participant's final study contact (Follow-Up Phone Assessment Visit/Termination Visit).

After the protocol-defined AE reporting period, unless otherwise noted, only suspected unexpected serious adverse reactions (SUSARs) as defined in Version 2.0 of the EAE Manual will be reported to DAIDS if the study staff become aware of the events on a passive basis (from publicly available information).

8.5 Pregnancy and Pregnancy Outcomes

Pregnant participants are excluded from this study. Urine testing is performed at screening, enrollment, and monthly study visits. If participants become pregnant at any time during the course of the study, study agents are discontinued, but participants will remain in the study and will continue with these assessments: acceptability assessments and safety bloods.

Pregnancy-related data will be collected using the pregnancy CRFs for all pregnancies detected during the study. Pregnancy outcomes will not be expeditiously reported to the DAIDS MO unless there is an associated AE in the pregnant participant that meets expedited reporting criteria or the pregnancy results in a congenital anomaly meeting the Manual for Expedited Reported of Adverse Events to DAIDS (Version 2.0, January 2010) guidelines for expedited reporting. Fetal losses without congenital anomalies or maternal complications that require expedited reporting will not be expeditiously reported but data will be captured via the pregnancy CRFs.

After the participant's final study contact (Follow-Up Phone Assessment Visit/Termination Visit), pregnancy outcomes that meet criteria for EAE reporting as described above (e.g., maternal complications, congenital anomalies) occurring among participants known to be pregnant at the Final Study Visit will continue to be expeditiously reported. The study coordinator will prepare a report on all pregnancies and their outcomes. The study coordinator will also prepare an annual summary report of all AEs for the annual IND and PPB reports.

8.6 Regulatory Requirements

Information on all reported AEs will be included in reports to the FDA, PPB and other applicable government and regulatory authorities. The study coordinator will submit AE information in accordance with local regulatory agencies' or other local authorities' requirements. The study coordinator also will submit AE information and any other relevant safety information to their IRBs in accordance with IRB requirements.

8.7 Social Harms Reporting

Although study sites 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-infected 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. Social harms that are judged by the Investigator of Record to be serious or unexpected will be reported to responsible site IRB at least annually, or according to their individual requirements. All social harms will be reported to the PL and will be reviewed by the PSRT. 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.

9 CLINICAL MANAGEMENT

Guidelines for clinical management and product discontinuation are outlined in this section. In general, the site investigator has the discretion to discontinue study product at any time if she/he feels that continued product use would be harmful to the participant or interfere with treatment deemed clinically necessary. Unless otherwise specified below, the investigator should immediately consult the PSRT for further guidance regarding permanent discontinuation. The site investigator or designee will document all discontinuations on applicable case report forms.

9.1 Grading System

The primary grading system is located in the Vaginal [and Rectal] Grading Table for Use in Microbicide Studies, which is labeled as Addendum 1 [and Addendum 3] in the DAIDS AE Grading Table, Version 1.0, December 2004 (Clarification dated August 2009), which can be found on the RSC website: http://rsc.tech-res.com/safetyandpharmacovigilance/.

9.2 Dose Modification Instructions

No dose modifications will be undertaken in this study.

9.3 Discontinuation of Study Product(s) in the Presence of Toxicity

Grade 1
Participants who develop a Grade 1 AE regardless of relatedness to study product that is not specifically addressed below may continue use of study products per protocol.

Grade 2
Participants who develop a Grade 2 genitourinary AE or toxicity that is not specifically addressed below and is judged to be related to study product should have that study product permanently discontinued. Participants who develop a Grade 2 systemic or non-genitourinary AE that is not specifically addressed below and is judged to be related to study product should be reviewed with the PSRT. Study product will be continued during the time required to conduct the review.

Grade 3 or 4
Participants who develop a Grade 3 or 4 AE or toxicity that is not specifically addressed below (regardless of relationship to study product) should have the current study product permanently discontinued.

Halting enrollment
In the circumstance where any of the following criteria are met and confirmed by the PL and DAIDS MO, enrollment in this study will be paused and the PSRT will be consulted to determine if the study should be permanently discontinued:

1. Two Grade 3 AEs that are similar and attributed to study product;
2. Any Grade 4 AE attributed to study product

9.4 General Criteria for Discontinuation of Study Product

Study participants will be permanently discontinued from product use by the Site Investigator or designee in the event of the following:

- Pregnancy
- Breastfeeding
- HIV seroconversion
- Report of use of pre- or post-exposure prophylaxis for HIV exposure

9.5 Specific Management of Other Clinical Events

Management of STIs and RTIs will be in accordance with local guidelines in Kenya and current CDC guidelines in the US, which are available at http://www.cdc.gov/std/treatment/. When clinically appropriate, investigators should use oral or parenteral (in the case of syphilis, for example) medications when at all possible to avoid intravaginal medication use. If study IVR use is temporarily discontinued due to STI and then reinstated, rings will be replaced and not reused.

In the absence of clinical evidence of cervicitis (as described below) and/or pelvic inflammatory disease, participants with gonorrhea and/or chlamydia detected during the study period may be treated with the study IVR in place. Participants with be re-evaluated in 48-72 hours and the PSRT will be consulted.

If suspected finding is reported by participant between scheduled visits, an interim visit may be scheduled at the discretion of the site investigator. Management of genital events observed at scheduled or interim visits will be in accordance with the following:

Superficial epithelial disruption, Grade 1 (abrasion/peeling)

- Continue study IVR use
- Perform naked eye evaluation
- Re-evaluate by speculum examination in 48-72 hours
- If condition worsens (≥Grade 2), temporarily hold study IVR use and consult the PSRT. Otherwise continue study IVR use

Deep epithelial disruption, ≥Grade 2 (ulceration)

- Remove study IVR for deep epithelial disruption confirmed by PL/designee
- Re-evaluate in 48-72 hours and reinstate study IVR use if resolved (≤Grade 1)
- If unresolved at 48-72 hours, re-evaluate in another 48-72 hours. If resolved at that time, may reinstate study IVR use. If unresolved at this second reevaluation, continue temporary product hold, consult with PSRT regarding permanent discontinuation, and provide care per local standard
- If there is reoccurrence with no identified etiology, continue temporary product hold and consult the PSRT regarding permanent discontinuation

Localized erythema or edema, Grade 1: area of less than 50% of vulvar surface or combined vaginal and cervical surface

- Continue study IVR use
- Perform naked eye evaluation
- If asymptomatic, re-evaluate at next regularly scheduled visit
- If symptomatic, re-evaluate by speculum examination in 48-72 hours
- If worsened significantly (≥Grade 2), temporarily hold study IVR use and consult the PSRT. Otherwise, continue study IVR use

Generalized erythema or severe edema, ≥Grade 2: area of more than 50% of vulvar surface or combined vaginal and cervical surface affected by erythema

- Remove study IVR
- Perform naked eye evaluation
- Re-evaluate in 48-72 hours and reinstate study IVR use if resolved (≤Grade 1)
- If unresolved at 48-72 hours, re-evaluate in another 48-72 hours. If resolved at that time may reinstate use. If unresolved at this second reevaluation, continue temporary product hold, consult with PSRT regarding permanent discontinuation, and provide care per local standard

Abnormal vaginal discharge, ≤Grade 1 (judged to be unrelated to cervicitis)

- Study IVR use may be continued without treatment in the presence of asymptomatic Candida vaginitis and/or asymptomatic BV
- Perform vaginitis evaluation, including assessment of signs, symptoms, vaginal pH and wet mount microscopy for Candida vaginitis, Trichomoniasis, and BV
- Provide or prescribe treatment and continue study IVR use for all cases of Trichomoniasis, symptomatic Candida vaginitis, and symptomatic BV. Re-evaluate in 48-72 hours and consult with PSRT.

Unexpected genital bleeding, Grade 1

- Continue study IVR use (at study clinician's discretion)
- Perform naked eye evaluation
- If determined to be due to deep epithelial disruption (≥Grade 2), refer to guidelines above, otherwise continue study IVR use

Cervicitis (including findings on exam such as inflammation and/or friability), ≥Grade 1

- Remove study IVR
- Evaluate for GC/CT
- If GC/CT detected, provide or prescribe treatment and consult PSRT
- If GC/CT is not detected, reevaluate in 72 hours. If all symptoms and signs are resolved at that time continue study IVR use

Genital petechia(e), Grade 1

- Continue study IVR use
- Perform naked eye evaluation
- No further evaluation or treatment is required

Genital ecchymosis, Grade 1

- Continue study IVR use
- Perform naked eye evaluation
- No further evaluation or treatment is required

9.6 HIV-1 Infection

A participant who has a positive test for HIV-1 must have study product permanently discontinued. Participants identified as infected with HIV will be managed or referred for management according to the local standard of care. An HIV genotype assay to assess for resistance will be obtained if subjects acquire HIV infection during study participation.

9.7 Pregnancy

All study participants are required to be using a copper IUD or any hormonal contraceptive method other than an IVR at Screening and intending to use the same method for the duration of study participation. Study staff will provide contraceptive counseling to enrolled participants as needed throughout the duration of study participation and in the US will facilitate access to contraceptive services through direct service delivery and/or active referrals to local service providers. Study participants in Thika will be offered contraception methods, including oral pills, long-acting injectables (DMPA), implants (Jadelle) or IUD. These methods are supplied by the government and are readily available at the Thika Clinic. Pregnancy testing will be performed at screening, enrollment, and at monthly visits. Participants will be encouraged to report all signs or symptoms of pregnancy to study staff during the course of the study. The investigator or designee will counsel any participants who become pregnant regarding possible risks of study IVR use. The investigator or designee also will refer the participant to all applicable services.

Participants who become pregnant during the course of the study will permanently discontinue study IVR use and will be instructed to return the study IVR. If participants become pregnant during the study, they will continue to complete study visits and procedures as described in Section 7.7. The study team will attempt to obtain pregnancy outcome data on any women who become pregnant while on study. Participants may be contacted to collect the outcome of pregnancies up

to one year after the birth of the infant. A participant who is pregnant at the Final Study/Early Termination Visit 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).

9.8 Criteria for Early Termination of Study Participation

Participants may voluntarily withdraw from the study for any reason at any time. The site investigators 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 the study sponsors, government or regulatory authorities (including the NIH, Office of Human Research Protections (OHRP), FDA, KEMRI Ethics and Research Committee, PPB, or Einstein IRB) terminate the study prior to its planned end date. Every reasonable effort is made to complete a final evaluation of participants who withdraw or are withdrawn from the study prior to completing follow-up. Study staff members will record the reason(s) for all withdrawals in participants' study records. In the event that participants who voluntarily withdraw from the study wish to re-join the study, they may resume product use (if applicable) and follow-up through their originally scheduled study exit date.

10 STATISTICAL CONSIDERATIONS

10.1 Overview and Summary of Design

This is a two-site, two-arm randomized single blind placebo-controlled trial to assess the safety and PK of IVRs containing TDF, when used continuously for 84 days by healthy, HIV-uninfected, sexually active women, as compared with a placebo IVR. A total of approximately 80 (40 US and 40 Kenyan) women will be randomized in a 3:1 (TDF:placebo) ratio to receive either TDF or placebo ring for 84 days. Randomization will be stratified by trial site.

10.2 Study Endpoints

Primary endpoints
Consistent with the primary study objective to assess the safety of an IVR containing TDF, when used continuously for 84 days by healthy, HIV-uninfected, sexually active women, as compared with a placebo IVR, the primary safety endpoints are the proportion of women experiencing AEs:

- Evidence of a Grade 2 or higher genitourinary events as defined by the DAIDS Table for Grading the Severity of Adult and Pediatric AEs, Version 1.0, Dec 2004 (Clarification dated August 2009), Addendum 1, (Female Genital Grading Table for use in Microbicide Studies) during the trial period judged to be related to study product

- Evidence of Grade 2 or higher systemic and local AEs as defined by the DAIDS Table for Grading the Severity of Adult and Pediatric AEs, Version 2.0, Nov 2014 during the trial period

Secondary endpoints
Consistent with the secondary study objective to examine systemic and local pharmacokinetics of TDF release during and after 84 days of continuous use of a reservoir-type IVR containing TDF in genital tract fluids, plasma, and cervical tissue the pharmacokinetic endpoints will be:

- Assessment of TDF and TFV levels in CVF, and TFV in plasma on days 28 (visit 4), 42 (visit 5), 56 (visit 6), 70 (visit 7), and 84 (visit 8) after TDF ring insertion, TFV-DP in DBS on days 28 (visit 4), 56 (visit 6), and 84 (visit 8) after TDF ring insertion in all participants, and TFV and TFV-DP in cervical tissue on days 28 (visit 4) and 84 (visit 8) in all participants and on day 56 (visit 6) in US participants only

Consistent with the secondary objective to evaluate the acceptability of the study IVR in HIV-uninfected sexually active women over 84 days of use:

- Participant report of acceptability including genitourinary and emotional (dis)comfort, awareness/feeling during daily activities, including sex and menses, and partner concerns about ring

Exploratory endpoints

Consistent with the exploratory objective to evaluate the systemic and genital tract PK of TDF and/or its metabolites (TFV, TFV-DP) soon after TDF ring insertion and removal to assess rapidity with which drug concentrations rise and fall 5-12 days post-removal:

- Assessment of TDF and TFV in CVF, TFV and TFV-DP in tissue at 1 hour (Visit 2a), 4 hours (Visit 2b) and 1 day (Visit 2c) after TDF ring insertion, TFV in plasma, TDF and TFV in CVF, and TFV and TFV-DP in tissue at 7 (Visit 2d), 14 (Visit 3a), and 21 (Visit 3b) days after TDF ring insertion, TFV-DP in tissue 5-7 days (Visit 9a) after ring removal, TDF and TFV in CVF and TFV in plasma 10-12 (Visit 10) days after ring removal in US participants only

Consistent with the exploratory objective to evaluate the impact of IVRs on the vaginal microbiome and assess the relationship between the vaginal microbiome and PK of TDF and/or its metabolites:

- Assess composition of vaginal microflora using broad-range 16S rRNA gene PCR with Illumina sequencing and species-specific qPCR before, during and after continuous TDF ring use and correlate changes in bacteria with concentrations of TDF and/or its metabolites in CVF and tissue

Consistent with the exploratory objective to evaluate the PD during and after 84 days of continuous use of a reservoir-type IVR containing TDF, the PD endpoints will be:

- Measure of anti-HIV activity in CVF

Consistent with the exploratory objective to examine the impact of the study IVR on genital tract mucosal immunity:

- Assessment of select innate immune and host defense mediators in vaginal swabs before and after IVR use. Selection of specific mediators to be evaluated will be based on the best available evidence at the time of measurement.

10.3 Primary Study Hypothesis

We hypothesize that both the TDF IVR and placebo IVR will be safe and well tolerated among healthy, sexually active women.

10.4 Sample Size and Power Calculations

10.4.1 Primary Endpoints

The proposed total sample size is approximately 80 women (40 US and 40 Kenyan) randomized in a 3:1 ratio giving 60 in TDF (30 at each site) and 20 in the placebo group (10 at each site). This sample size was chosen with the goal of achieving 32 women with complete data per site (24 TDF and 8 placebo) and includes additional women who may not complete the study or are lost to follow-up. The sample size is based upon the size of similar Phase 1 studies of vaginal microbicide products.

As a means to characterize the statistical properties of this study Table 12 below presents the probability of observing zero, at least one, and two or more safety endpoints among the 24 women in the TDF group and 8 women in the placebo group for various "true" event rates:

Table 12: Analysis of Safety Event Frequency.

Event Rate in TDF Arm	P (0 events | n=24)	P (≥1 event | n=24)	P (≥2 events | n=24)
1%	78.6%	21.4%	2.4%
5%	29.2%	70.8%	33.9%
10%	8.0%	92.0%	70.8%
15%	2.0%	98.0%	89.4%
25%	0.1%	99.9%	99.1%
35%	0%	100.0%	99.9%
45%	0%	100.0%	100.0%

Event Rate in Placebo Arm	P (0 events | n=8)	P (≥1 event | n=8)	P (≥2 events | n=8)
1%	92.3%	7.7%	0.3%
5%	66.3%	33.7%	5.7%
10%	43.1%	56.9%	18.7%
15%	27.2%	72.8%	34.3%
25%	10.0%	90.0%	63.3%
35%	3.2%	96.8%	83.1%
45%	0.8%	99.2%	93.7%

An alternative way of describing the statistical properties of the study design is in terms of the 95% confidence interval for the true rate based on the observed data. Table 13 below shows the exact 2-sided 95% confidence intervals for the probability of an event based on a particular observed rate. If none of the 24 participants receiving TDF experiences a safety event, the exact 2-sided upper 95% confidence bound for the true rate of such events in a particular arm of the study is 14.3%.

Table 13: Exact 2-sided 95% Confidence Intervals Based on Observing a Particular Rate of Safety Endpoints for TDF Arm of Size 24 and Placebo Arm Size of 8.

Observed event rate in TDF Arm	Confidence interval (%)
0/24	0, 14.3
1/24	0.1, 21.1
2/24	1.0, 27.0

Observed event rate in Placebo Arm	Confidence interval (%)
0/8	0, 36.9
1/8	0.3, 52.7
2/8	3.2, 65.1

An additional aim of the study is to compare the safety between the TDF IVR and the placebo IVR. Assuming a one-sided test with α=0.05 and 80% power, Table 14 below provides the difference in the rates of safety events (proportion of women experiencing the safety event of interest) between the TDF IVR and the placebo IVR that is detectable with 80% power for a given rate in the placebo IVR arm. For example, if the true rate of a given safety endpoint in the placebo IVR arm is 25% (2 of 8 women experiencing a safety event), the proposed sample size provides 80% power to exclude safety endpoint rates greater than 78.4%. Hence, while comparisons will be made between the TDF IVR and the placebo IVR, the study will only have power to detect very large differences in safety event rates.

Table 14: Difference in Rates of Safety Events.

Rate in Placebo IVR Arm	Rate in the TDF IVR Arm Detectable with 80% Power
12.5%	64.5%
25.0%	78.4%
37.5%	88.1%
50.0%	96.1%

10.4.2 Secondary Endpoints

Pharmacokinetic endpoints will include concentration-time course, maximum concentration (C_{max}), and time to maximum concentration (T_{max}), assessed in blood, swabs and cervical tissue. Drug concentration at time of ring removal will be described and drug half-life after ring removal will be estimated in blood, swabs, and cervical tissue. The sample size of the study is driven by the safety endpoints as described above.

Several components of acceptability (e.g., genitourinary discomfort, awareness/feeling the study IVR during daily activities, sex and menses, emotional comfort wearing the ring continuously for 84 days, partner-related concerns about the ring) will be used to assess overall acceptability. Each component will be assessed by a combination of dichotomous measures and rating scales where women will be categorized into (1) those reporting no acceptability issues during the 84 days of study IVR use (e.g., those reporting no genitourinary discomfort) and (2) those reporting at least one issue during the 84 days of study IVR use (e.g., those reporting some discomfort), and if yes, the intensity or severity of the issue (on a scale from 1 to 10). An acceptability endpoint is defined as a negative report by a participant, on any of the above components for acceptability. A sample size of 32 women will provide a precision of 16% (i.e., half the width of the 95% confidence interval) assuming an observed acceptability of 75%.

10.5 Participant Accrual, Follow-up and Retention

Based on previous studies of vaginal products with similar eligibility requirements, the accrual of 80 eligible participants will take approximately 12 months. Women lost to follow-up and/or on temporary hold or permanent product discontinuation will not be replaced. However, every effort will be made to complete their regularly scheduled safety evaluations. We will target retention of 90% of enrolled participants over the study period.

10.6 Randomization

Participants will be randomized in a 3:1 ratio (TDF:placebo). A block randomization scheme with a block size of 4 will be generated and maintained by the study statistician. The statistician will provide the sites with a set of randomization envelopes to be used in the study clinics. The

randomization envelopes will be stored by the study coordinators in a locked cabinet. Study staff, not including the investigator who will perform clinical evaluations after ring insertion, will assign these envelopes in sequential order, by envelope number, to eligible participants. Each randomization envelope will include study product arm randomization assignment. Assignment of the randomization envelope is considered the effective act of participant enrollment/randomization. Pharmacy staff will store assigned randomization envelopes. All US participants will undergo additional sample collection for PK studies at only 1 of 7 time-points (either 1 hour, 4 hours, 1, 7, 14, or 21 days after ring insertion, or 5-12 days after final ring removal). The selection of participants for each time-point will be randomized. In-depth qualitative interviews will be conducted in 20 female participants and up to 20 male partners at 2 time-points in the study at the Thika site. A trained qualitative researcher will assist in randomly selecting couples and will conduct the interviews.

10.7 Blinding

The study will be single-blinded because the TDF and placebo rings are not identical in appearance (Figure 1). However, all study participants and laboratory staff will be blinded to the treatment assignments. In order to objectively perform safety assessments, every effort will be made to have the clinician performing safety assessments be different from the clinician who inserts and changes rings. All IVRs will be individually packaged and labeled. Multiple codes will be utilized to conceal and protect randomization assignments and the identity of the content of the ring.

Blinding will be maintained until all data are entered into the study database, all study endpoint data and other data included in the final analysis have been cleaned and verified, and the data are ready for final analysis. This will be explained to participants as part of the study.

There are no circumstances under which it is expected that unblinding will be necessary for the provision of medical treatment or to otherwise protect the safety of study participants. As described in Section 9, in the event that an Investigator is concerned that a participant might be put at an undue risk by continuing product use, the Investigator may discontinue study product use by this participant; however, knowledge of the specific product to which the participant was assigned should not be necessary to guide further follow-up and/or treatment. If an Investigator feels that specific product knowledge is necessary to protect participant safety, the Investigator will notify the PSRT to consider and rule upon the request.

10.8 Data and Safety Monitoring and Analysis

10.8.1 Protocol Safety Review Team

No Data and Safety Monitoring Board oversight is planned for this study. The PL will conduct interim reviews of study progress, including rates of participant accrual, retention, completion of primary and main secondary endpoint assessments, study or lab issues, etc. A safety report will be prepared by the study team for review by the PSRT. These reviews will take place every month, and ad-hoc as needed. At the time of these reviews, or at any other time, the PSRT may recommend that the study proceed as designed, proceed with design modifications, or be discontinued.

10.8.2 Interim Analysis

The study will be initiated at the US site only. US participants will be instructed to wear a TDF or placebo IVR continuously for 84 days without interruption. An interim safety analysis will be performed after 20 US women (15 in the TDF IVR and 5 in the placebo IVR) have completed 30 days of ring use. If there are 4 or more women with any Grade 2 or higher adverse events, the

Kenya site will not initiate enrollment and the PSRT will evaluate all safety data available in US participants to assess whether enrollment in the US should continue. Table 15 below presents the probability of observing four or more safety endpoints among the 20 US women:

Table 15: Analysis of Safety Event Frequency in 20 US women (15 in TDF and 5 in placebo) completing 30 days of ring use

Event Rate	P (≥4 event | n=20)
1%	0%
5%	1.6%
10%	13.3%
15%	35.2%
25%	77.4%
35%	95.6%
45%	99.5%

10.8.3 Primary Analysis

When the use of descriptive statistics to assess group characteristics or differences is required, the following methods will be used: for categorical variables, the number and percent in each category; for continuous variables, the mean, median, standard deviation, quartiles and range (minimum, maximum). To assess the adequacy of the randomization, participants in each of the two arms will be compared for baseline characteristics including demographics and laboratory measurements using chi-square or Fisher's exact tests for categorical variables and t-tests or Wilcoxon rank-sum tests for continuous variables. Within-treatment group assessment of the change from the baseline measurement to a follow-up measurement will be analyzed using McNemar's test (for categorical response variables) or the paired t-test or Wilcoxon signed-ranks test (for continuous variables). Mixed effects models will be used to examine whether changes in response variables over time vary with the TDF or placebo IVR.

All visits in which a woman has been exposed to the study product will be included in the primary analyses of safety. Secondary intent to treat analyses may also be performed. To assess genitourinary safety, the number and the percentages of participants experiencing each safety endpoint (see section 10.2) will be tabulated by study arm. Each participant will contribute once in each category (i.e., only for highest severity AE for each participant) for the calculation of event rates. Exact binomial confidence intervals will be calculated for each safety endpoint in all women and for each arm. Cochran-Mantel-Haenszel chi-square tests will be used to test for differences in event rates between TDF IVR arm and the placebo IVR arm adjusted for study site.

10.8.4 Secondary Analyses

PK

Blood, CVF and tissue will be analyzed for routine PK parameters - C_{max}, T_{max}, and area under the curve (AUC) – and described using descriptive statistics. Half-life will be estimated for drug in blood, CVF, and cervical biopsies during the period following ring removal.

Acceptability

To assess acceptability of the study IVR, the number and percentage of participants experiencing at least one negative report of acceptability, including genitourinary discomfort and ring removal issues will be presented. This binomial proportion will be used to assess the acceptability of the study IVR along with its corresponding 95% confidence interval. This analysis will be supplemented by presenting the above proportion by randomization arm along with its corresponding 95% confidence interval and compared using the Cochran-Mantel-Haenszel test

adjusting for the randomization strata. Exploratory age-stratified analyses will be conducted with the full and the qualitative sample.

Qualitative data
Interviews will be audio recorded (with consent), transcribed and entered into Dedoose online qualitative analysis tool to facilitate retrieving text, reorganizing conceptual associations among data concepts and identifying themes. The analysis team will begin by reading the same 3 interviews independently to identify repeating ideas. The team will then meet to compare observations, formulate codes, and apply these to the next few interviews in an iterative process until the team agrees that we have a complete, replicable codebook. The qualitative researchers will then code the transcripts (n=40 in women and up to 40 in men) in Dedoose using the codebook; 10% of interviews will be double coded to assure accuracy. Coded data can then be retrieved using Dedoose to generate summaries of the material and to facilitate comparisons across cases and groups of cases over time.

We will simultaneously assign ratings to individual cases to create theoretically relevant subgroups for comparison (e.g., age, race/ethnicity, partnered or not, acceptability from the questionnaire data). We can also use *quantitated data* derived from the interview text that is converted to ratings. Through the matrix function, codes can be compared between groups defined by the ratings.

10.8.5 Exploratory Analyses

Impact of IVRs on the vaginal microbiome and relationship between the microbiome and PK
To determine the impact of TDF and placebo IVRs on the vaginal microbiome, the composition of bacterial communities will be determined by broad-range 16S rRNA gene PCR with Illumina sequencing in women before, during, and after TDF or placebo IVR use. To assess potential effects of drug on the vaginal microbiome, we will apply species-specific qPCR assays [41, 42] based on the broad range results to correlate changes in bacteria with concentrations of TDF and/or its metabolites measured in CVF and tissue.
.
Pharmacodynamics and genital tract mucosal immunity
To examine pharmacodynamics, anti-HIV activity in genital secretions will be measured. To assess mucosal immunity, soluble immune mediators will be measured at least 3 points in time from each subject. Linear mixed effects models will be used to assess whether mediators change over time and whether effects of TDF or placebo IVR on mediators vary with time accounting for within subject correlation. Treatment, time, and treatment-by-time interaction will be included as fixed effects and subjects will be included as random effects in our modeling.

10.8.6 Missing Data

We are targeting a retention rate of 90%. If missing data rates are higher than anticipated (over 20%), robust methods such as nonparametric tests and generalized estimating equations using all available baseline predictors of the missing outcomes as covariates will be used to obtain less biased estimates of the treatment effect.

11 DATA HANDLING AND RECORDKEEPING

11.1 Data Management Responsibilities

CRFs will be developed by the PL in conjunction with the study coordinators using the secure, web-based application REDCap (Research Electronic Data Capture). REDCap is designed exclusively to support data capture for research studies. It provides an intuitive interface for users

to enter data and has real time validation rules (with automated data type and range checks) at the time of entry. REDCap allows different members of the study team to have different levels of access to data entry forms, database management and data export tools. REDCap provides automated export procedures for seamless data downloads to Excel, SPSS, SAS, and Stata. REDCap servers are housed in a data center at Einstein and all web-based information transmission is encrypted. REDCap was developed specifically around HIPAA-Security guidelines. There are provisions to restrict access to data export to allow export of de-identified data only. REDCap includes full audit trail, recording all operations on the data, including viewing and exporting. The audit log records operation, date and time, and the user performing the operation, permitting review of the audit trail as necessary. Electronic data capture will be utilized to ensure the completeness, reliability, and accuracy of the data. Data captured by a member of the study team will be reviewed for discrepancies and incompleteness and any inconsistencies or missing data will be resolved prior to locking the form. Any change to a locked and verified form will be captured by the audit trail, and the system will prompt the user to document a reason for the change. Data are preserved in REDCap indefinitely until deleted by PL request. Electronic study questionnaires will also be developed using REDCap. Prior to study start, the PL will identify all CRFs to be used as source documents.

11.2 Source Documents and Access to Source Data/Documents

The study site will maintain source data/documents in accordance with Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials (http://www3.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/PDF/SourceDocPolicy.pdf).

Members of the study team will capture data initially into REDCap. Source data not captured electronically will be retained in the participant's paper chart. In-depth interviews and focus groups will be audio recorded and the interviews will be transcribed. The recordings and transcripts will be stored securely. Study clinicians will have access to both REDCap and paper charts; this will ensure good clinical practice, participant safety and continuum of care. The PL will maintain, and store securely, complete, accurate and current study records throughout the study. In accordance with US regulations, for the investigational product tested, the investigator will retain all study records for at least two years following the date of marketing approval for the study product for the indication in which it was studied. If no marketing application is to be filed or if the application is not approved, the records must be retained until two years after the investigation is discontinued and the US FDA is notified. Study records must be maintained on site for the entire period of study implementation. No study records may be moved to an off-site location or destroyed prior to receiving approval from DAIDS.

11.3 Quality Control and Quality Assurance

The study site will conduct quality control and quality assurance procedures in accordance with Requirements for Clinical Quality Management Plans at DAIDS Funded and/or Supported Clinical Research Sites available at: (http://www3.niaid.nih.gov/research/resources/DAIDSClinRsrch/PDF/QMPPolicy.pdf).

11.4 Study Coordination

Einstein holds the IND for this study (IND# 116945). Assignment of all sponsor responsibilities for this study will be specified in a Clinical Trials Agreement (CTA).

Study implementation will be directed by this protocol, which may not be amended without prior written approval from the PL and DAIDS MO. Study implementation will also be guided by a common study-specific procedures 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. All study staff will receive standardized study-specific training. Close coordination between the study 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. The team as well as the PSRT will monitor rates of accrual, follow-up, and AE incidence closely.

12 CLINICAL SITE MONITORING

Study monitoring will be carried out by Pharmaceutical Product Development, Inc. (PPD) (Wilmington, NC) in accordance with current DAIDS policies. Study monitors will visit the sites to do the following:

- Review informed consent forms, procedures, and documentation.
- Assess compliance with the study protocol, GCP guidelines, and applicable regulatory requirements (US and non-US), including CFR Title 45 Part 46 and Title 21 Parts 50, 56, and 312.
- 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.

The PL 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 PL also will allow inspection of all study-related documentation by authorized representative of NIAID, Gilead, FDA, US OHRP, IRBs, EC, PPB and other local and US regulatory authorities. A site visit log will be maintained at the study site to document all visits.

13 HUMAN SUBJECTS PROTECTIONS

The investigators will make efforts to minimize risks to participants. Volunteers and study staff members will take part in a thorough informed consent process. Before beginning the study, the investigators will have obtained IRB and EC approval and the protocol will have been submitted to the FDA and PPB. The investigators will permit audits by the NIH, the sponsor, the FDA, EC, PPB or any of their appointed agents.

13.1 Institutional Review Boards

The participating institution is responsible for assuring that this protocol and the associated site-specific informed consent documents and study-related documents (such as participant education and recruitment materials) are reviewed by an IRB responsible for oversight of research conducted

at the study sites. Any amendments to the protocol must be approved by the responsible IRB and DAIDS prior to implementation. Subsequent to the initial review and approval, the responsible IRB must review the study at least annually. The investigator will make safety and progress reports to the IRB 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 PSRT reviews of the study will be provided to the IRBs. Study sites will submit documentation of continuing review to the DAIDS Protocol Registration Office (PRO) in accordance with the DAIDS Protocol Registration Policy and Procedures Manual.

13.2 Protocol Registration

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

Site-specific informed consent forms (ICFs) *WILL NOT* be reviewed or approved by the DAIDS PRO, and sites will receive an Initial Registration Notification when the DAIDS PRO receives a complete registration packet. Receipt of an Initial Registration Notification indicates successful completion of the protocol registration process. Sites will not receive any additional notifications from the DAIDS PRO for the initial protocol registration. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

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

13.3 Risk-Benefit Statement

13.3.1 Risks

General

Phlebotomy may lead to excessive bleeding, discomfort, feelings of dizziness or faintness, and/or bruising, swelling and/or infection. Pelvic examination may cause mild discomfort and/or vaginal bleeding or spotting. 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 counselors 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.

Participants could have problems in their relationships with their sexual partners after partner notification in response to a diagnosed STI or HIV infection. Participants also could have problems in their partner relationships associated with use or attempted use of study product and because of the study requirement to abstain from receptive vaginal and anal sex for 1 week after biopsy visits.

Use of the study IVR may lead to vaginal symptoms, including irritation, increased discharge, and discomfort.

Pelvic exam and specimen collection carry the risk of discomfort during the exam and collection. Cervical biopsies carry the risk of discomfort or pain during the procedure and for a few hours afterwards. Participants may have mild vaginal spotting (bleeding) for one or two days. Participants may take acetaminophen for pain related to biopsy before and/or after the procedure. Non-steroidal anti-inflammatory drugs and other drugs known to affect coagulation will not be permitted. All participants will be instructed to not have sexual intercourse for 1 week after Visits 4, 6, and 8. Participants who undergo biopsy at Visits 2a, 2b, 2c, 2d, 3a, 3b or 9a will also be instructed to not have sex for 1 week after biopsy collection to allow for biopsy sites to heal. Participants will also be restricted from vaginal product use for the duration of the study. Participants will be sexually active and may be at increased risk for STIs and HIV acquisition, if exposed. Also some temporary discomfort with sexual intercourse may occur if the biopsy areas are still healing. There is a small risk of infection and heavier bleeding. Participants will be instructed to contact the clinic if symptoms are bothersome, if bleeding is noted (anything more than spotting) or if the participant develops any abnormal odor or discharge from the vagina.

The following side effects have been associated with the use of oral TDF:

- Upset stomach, vomiting, gas, loose or watery stools
- Generalized weakness
- Dizziness
- Headache
- Abdominal pain
- Worsening or new kidney damage or failure
- Inflammation or swelling and possible damage to the pancreas and liver
- Shortness of breath
- Rash
- Allergic reaction: symptoms may include fever, rash, upset stomach, vomiting, loose or watery stools, abdominal pain, achiness, shortness of breath, a general feeling of illness or potentially serious swelling of the face, lips, and /or tongue
- Bone pain and bone changes such as thinning and softening which may increase the risk of breakage
- Muscle pain and muscle weakness

These side effects may or may not be associated with TDF when formulated in a vaginal ring.

13.3.2 Benefits

Participants and others may benefit in the future from information learned from this study. Specifically, information learned in this study may lead to the development of safe and effective interventions to prevent HIV transmission. Participants also may appreciate the benefit of earlier diagnosis of STIs in addition to the opportunity to contribute to the field of HIV prevention research.

Additionally, participants will be referred for treatment for any incidental findings detected during screening and other examinations.

13.4 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 investigators 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 the Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials available at: (http://www3.niaid.nih.gov/research/resources/DAIDSClinRsrch/PDF/SourceDocPolicy.pdf).

Participants will be provided with copies of the informed consent forms if they are willing to receive them. The study site is responsible for developing study informed consent forms for local use, based on the templates in the Appendices that describe the purpose of screening and of the study, the procedures to be followed, and the risks and benefits of participation, in accordance with all applicable regulations. 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 import to this study:

- The unknown safety and unproven efficacy of the study products
- The need to practice safer sex behaviors regardless of study treatment group
- The importance of participants 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

The informed consent process will include an assessment of each potential participant's understanding prior to enrollment and randomization of concepts identified by the protocol team as essential to the informed consent decision. Participants who are not able to demonstrate adequate understanding of key concepts after exhaustive educational efforts will not be enrolled in the study.

Young women in Thika have high literacy levels compared with many regions in Kenya and thus can read the consent form as part of the consent process. In addition, we will use the "teach back" method to ascertain that all of the women understand what they are consenting to. During the

consent process, each woman will be asked these questions: 1. How would you explain this study to a friend? 2. What are some of the things that will happen to you in this study? 3. What do you need to avoid doing after biopsies are taken? The consenting investigator will document that the responses provided reflect a thorough understanding of the study prior to initiating any procedures.

13.5 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. Each study site 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. In addition to local considerations, the protections described below will be implemented.

All study-related information will be stored securely at the study site. All participant information will be stored in locked file cabinets in areas with access limited to study staff. Data collection, process, and administrative forms, laboratory specimens, and other reports will be identified by a coded number only to maintain participant confidentiality. All local databases will be secured with password-protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link participant ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access. Participants' study information will not be released without their written permission, except as necessary for review, monitoring, and/or auditing by:

- Representatives of the US Federal Government, including the US FDA, the OHRP, NIH, and/or contractors of the NIH, PPB
- Representatives of Gilead
- KEMRI Ethics and Research Committee or Einstein IRB

The in-depth interviews and focus groups will be audiotaped. This is so researchers can analyze the ideas brought up during the discussions. Only voice will be recorded; participant faces and names will not be identifiable. The audiotape will not be used for commercial or media purposes. The recordings will be stored, along with other study records, in a secured manner by the research team until they are transcribed. Audiotapes will be destroyed once transcription is completed.

13.6 Special Populations

This section outlines considerations made for the inclusion or exclusion of special populations in this study.

13.6.1 Pregnant Women

Women who test positive for pregnancy at screening or enrollment visits will not be eligible to participate in this study. In addition, a urine pregnancy test will be performed on all women at monthly visits and at unscheduled visits, if needed. Investigators will discontinue study product among participants who test positive for pregnancy. During the informed consent process, women will be informed that the study product is not a method of contraception and that the effects of this product on a developing human fetus are unknown.

All potential participants are required by the Eligibility Criteria for Screening and Enrollment to be currently using a copper IUD or any hormonal contraceptive method other than an IVR, with no change in the prior 2 months and intending to use same method for the duration of study

participation. Women who become pregnant during the study period following randomization will discontinue product use but not be excluded from analysis.

13.6.2 Children

The NIH has mandated that children be included in research trials when appropriate. Approximately half of all new HIV infections each year occur in people under the age of 25. Adolescent girls and young women account for 1 in 4 new HIV infections in Sub-Saharan Africa. This study will include females 18 years of age and older.

13.7 Compensation

Pending IRB approval, participants will be compensated for their time and effort in this study, and/or be reimbursed for travel to study visits, child care, and time away from work.

13.8 Communicable Disease Reporting

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

13.9 Access to HIV-related Care

13.9.1 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 and additionally will emphasize the unknown efficacy of the study products in preventing HIV infection. In accordance with the policies of the US NIH, participants must receive their HIV test results to take part in this study.

13.9.2 Care for Participants Identified as HIV-Infected

Participants will be provided with their HIV test results in the context of post-test counseling. Participants found to be HIV-infected will be referred to available sources of medical and psychosocial care and support, and local research studies for HIV-infected adults.

13.10 Study Discontinuation

This study may be discontinued at any time by NIAID, US FDA, OHRP, PPB, KEMRI Ethics and Research Committee, or the Einstein IRB.

14 PUBLICATION POLICY

Publication of the results of this study will be governed by NIAID policies. Any presentation, abstract, or manuscript will be submitted by the Investigator to DAIDS for review prior to submission.

15 REFERENCES

1. (UNAIDS), J.U.N.P.o.H.A., *Global report: UNAIDS report on the global AIDS epidemic 2013*, 2013.
2. Abdool Karim, Q., et al., *Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women.* Science, 2010. **329**(5996): p. 1168-74.
3. Rees, H., et al. *FACTS 001 Phase III trial of pericoital tenofovir 1% gel for HIV prevention in women*. in *Conference on Retroviruses and Opportunistic Infections 2015*. 2015. Seattle, WA.
4. Marrazzo, J.M., et al., *Tenofovir-based preexposure prophylaxis for HIV infection among African women.* N Engl J Med, 2015. **372**(6): p. 509-18.
5. Kiser, P.F., T.J. Johnson, and J.T. Clark, *State of the Art in Intravaginal Ring Technology for Topical Prophylaxis of HIV Infection.* AIDS Rev, 2012. **14**(1): p. 62-77.
6. Brache, V. and A. Faundes, *Contraceptive vaginal rings: a review.* Contraception, 2010. **82**(5): p. 418-27.
7. Hardy, E., et al., *Delivery of microbicides to the vagina: difficulties reported with the use of three devices, adherence to use and preferences.* Contraception, 2007. **76**(2): p. 126-31.
8. Smith, D.J., et al., *An evaluation of intravaginal rings as a potential HIV prevention device in urban Kenya: behaviors and attitudes that might influence uptake within a high-risk population.* J Womens Health (Larchmt), 2008. **17**(6): p. 1025-34.
9. Robbins, B.L., et al., *Anti-human immunodeficiency virus activity and cellular metabolism of a potential prodrug of the acyclic nucleoside phosphonate 9-R-(2-phosphonomethoxypropyl)adenine (PMPA), Bis(isopropyloxymethylcarbonyl)PMPA.* Antimicrob Agents Chemother, 1998. **42**(3): p. 612-7.
10. Mesquita, P.M., et al., *Intravaginal ring delivery of tenofovir disoproxil fumarate for prevention of HIV and herpes simplex virus infection.* J Antimicrob Chemother, 2012. **67**(7): p. 1730-8.
11. Keller, M.J., et al., *A phase 1 randomized placebo-controlled safety and pharmacokinetic trial of a tenofovir disoproxil fumarate vaginal ring.* AIDS, 2015.
12. Kashuba, A.D., et al., *Genital Tenofovir Concentrations Correlate With Protection Against HIV Infection in the CAPRISA 004 Trial: Importance of Adherence for Microbicide Effectiveness.* J Acquir Immune Defic Syndr, 2015. **69**(3): p. 264-9.
13. Hendrix, C.W., et al., *MTN-001: randomized pharmacokinetic cross-over study comparing tenofovir vaginal gel and oral tablets in vaginal tissue and other compartments.* PLoS One, 2013. **8**(1): p. e55013.
14. Haberer, J.E., et al., *Adherence to antiretroviral prophylaxis for HIV prevention: a substudy cohort within a clinical trial of serodiscordant couples in East Africa.* PLoS Med, 2013. **10**(9): p. e1001511.
15. Smith, J.M., et al., *Intravaginal ring eluting tenofovir disoproxil fumarate completely protects macaques from multiple vaginal simian-HIV challenges.* Proc Natl Acad Sci U S A, 2013. **110**(40): p. 16145-50.
16. Nixon, B., et al., *Vaginally delivered tenofovir disoproxil fumarate provides greater protection than tenofovir against genital herpes in a murine model of efficacy and safety.* Antimicrob Agents Chemother, 2014. **58**(2): p. 1153-60.
17. Seay, K., et al., *The Vaginal Acquisition and Dissemination of HIV-1 Infection in a Novel Transgenic Mouse Model Is Facilitated by Coinfection with Herpes Simplex Virus 2 and Is Inhibited by Microbicide Treatment.* J Virol, 2015. **89**(18): p. 9559-70.
18. Johnson, T.J., et al., *A 90-day tenofovir reservoir intravaginal ring for mucosal HIV prophylaxis.* Antimicrob Agents Chemother, 2012. **56**(12): p. 6272-83.

19. Smith, J.M., et al., *Tenofovir disoproxil fumarate intravaginal ring protects high-dose depot medroxyprogesterone acetate-treated macaques from multiple SHIV exposures.* J Acquir Immune Defic Syndr, 2015. **68**(1): p. 1-5.
20. Srinivasan, P., et al., *Pharmacokinetic evaluation of tenofovir disoproxil fumarate released from an intravaginal ring in pigtailed macaques after 6 months of continuous use.* J Med Primatol, 2014. **43**(5): p. 364-9.
21. Van Damme, L., et al., *Lack of effectiveness of cellulose sulfate gel for the prevention of vaginal HIV transmission.* N Engl J Med, 2008. **359**(5): p. 463-72.
22. Van Damme, L., et al., *Effectiveness of COL-1492, a nonoxynol-9 vaginal gel, on HIV-1 transmission in female sex workers: a randomised controlled trial.* Lancet, 2002. **360**(9338): p. 971-7.
23. Segarra, T.J., et al., *Bridging the gap between preclinical and clinical microbicide trials: blind evaluation of candidate gels in murine models of efficacy and safety.* PLoS One, 2011. **6**(11): p. e27675.
24. Wilson, S.S., et al., *Susceptibility to genital herpes as a biomarker predictive of increased HIV risk: expansion of a murine model of microbicide safety.* Antivir Ther, 2009. **14**(8): p. 1113-24.
25. Galen, B.T., et al., *A comprehensive murine model to evaluate topical vaginal microbicides: mucosal inflammation and susceptibility to genital herpes as surrogate markers of safety.* J Infect Dis, 2007. **195**(9): p. 1332-9.
26. Taneva, E., et al., *Differential Mechanisms of Tenofovir and Tenofovir Disoproxil Fumarate Cellular Transport and Implications for Topical Preexposure Prophylaxis.* Antimicrob Agents Chemother, 2015. **60**(3): p. 1667-75.
27. Uwai, Y., et al., *Renal transport of adefovir, cidofovir, and tenofovir by SLC22A family members (hOAT1, hOAT3, and hOCT2).* Pharm Res, 2007. **24**(4): p. 811-5.
28. Hijazi, K., et al., *Expression of Genes for Drug Transporters in the Human Female Genital Tract and Modulatory Effect of Antiretroviral Drugs.* PLoS One, 2015. **10**(6): p. e0131405.
29. Nicol, M.R., et al., *Expression of six drug transporters in vaginal, cervical, and colorectal tissues: Implications for drug disposition in HIV prevention.* J Clin Pharmacol, 2014. **54**(5): p. 574-83.
30. Mayer, K.H., et al., *Safety and tolerability of tenofovir vaginal gel in abstinent and sexually active HIV-infected and uninfected women.* Aids, 2006. **20**(4): p. 543-51.
31. Schwartz, J.L., et al., *A multi-compartment, single and multiple dose pharmacokinetic study of the vaginal candidate microbicide 1% tenofovir gel.* PLoS One, 2011. **6**(10): p. e25974.
32. Herold, B.C., et al., *Impact of Sex on the Pharmacokinetics and Pharmacodynamics of 1% Tenofovir Gel.* Clin Infect Dis, 2016. **62**(3): p. 375-82.
33. Chittick, G.E., et al., *Pharmacokinetics of tenofovir disoproxil fumarate and ritonavir-boosted saquinavir mesylate administered alone or in combination at steady state.* Antimicrob Agents Chemother, 2006. **50**(4): p. 1304-10.
34. Blum, M.R., et al., *Steady-state pharmacokinetics of emtricitabine and tenofovir disoproxil fumarate administered alone and in combination in healthy volunteers.* J Clin Pharmacol, 2007. **47**(6): p. 751-9.
35. Gilead Sciences, I., *Investigator's Brochure Tenofovir Disoproxil Fumarate Human Immunodeficiency Virus Type 1 Infection*.
36. Nel, A., et al., *A safety and pharmacokinetic trial assessing delivery of dapivirine from a vaginal ring in healthy women.* AIDS, 2014. **28**(10): p. 1479-87.
37. Chen, B.A., et al., *Phase 1 Safety, Pharmacokinetics, and Pharmacodynamics of Dapivirine and Maraviroc Vaginal Rings: A Double-Blind Randomized Trial.* J Acquir Immune Defic Syndr, 2015. **70**(3): p. 242-9.
38. Baeten, J.M., et al., *Use of a Vaginal Ring Containing Dapivirine for HIV-1 Prevention in Women.* N Engl J Med, 2016.

39. Nel, A., et al. *Safety and efficacy of dapivirine vaginal ring for HIV-1 prevention in African women*. in *Conference on Retroviruses and Opportunistic Infections*. 2016. Boston, MA.
40. Mugwanya, K.K., et al., *Circumcision of male children for reduction of future risk for HIV: acceptability among HIV serodiscordant couples in Kampala, Uganda.* PLoS One, 2011. **6**(7): p. e22254.
41. Fredricks, D.N., et al., *Changes in vaginal bacterial concentrations with intravaginal metronidazole therapy for bacterial vaginosis as assessed by quantitative PCR.* J Clin Microbiol, 2009. **47**(3): p. 721-6.
42. Fredricks, D.N., et al., *Targeted PCR for detection of vaginal bacteria associated with bacterial vaginosis.* J Clin Microbiol, 2007. **45**(10): p. 3270-6.

APPENDICES

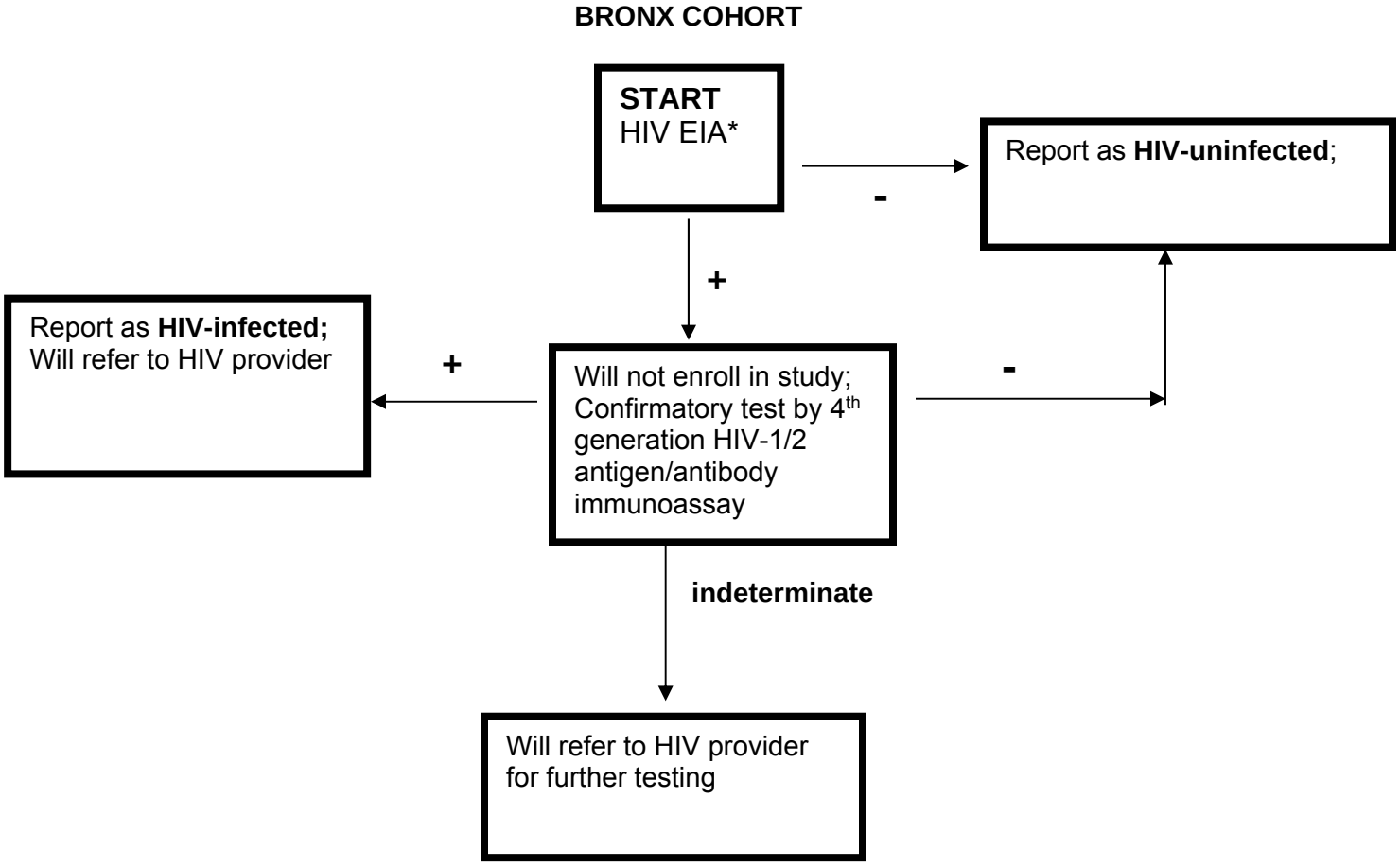
APPENDIX I: SCHEDULE OF STUDY VISITS AND EVALUATIONS
APPENDIX II: ALGORITHM FOR HIV ANTIBODY TESTING
APPENDIX III: PHARMACY PLAN

APPENDIX I: SCHEDULE OF STUDY VISITS AND EVALUATIONS

	Visit 1: Scr	Visit 2: Enr IVR insertion	Visit 2a, 2b, 2c: 1 hr, 4hr, Day 1	Visit 2d: Day 7 (Day 7-9)	Visit 3, 3a: Day 14 (Day 14-16)	Visit 3b: Day 21 (Day 21-23)	Visit 4: Day 28 (Day 28-30) IVR change[1]	Visit 5: Day 42 (Day 42-44)	Visit 6: Day 56 (Day 56-58) IVR change[1]	Visit 7: Day 70 (Day 70-72)	Visit 8: Day 84 (Day 84-86) IVR removal[1]	Visit 9, 9a: Day 89 (Day 89-91)	Visit 10: Day 94 (Days 94-96)
Informed consent	X												
Demographics/ Medical History	X												
Vital Signs	X												
Urine Pregnancy	X	X		X	X	X	X		X		X	X	
HIV test with counseling	X	X					X		X		X		
Syphilis testing	X[2]												
HSV-1 & 2 IgG	X										X		
Hepatitis B surface antigen	X[2]												
CBC, serum chemistries	X[2]						X		X		X		
Plasma for PK		X			X	X[3]	X	X	X	X	X	X	X[3]
DBS for PK							X		X		X		
Physical exam	X[2]												
Pelvic/speculum exam	X	X	X	X	X	X	X	X[2]	X	X[2]	X	X	X[3]
NAAT for GC/CT/Trich	X[2]												
Pap test	X[2]												
Vaginal swab for Gram stain and pH	X	X	X[3]	X[3]	X	X[3]	X	X	X	X	X	X	X[3]
Swab for RSID		X	X[3]	X[3]	X	X[3]	X	X	X	X	X	X	X[3]
Vaginal swabs for flora		X	X[3]				X		X		X	X	
Endocervical swab for mediators and antimicrobial activity		X					X		X		X		
CVF swabs for drug levels		X	X[3]	X[3]	X	X[3]	X	X	X	X	X	X	X[3]
Cervical biopsy for PK			X[3]	X[3]	X[3]	X[3]	X[4]		X[4]		X[4]	X[3]	
Assess for AEs	X	X	X	X	X	X	X	X	X	X	X	X	X[3]
Acceptability questionnaire							X				X		
In-depth qualitative interview							X[5]				X[5]		
[6]Focus groups													

[1]Rings will be collected and assessed for residual drug levels
[2]Will be repeated at follow-up visits if clinically indicated
[3]Only US participants will be randomized to additional sampling at 1 hour (Visit 2a), 4 hours (Visit 2b), 1 (Visit 2c), 7 (Visit 2d), 14 (Visit 3a) or 21 days (Visit 3b) after IVR insertion or 5-7 days after IVR removal (Visit 9a). There will be 5 or 6 US participants per time-point. The participants randomized to Visit 9a will have a final study visit 5-7 days later (Visit 10)
[4]All participants will undergo cervical biopsy collection at Visits 4 and 8 and at visit 6 in US participants only
[5]An in-depth qualitative interview will be conducted in 20 female participants and up to 20 male partners at the Thika site at Visit 4 and Visit 8
[6]Focus groups will be conducted in female participants at the Thika site after study completion

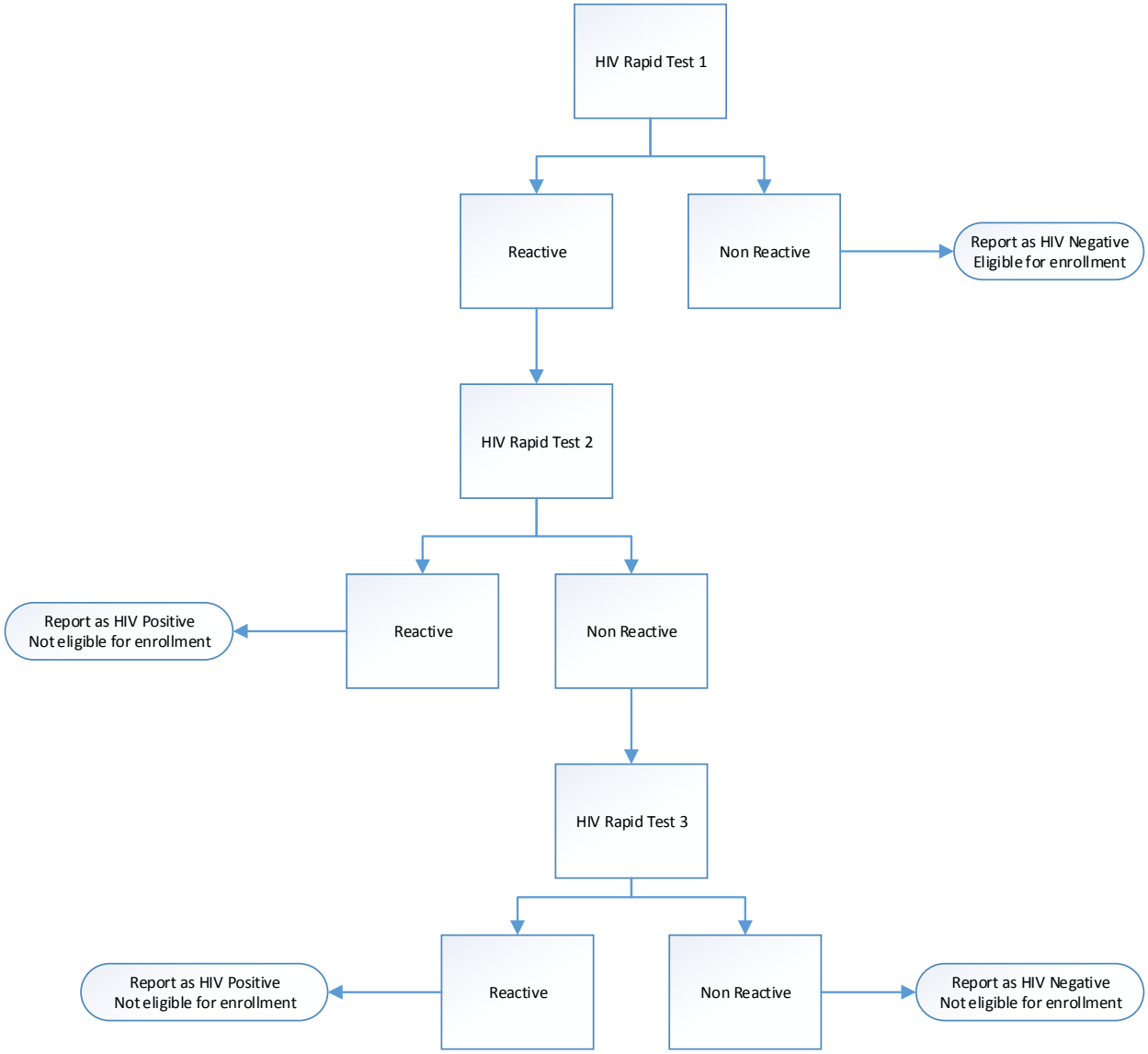
APPENDIX II: ALGORITHM FOR HIV ANTIBODY TESTING



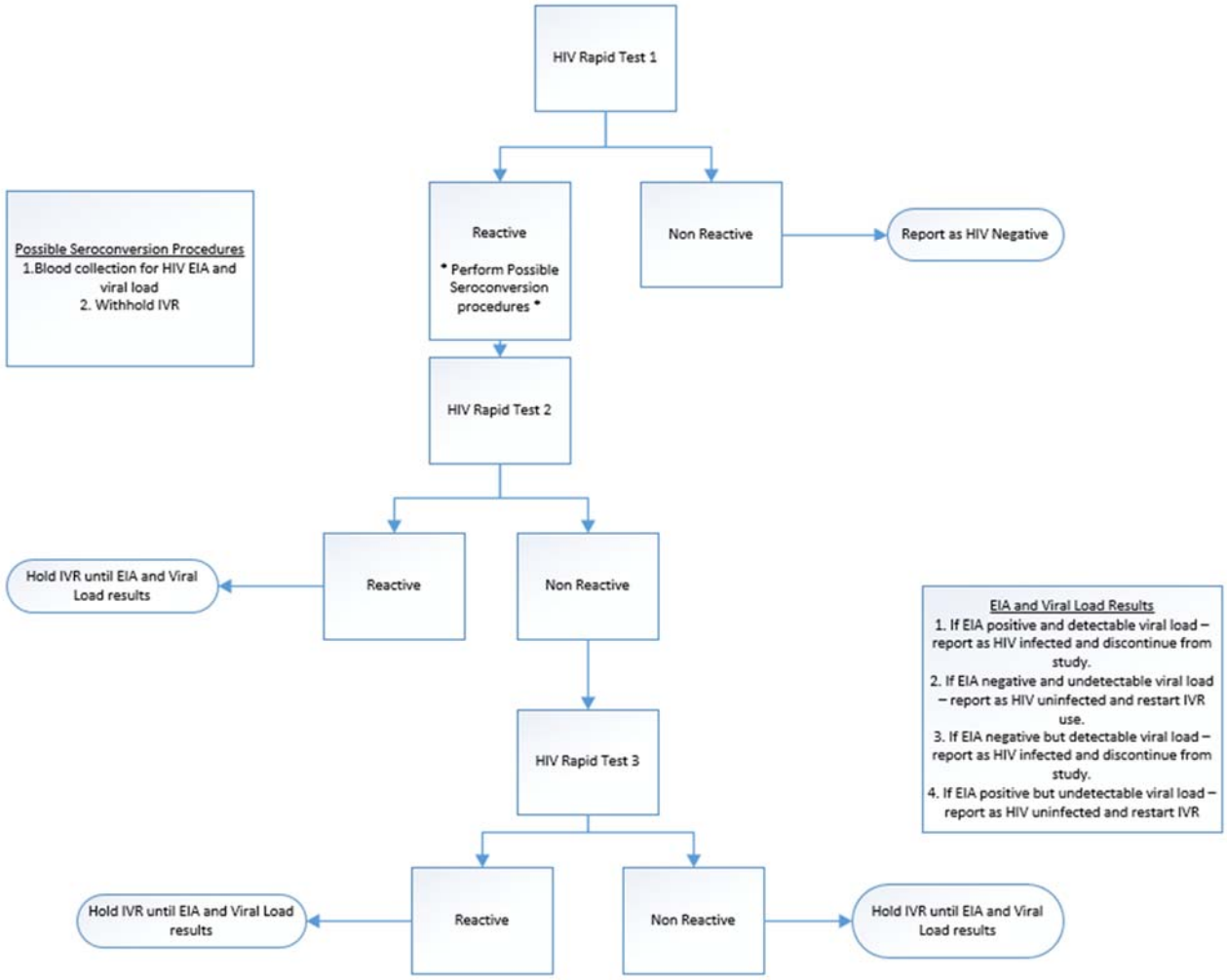
* OraQuick *ADVANCE*® Rapid HIV-1/2 Antibody Test

If HIV EIA is negative, will report as HIV-uninfected and enroll in study.
If HIV EIA is positive, will not enroll in study; will obtain confirmatory test by 4th generation HIV-1/2 antigen/antibody immunoassay.
If confirmatory test is positive for HIV-1 and HIV-2 antibodies and p24 antigen, will report as HIV-infected, and will refer to an HIV provider.
If confirmatory test is negative for HIV-1 and HIV-2 antibodies and p24 antigen, will report as HIV-uninfected.

KENYA COHORT – SCREENING VISIT



KENYA COHORT – FOLLOW-UP VISITS



APPENDIX III: PHARMACY PLAN

The following describes the pharmacy plan for:

Phase 1 Safety and Pharmacokinetic Study of a Polyurethane Tenofovir Disoproxil Fumarate Vaginal Ring in Sexually Active Women (TDF IVR-002)

TDF and placebo rings for this study will be stored in and distributed by the research pharmacy located at the Weiler Hospital at the Albert Einstein College of Medicine and by the pharmacist at the Partners in Health Research and Development site in Thika, Kenya. The pharmacy at Weiler Hospital is located in a building neighboring the Clinical Research Center where study visits take place. The pharmacy is accessible through adjoining buildings. The pharmacy in Thika is located at the clinic where study participants will be seen (Figure 1). Each pharmacy has adequate space to store sufficient quantities of study agent to assure continuous access to all study participants.

The handling of all study rings shall be in compliance with the policies and procedures of the Albert Einstein College of Medicine and Kenya Medical Research Institute Institutional Review Boards, the Albert Einstein College of Medicine Research Pharmacy, the pharmacy in Thika as well as all state, federal, and Pharmacy and Poisons Board (PPB) regulations in Kenya. All study rings for participant use shall be stored in a locked, limited access area in the pharmacy at each site. The Pharmacist of Record (PoR) at each site is responsible for receiving, storing, and dispensing study rings according to protocol requirements.

All drug shipment receipts will be stored in a binder in the pharmacy at each site. The PoR at each site will oversee the proper disposition of the drug supplies: inventory, labeling, accountability, storage, and distribution.

Study rings will be shipped by Particle Sciences, Inc. to:

Yvonne Gayle, Pharm D
Director, Pharmacy Operations
Jack D. Weiler Hospital of the Albert Einstein College of Medicine
1825 Eastchester Road
Bronx, NY 10461
Tel: (718) 904-2825
Fax: (718) 904-2158
ygayle@montefiore.org

George Mugendi, MPharm
Research Pharmacist
Partners in Health Research and Development
Section 9, OAU Road, Thika
P.O Box 19865 -00202, Nairobi
+254-727 961803; +254 (067) 22561
gmugendi@pipsthika.org

All shipments will be inventoried by the PoR at each site. Study rings will be stored at 20 degrees Celsius (°C) to 25°C (68° to 77°Fahrenheit) with excursions permitted between 15° and 30°C (between 59° and 86°F). Temperature and humidity will be recorded twice a day in logs maintained by the PoR at each site to ensure adequate storage conditions. Each site will use a digital thermometer that continuously reads temperatures and captures data, which can be downloaded or printed. At both sites, temperature excursions will be identified by an alarm system. The alarm system will page the pharmacist if temperature excursions occur. The PoR or designee at each site will answer the temperature excursions. Weiler Hospital and the Thika clinic, where the pharmacies are located, have back-up generators in the event of power outage.

Upon receipt of study product, the shipment will be inspected for accountability and condition of contents. Shipment logs included in the package will be completed, signed and dated. A copy of these documents will be maintained in the pharmacy binder.

RING DISPENSING

One TDF or placebo ring will be dispensed per participant by the PoR after receiving a written prescription signed by an authorized prescriber. The study clinician or designee will bring a prescription and a copy of the signed consent form to the PoR after the participant has been evaluated per the protocol as eligible for ring dispensation and insertion.

Prescriptions are used to document ordering of the study drugs. Original prescriptions are filed in the pharmacy and a copy of the prescription is filed in the participant's research record.

CHAIN OF CUSTODY OF PRODUCT

The PoR at each site will dispense the study product upon receipt of a prescription from the study clinician and based on the randomization sequence. The study product will be transported from the pharmacy to the Clinical Research Center at Einstein by a study team member to be inserted by the study clinician. Upon arrival to the clinic, a transportation log will be signed with the date, time, and initials of the study team member delivering the ring. Ring placement will then be checked per the protocol and documented. The PoR at each site will maintain a log documenting all rings dispensed to study staff members; this log will include the enrollment/randomization number, the type of ring dispensed, the date and time the ring was dispensed, and the initials of the study staff member transporting the ring. Study staff will also maintain a record of rings inserted by the study clinician and collected or removed from the subject.

DRUG ACCOUNTABILITY

All drug accountability records include receipt of study product, use and disposition of each ring. Each time study product is removed from the inventory, it is documented using the perpetual inventory method. The Investigational Drug Accountability Record is used to document the receipt and disposition of all study product received from the Sponsor. This accountability record provides for recording data on the disposition of the protocol-specific study product as follows:

- Date
- Study subject identifiers
- Quantity dispensed or received

- Current balance
- Manufacturer and lot number
- Research pharmacist initials

The drug accountability records are retained for two years following the date that a New Drug Application (NDA) is approved or, if an NDA is not approved, until two years after the Investigational New Drug Application or study is closed. These drug accountability records are available, upon request, for inspection and copying by a properly authorized employee, representative or monitor of the FDA, PPB or NIAID. CRFs will include the date and time of ring insertion, the participant identification number (PTID), the randomization number, the type of ring dispensed, and the name of the clinician that inserted the ring. Drug accountability records will only be destroyed with DAIDS permission.

STORAGE OF PROTOCOL AND 1572 FORM

The protocol and copy of the 1572 form will be stored in a binder in the pharmacy.

STUDY INITIATION

Authorized prescribers are listed in the delegation of responsibilities or study responsibility log as well as an authorized prescriber log in the pharmacy regulatory binder. Copies of the IRB approved protocol, the informed consent documents, NIH Prevention Science Review Committee (PSRC) approval and DAIDS PSP activation notice are maintained in the Regulatory Binder. In addition, dispensing logs, accountability records, correspondence, study product receipts and acknowledgements are maintained in the Regulatory Binders as well.

Prior to study initiation, the PoR at each site will complete human subjects protection training, good clinical practice (GCP), and protocol training. Records of completed training will be maintained in the Regulatory binder. The pharmacist will also complete Clinical Site Monitoring (CSM) training prior to the first monitoring visit.

QUARANTINE

Any ring that is found to be defective will be placed in a box to separate it from the rest of the study drug. A note will be made in the comments section on the accountability log. The DAIDS Clinical Operations Manager will be notified by email within 24 hours of becoming aware of a defective ring or temperature excursion. Any ring that is found to be defective will be handled and packaged appropriately and returned to Particle Sciences.

STUDY CLOSURE

When a study is closed, all remaining study supplies are audited. Notification from Particle Sciences will provide guidelines for product return. Notifications should be maintained in the Regulatory Binders. All records pertaining to product dispensing and correspondence are compiled and archived in a secured area in the office of the PL.

DRUG/DEVICE RETURN/DISPOSAL

Study product will be returned to Particle Sciences for the following reasons:

- The protocol is completed or terminated

- The product has been stored improperly
- Return has been requested

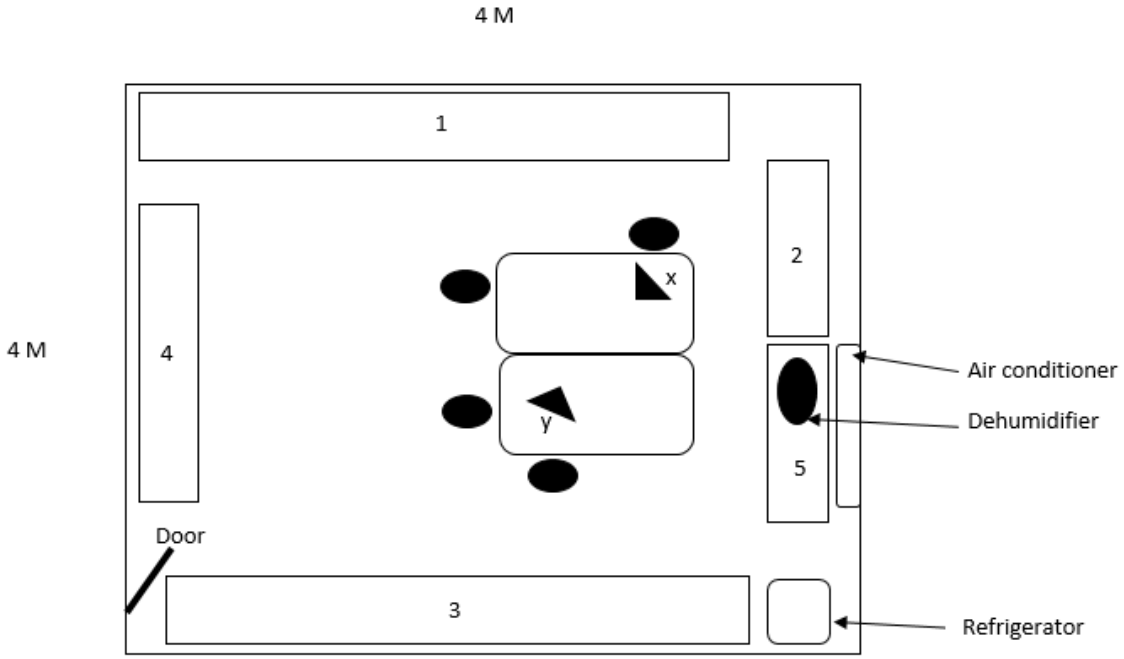
Return or disposal of study product should include a memo that states the protocol title, protocol number, name of study product being returned or destroyed, amount of product being returned or destroyed. This documentation should be filed with the Regulatory Binders for the study.

After the study is completed (or otherwise terminated), all unused study product will be shipped to Particle Sciences.

REVIEW AND ADJUDICATION OF MONITORING REPORTS

Study monitors will verify proper storage, dispensing, and accountability of investigational study products. The PoR at each site will review all monitoring reports and address all items requiring action per the study monitor via the CSM.

Figure 1: Thika Pharmacy Layout



Key
1, 2, 3, and 4; cabinets with 6 shelves each for storage of study drug
5; filing cabinet for study-related documents
X & y – desktop computers stationed on two office tables with 4 chairs