HPTN 059
Phase II Expanded Safety and Acceptability Study of the Vaginal Microbicide 1% Tenofovir Gel

A Study of the HIV Prevention Trials Network

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LIST OF ABBREVIATIONS AND ACRONYMS

AE	adverse event
AIDS	Acquired Immunodeficiency Syndrome AIDS
ALP	alkaline phosphatase
ALT	alanine transaminase
AST	aspartate aminotransferase
BLHC	Bronx-Lebanon Hospital Center
BMD	bone mineral density
BV	bacterial vaginosis
CAB	community advisory board
CBO	community based organization
CBC	complete blood count
CHBV	chronic hepatitis B virus
CDC	Centers for Disease Control and Prevention
CL	(HPTN) Central Laboratory
Cmax	maximum serum concentration
Cmax, ss	maximum serum concentrations at steady state
CORE	(HPTN) Coordinating and Operations Center
CRF	case report form
CRPMC	(NIAID) Clinical Research Products Management Center
CT	*Chlamydia trachomatis,* chlamydia
DAIDS	Division of AIDS
DSMB	Data and Safety Monitoring Board
EC	ethics committee
EAE	expedited adverse event
EIA	enzyme immunoassay
FDA	(United States) Food and Drug Administration
GC	*Neisseria gonorrhoeae,* gonorrhea
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HIV	Human Immunodeficiency Virus
HPTN	HIV Prevention Trials Network
HSV-1, HSV-2	Herpes simplex virus type 1, type 2
IFA	Immuno-fluorescent antibody
IND	investigational new drug
IRB	Institutional Review Board
LDMS	Laboratory Data Management System

LIST OF ABBREVIATIONS AND ACRONYMS continued

LFT	liver function test
LL	local laboratory
mL	Milliliter
N-9	Nonoxynol 9
NARI	National AIDS Research Institute
NAAT	nucleic acid amplification testing
NGO	Non-Governmental Organization
NIAID	(United States) National Institute of Allergy and Infectious Disease
PCR	polymerase chain reaction
PK	Pharmacokinetic
PMPA	9-[(R)-2-(phosphonomethoxy) propyl] adenine monohydrate
PMPAp	PMPA monophosphate
PMPApp	PMPA diphosphate
PSRT	Protocol Safety Review Team
RCC	(DAIDS) Regulatory Compliance Center
RFP	renal function parameter
RTI	reproductive tract infection
SDMC	(HPTN) Statistical and Data Management Center
SIV	Simian Immunodeficiency Virus
SMC	(HPTN) study monitoring committee
SOP	standard operating procedure
STI	sexually transmitted infection
TDF	tenofovir disoproxil fumarate (oral tenofovir)
UAB	University of Alabama at Birmingham (site)
ULN	upper limit of normal
µM	Micromole
WB	western blot

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Phase II Extended Safety and Acceptability Study of the Vaginal Microbicide 1% Tenofovir Gel
Investigator Signature Form

Final Version 2.0
13 March 2006

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US National Institute on Drug Abuse
US National Institutes of Health

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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 gel for the indication in which it was studied, unless otherwise specified by the Division of AIDS (DAIDS), Gilead Sciences Inc., or the HIV Prevention Trials Network (HPTN) Coordinating and Operations Center. If no marketing application is filed, or if the application is not approved, the records will be retained for two years after the FDA is notified that the Investigational New Drug application (IND) is discontinued. Publication of the results of this study will be governed by HPTN policies. Any presentation, abstract, or manuscript will be submitted to the HPTN Manuscript Review Committee, DAIDS, and Gilead Sciences, Inc. for review prior to submission.

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.

_____
Name of Investigator of Record

_____ _____
Signature of Investigator of Record Date

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Phase II Expanded Safety and Acceptability Study of the Vaginal Microbicide 1% Tenofovir Gel

SCHEMA

Purpose: To assess the safety of tenofovir gel for vaginal use in HIV uninfected women versus a placebo gel over 24 weeks of study gel use

Design: Phase II, four arm, three site, randomized, double blind, controlled trial comparing tenofovir 1 % vaginal gel or placebo gel used once daily and tenofovir 1% vaginal gel or placebo gel used prior to intercourse, with 24 weeks of product exposure and follow up. Participants with chronic hepatitis B virus (CHBV) will have an additional 12 weeks follow up.

Study Population: US and non-US sexually active, HIV uninfected women with a normal lower genital tract between the ages of 18 and 50

Study Size: Approximately 100 participants will be enrolled at the Pune site and approximately 100 participants will be enrolled between the Birmingham site and the Bronx site, for a total of 200 women. The US sites will competitively enroll the 100 participants.

Treatment Regimen:

Arm	Description	N	Frequency
1	Tenofovir gel coitally dependent use	50	Up to two hours before each act of penile-vaginal sexual intercourse to a maximum of twice daily
2	Placebo gel coitally dependent use	50	Up to two hours before each act of penile-vaginal sexual intercourse to a maximum of twice daily
3	Tenofovir gel daily use	50	Once daily at bedtime or longest period of rest
4	Placebo gel daily use	50	Once daily at bedtime or longest period of rest

Participants in Arms 1 and 2 will use the study gel before each act of penile-vaginal sexual intercourse (to a maximum of two doses daily). Participants in Arms 3 and 4 will use the study gel once daily (before bedtime or longest period of rest).

Study Duration: Accrual will require approximately six calendar months. Each participant will be followed for up to 24 weeks. CHBV infected participants will additionally return to site at 4, 8 and 12 weeks after completion of study gel use. Therefore the entire study should be completed within 15 calendar months.

SCHEMA continued

Primary Objective:

- To assess the local and systemic safety of tenofovir 1% gel for vaginal use in HIV uninfected women versus a placebo gel over 24 weeks of daily and coitally dependent use

Secondary Objective:

- To assess the acceptability of, and adherence to tenofovir gel for vaginal use in HIV uninfected women versus placebo gel over 24 weeks of daily or coitally dependent use

Exploratory Objectives:

- To measure vaginal flora characteristics, and to descriptively examine changes in these characteristics over the course of prolonged study gel use
- To assess the effects of study gel on cytokine and chemokine expression in cervical secretions
- To evaluate the association between cytokine and chemokine expression
- To correlate cytokine and chemokine expression with colposcopic evidence of inflammation and epithelial disruption

Study Sites:

- National AIDS Research Institute (NARI), Pune, India
- University of Alabama at Birmingham, Birmingham, Alabama, USA (UAB)
- Bronx-Lebanon Hospital Center, Bronx, New York, USA (BLHC)

1. INTRODUCTION

1.1 Microbicides and Human Immunodeficiency Virus (HIV) Prevention

While the male condom is effective in preventing sexual transmission of HIV, its use is hampered by deeply rooted cultural and social barriers. About half of all HIV infections worldwide are among women, yet the only available female-controlled method of HIV prevention is the female condom. Alternative prevention tools, such as vaginal microbicides, are urgently needed to slow the rapid spread of heterosexual HIV infection.[1]

Effectiveness trials of vaginal microbicides completed to date have all involved products containing nonoxynol-9 (N-9). Marketed as a spermicide, N-9 is a surfactant, or surface-acting agent, that destroys cellular and microbial membranes.[2, 3] Several formulations and various concentrations of N-9 have been tested in humans for safety and effectiveness. Four randomized controlled trials showed that use of N-9 does not protect against HIV infection, and higher doses and more frequent use of N-9 have been linked to increased findings of genital lesions, which increase the risk of HIV infection.[2, 3] Therefore, N-9 can not be considered a viable option for HIV prevention.

Tenofovir gel may be an alternative method for preventing HIV transmission. Evaluating the safety of new microbicide candidates such as tenofovir gel is the first step in the critical path to effectiveness trials.

1.2 Tenofovir Gel

Tenofovir is currently approved as antiretroviral therapy in oral form. It is an adenosine nucleoside monophosphate (nucleotide) analog belonging to the class of acyclic phosphonomethylether nucleosides tenofovir, the generic name for the active compound, is interchangeable with its chemical name 9-[(R)-2-(phosphonomethoxy) propyl] adenine monohydrate, or PMPA.

Refer to Sections 1.3 through 1.3.6.2 below, Section 4.1.1, the Investigator's Brochure for Tenofovir Gel (GS-1278) Second Edition, and, the Investigator's Brochure for Viread: Tenofovir Disoproxil Fumarate (PMPA Prodrug) (GS 4331-05) Tenth Edition for additional information on Tenofovir Gel and gel formulation.

1.3 Rationale

Topical agents under development for the prevention of vaginal HIV transmission include non-specific inhibitors of HIV (detergents, acid pH buffering agents, and anti-microbial peptides) and agents that work specifically by inhibiting virus-cell attachment. Another promising approach is using a topical product to block virus replication once infection has occurred. Topical tenofovir gel was chosen to test this approach for a number of reasons, including its activity in target cells for HIV infection (Langerhans-

dendritic cells; monocyte/macrophages, and T cells) of the vagina and cervix and the low frequency of local and systemic toxicity observed in the HPTN 050 phase I study of tenofovir 1% gel. In addition, animal studies have demonstrated that tenofovir gel prevents establishment of systemic infection in macaques when administered prior to or following intravenous challenge with simian immunodeficiency virus (SIV) and that it inhibits vaginal transmission of SIV in macaques.

The goal of this three-center phase II study is to determine the safety of tenofovir 1% gel as a vaginal microbicide over 24 weeks of use, and to gain additional information about the product's acceptability. The HPTN 050 study evaluated 14 days of gel use dosed once or twice daily, (i.e., up to 28 doses). Over 24 weeks, a probable maximum of 168 consecutive daily doses will provide cumulative multiple dose data to assess the long-term safety of tenofovir 1% gel. Additionally, with 24 weeks of gel use, the team intends to identify the length of time beyond two weeks that the participants are willing use the study gel.

The study will be a four-arm, randomized, double-blind, controlled trial, comparing two frequencies of study gel use (daily and coitally dependent) and corresponding study arms in which participants will use a placebo gel. Participants will:

- have pelvic exams with colposcopy and specimen collection for cytokine and chemokine testing
- have blood and urine testing
- have blood pharmacokinetic (PK) sampling
- be provided with and counseled to use water or silicone base lubricated, non-N9 or spermicide containing, male, latex condoms
- be counseled to use the study gel either once daily or with each act of vaginal intercourse up to twice a day, depending on the study arm to which they are randomized

This study will collect data on the use and acceptability of a product that might prove to be effective against HIV and other sexually transmitted infections (STIs) in future larger studies. Information about sexual behavior, including previous vaginal gel use, sexual relationships and negotiation, and use of male condoms, within each site is important background information for proceeding to Phase II/III studies. Moreover, any information obtained about the product's characteristics, packaging, and methods of administration that increases the likelihood that it will be used as recommended for effective protection is relevant to further studies.

1.3.1 Justification of 1% Dosing

Choice of tenofovir gel concentration for the Phase II clinical study is based on the following. First is the demonstration of minimal vaginal irritation in animal and human studies. A rabbit vaginal irritation test identified 1% tenofovir gel as being histopathologically identical to sham or control treatment, while on a qualitative basis 3% gel was more irritating to vaginal epithelia.[4] The tolerability of the 1% gel was confirmed in the HPTN 050 Phase I study, the Phase I dose ranging

study of tenofovir gel (0.3% once daily, then 1.0% once daily, then 0.3% twice daily followed by 1% twice daily). In this study, of the two doses and frequencies studied in the dose finding cohort, the 1% gel applied intravaginally twice daily for 14 days was well tolerated and was identified as the highest practical dose and frequency for further study in subsequent cohorts.

The second line of evidence is from vaginal transmission inhibition studies performed in non-human primates.[4] Six separate studies provided evidence for efficacy of the gel over a range of tenofovir concentrations of 0.3% to 10%. Although the total data are limited and a powered statistical determination as to the efficacy of 1% tenofovir gel versus 0.3% and 10% cannot be made, empirical examination of the efficacy data identifies 1% tenofovir gel as the lowest efficacious concentration tested when given within two hours of infection. See Table 1 summarizing the vaginal transmission inhibition studies of tenofovir gel performed in non-human primates.

Finally, limited vaginal PK tenofovir data in primates and humans demonstrate that tenofovir gel is broadly distributed in vaginal tissues following vaginal application and can penetrate to epithelial tissues.[5] The amount of tenofovir administered by intravaginal application of 4 grams of a 1% dose (40 mg) is comparable to the amount of tenofovir systemically absorbed following ingestion of a 300 mg dose of tenofovir disoproxil fumarate (TDF), the oral form of tenofovir. This dose of tenofovir is highly active against HIV and results in a reduction of plasma HIV ribonucleic acid (RNA) of 1.5 $\log_{10}$ copies / mL after daily administration for 21 days.

Comparison of the predicted cervicovaginal concentrations of tenofovir gel delivered to those achieved systemically at the standard treatment dose of 300 mg oral TDF, and tenofovir's characteristic prolonged intracellular half-life (diphosphate form, nine to 50 hours depending upon cell type), suggest that an initial and potentially durable barrier to HIV transmission may be possible. In terms of weighing potential risks and benefits, the 1% tenofovir gel minimizes the potential risks of vaginal epithelial toxicity while providing the potential benefit of delivering sufficient tenofovir to achieve an initial and possibly durational barrier to infection.

Table 1: Use of Topical Tenofovir to Prevent Vaginal Transmission of SIV

Study	Number of Exposures	Treatment	Time of administration	Number Infected	Progesterone Pretreatment
1	2	1 mL vehicle	-24 h, 0 h, 24 h, 48 h	2 of 2	No**
		10% tenofovir	-24 h, 0 h, 24 h, 48 h	0 of 4	
2	1	untreated control	N/A	5 of 5	No**
		10% tenofovir	-24 h , -15 m, +24 h	1 of 5	
		1% tenofovir	-24 h , -15 m, +24 h	1 of 5	
		1% tenofovir	-15 m	2 of 5	
3	1	untreated control	N/A	2 of 5	No**
		vehicle	-15 m	1 of 5	
		1% tenofovir	-15 m	1 of 5	
		1% tenofovir	- 2 h	3 of 5	
		1% tenofovir	- 8 h	1 of 5	
4	1	untreated control	N/A	4 of 5	No**
		vehicle	-15 m	2 of 5	
		1 % tenofovir	-15 m	1 of 5	
		1 % tenofovir	- 2 h	1 of 5	
		1 % tenofovir	- 8 h	2 of 5	
5	1	untreated control	N/A	2 of 5	No**
		vehicle	- 2 h	2 of 5	
		1 % tenofovir	- 2 h	0 of 5	
6	1	1% tenofovir	-12 h	5 of 8	Yes***
		Vehicle	-12 h	8 of 8	
		1% tenofovir	-24 h	8 of 8	
		Vehicle	-24 h	8 of 8	
		Untreated control	N/A	8 of 8	
		1% tenofovir	-72 h, -48 h, - 24 h	6 of 8	

***** All studies were performed with the SIVmac251 isolate of SIV, and female rhesus macaques were inoculated intravaginally. Virus challenges were performed without progesterone pretreatment in studies 1-5, macaques in Study 6 were pretreated with 30 mg Depo-Provera 30 days prior to viral challenge. The indicated studies were performed by 3 independent investigators with studies 2, 3, 4, and 5 being performed by the same laboratory.

**Indian rhesus
***Chinese rhesus

h = hours m = minutes mL = milliliters

1.3.2 Clinical Research

Tenofovir (0.3% and 1%) gel was recently tested in the HPTN 050 Phase I study. In this trial, tenofovir gel was administered intravaginally in four groups of women: sexually abstinent HIV-uninfected and HIV infected women, and sexually active HIV-uninfected and HIV-infected women. The women and their male partners (in the sexually active cohorts) were also asked to assess the acceptability of the product. The study did not include a placebo or comparison arm. Results from the HPTN 050 Phase I study have shown tenofovir 1% gel to be safe and acceptable.

A total of 60 HIV-uninfected and 24 HIV infected women completed the HPTN 050 Phase I study. Safety and acceptability of both formulations were initially tested in the HIV-uninfected, sexually abstinent women at varying doses and frequencies. Since 1% tenofovir gel twice a day was as well tolerated as lower dose/frequency combinations, this regimen was used in sexually active HIV-uninfected and, sexually abstinent/sexually active HIV-infected women. Although 92% reported at least one adverse event (AE) the majority of these events were mild (87%) and limited to the genitourinary tract (70%).[6] Four severe AEs were reported, but only one lower abdominal pain was thought to be product-related[6].

Of 76 participants who had bacterial vaginosis (BV) evaluation (by using Nugent's score criteria) at both enrollment and Day 14, 30 women had asymptomatic BV at baseline and 15 of them became BV negative after 14 days of tenofovir gel use, while one out of 46 women without BV at baseline had BV detected at 14 days. Overall, 40% of the women had asymptomatic BV at baseline compared to 21% of the women after fourteen days of tenofovir gel use (p=0.0005).

Fourteen of 25 women (56%) with PK results had low, but detectable, serum tenofovir levels (limit of quantitation: 3.0 ng/mL) at some point in the 12 hours after dosing on either Day 0 (following the first dose) or on Day 13 (after daily dosing); three of the 14 had detectable levels on both days. The maximum tenofovir concentrations (Cmax) ranged from 3.0 to 25.8 ng/mL, with no clear dose-concentration relationship identified. For the woman with the 25.8 ng/mL level, this peak level occurred 2 hours following the dose; the level rapidly declined to 10.9 ng/mL at 4 hours and was undetectable at 12 hours following the dose. Besides the outlier with the highest tenofovir level, the next highest Cmax was 7.1 ng/mL. Considering all women in the PK cohort, the median tenofovir Cmax was 3.4 ng/mL (interquartile range: below limit of quantitation [3.0 ng/mL] to 4.7 ng/mL)[6]. The median Cmax for all subjects (3.4 ng/mL) corresponds to approximately 1% of the maximum (Cmax, ss) and 7% of the minimum (C_{24} single dose) blood concentrations at steady-state with 300 mg daily oral tenofovir dosing[7].

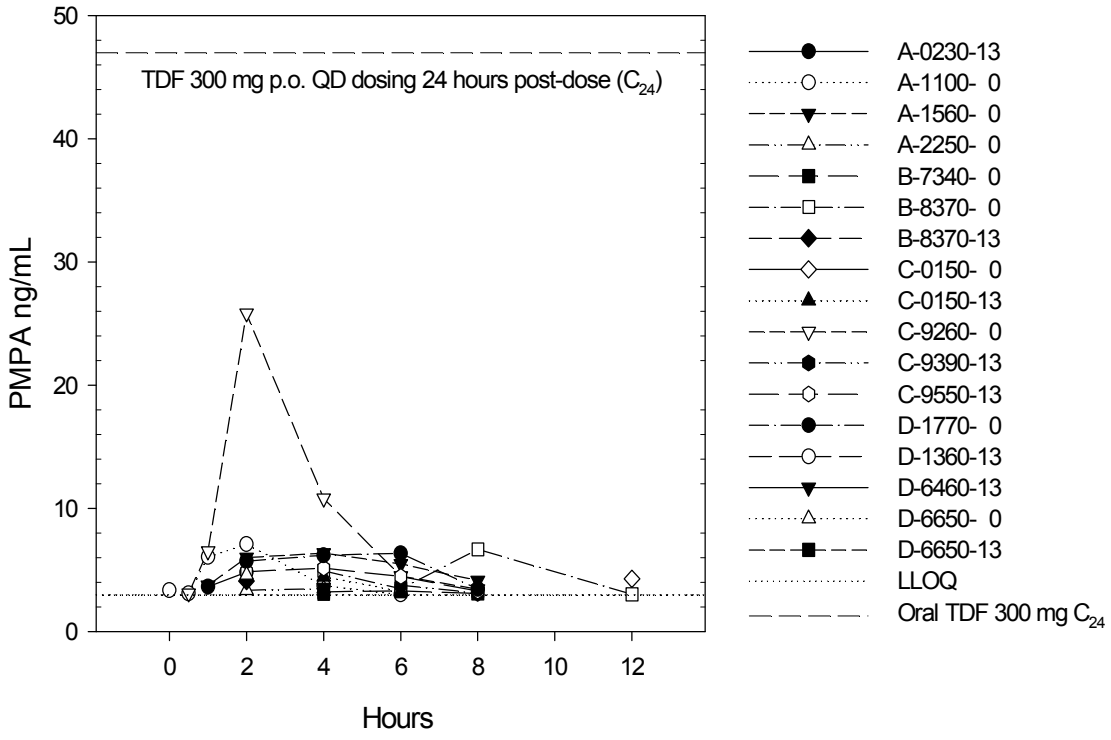
Figure 1 presented below demonstrates tenofovir blood concentration following vaginal administration of 1% tenofovir gel. All levels for all women with measurable tenofovir levels in the blood are shown. (14 of 25; lower limit of

quantitation (LLOQ) approximately 3.0 ng/mL [dotted line]). Legend indicates "cohort" – "ID" – "study day". For reference the tenofovir level associated with the median 24 hour post-dose blood concentration following an oral 300 mg tenofovir dose is indicated with dashed line.[7]

No clinically significant systemic toxicity was detected. Therefore, it can be concluded that tenofovir 1% vaginal gel used twice daily was well tolerated in abstinent and sexually active HIV uninfected and HIV infected women, with limited systemic absorption and with possible beneficial effects on vaginal microflora. Extended safety and effectiveness studies are warranted based on these initial data.

Additional PK data will be evaluated in this study. Refer to Section 1.3.3 below.

Figure 1 - Tenofovir Blood Concentrations vs. Time after Vaginal Administration



First letter = Cohort
Cohort A - HIV -uninfected/sexually abstinent
Cohort B - HIV -uninfected/sexually active
Cohort C - HIV-infected/sexually abstinent
Cohort D - HIV-infected/sexually active

4 digits = last 4 digits of participant identifier
Last digit = day of study

1.3.3 Mechanism of Action/Frequency of Dosing

One of the concerns related to the use of tenofovir as a microbicide in gel form is the uncertainty about how long it will take for the prodrug to be converted to the active antiviral metabolite and therefore how soon after application it can be expected to provide protection during coitus.

Activation of tenofovir is dependent upon anabolic phosphorylation by intracellular nucleoside kinases, whose activity and availability are dependent upon the activation state of the cell. It is unknown how long it will take the lymphocytes, dendritic cells and monocyte/macrophages in the vaginal mucosa to convert the tenofovir to its di-phosphorylated antiviral metabolite. Thus, it is possible that the absolute rate of tenofovir activation will depend upon a wide range of cervicovaginal health factors (e.g., inflammation, stage in menses etc.), as well as the physiochemical properties of the tenofovir gel in the vagina.

Intracellularly, constitutively expressed enzymes convert tenofovir through two phosphorylation reactions to an active triphosphorylated anabolite, tenofovir diphosphate (PMPApp). Tenofovir diphosphate inhibits viral polymerase by direct binding competition with the natural deoxyribonucleotide substrate and, after incorporation into DNA, by DNA chain termination.

Some intracellular nucleoside kinase concentrations, particularly thymidine kinase, which is responsible for the initial phosphorylation reaction of zidovudine and d4T, are cell cycle-dependent such that non-proliferating lymphocytes and macrophages express low levels. Because tenofovir is a nucleotide analogue and, therefore, does not require the initial phosphorylation reaction (which is often rate limiting), it may be a more effective inhibitor of HIV in macrophages and other non-dividing cells as compared to some nucleoside analogs.[8]

A number of studies have indicated that the uptake and metabolism of tenofovir to its active metabolite PMPApp by immune cells is believed to be a rapid process, giving rise to potentially antiviral levels of PMPApp with intracellular half-lives in the 10 to 50 hour range. The IC_{50} (50% inhibitory concentrations for tenofovir ranges from 0.04 micromoles (µM) to 8.5 µM. Radiolabeled (^3H) tenofovir is rapidly taken up by resting (3 to 4 µM) and activated (1-2 µM) peripheral blood mononuclear cells (PBMC), suggesting that cellular uptake is via endocytosis. Endocytosis is a process of cellular ingestion by which the plasma membrane folds inward to bring substances into the cell.

Subsequent metabolism of tenofovir appears to proceed quickly. The mono-and di-phosphate metabolites of tenofovir accumulate rapidly, reaching approximately 0.1 micromolar and approximately 0.3 micromolar, respectively, at six hours after exposure, whereas the tenofovir is not detectable (within 0.5 to 24 hours). This suggests that upon entry to the cell, tenofovir is rapidly processed to PMPAp and then to PMPApp, facilitating the formation of a barrier to HIV infection. Therefore, the metabolic properties of tenofovir facilitate the rapid formation and

maintenance of a barrier to virus replication that could hypothetically facilitate its deployment for both coital-associated and coital-dissociated microbicide applications.[8]

Given that tenofovir gel may be effective when administered up to two hours prior to exposure and has a long half life, the suggested timing of coitally associated dosing will be a maximum of twice daily, up to two hours prior to coitus.

1.3.4 Safety

Given that Phase I data demonstrates measurable plasma concentrations of tenofovir in some participants[6], participants with CHBV might be at risk for development of tenofovir resistant HBV.[9] Participants with CHBV will be eligible for enrollment; however they will be monitored through HBV viral load, and LFTs.

It is not known what effect tenofovir gel could have on the HIV virus or HIV disease progression in HIV infected participants or their partners. There is a theoretical risk that tenofovir absorbed systemically from tenofovir gel could result in mutations of the HIV virus in participants who become infected with HIV during the study, or their partner, if the partner is infected with HIV. Limited resistance data from the HPTN 050 study show that no new resistance mutations evolved in plasma or cervicovaginal lavage specimens after 14 days of tenofovir gel use. No participant had high level tenofovir mutations (e.g., K65R).[6]

Some of the possible side effects of the study gel are dryness, itching, burning, or pain in the genital area. [6] Refer to Section 8.3 for risks associated with oral tenofovir.

Changes in bone growth and strength were seen in study animals given tenofovir. Tenofovir and its oral form administered in systemic dose toxicology studies to rats, dogs and monkeys at exposures (based on areas under the plasma concentration curve (AUC)) greater than or equal to six fold those observed in humans caused bone toxicity. In monkeys the bone toxicity was diagnosed as osteomalacia. Osteomalacia observed in monkeys appeared to be reversible upon dose reduction or discontinuation of tenofovir. In rats and dogs, the bone toxicity manifested as reduced bone mineral density (BMD). The mechanism(s) underlying bone toxicity is unknown. Bone thinning has been seen in adults and children taking tenofovir. However, systemic absorption of tenofovir from the vaginal gel appears to be minimal, (approximately 1% of the therapeutic oral dose) and the perceived risk of an effect on BMD is low.

1.3.5 Pharmacokinetic Evaluation

The purpose of this PK evaluation is to evaluate the levels of systemic tenofovir observed among women using study gel daily for up to five months.

All women will have phlebotomy at the Week 4, 12 and 20 Visits for PK evaluation of tenofovir in the blood. In order to obtain an assay for one two to six hour timepoint from each participant, participants in the daily dosing arm will be asked to insert their daily study gel dose the morning of the visit, and participants in the coitally dependant arm will be asked to insert a dose the morning of the visit at the Week 20 Visit. PK specimens from participants randomized to the placebo gel arm will not be analyzed.

All PK specimens will be shipped to the HPTN Central Laboratory (HPTN CL) for batched assay. Specimens from each participant receiving daily tenofovir gel from all three PK specimen collections will be evaluated simultaneously at the end of the study.

1.3.6 Colposcopy, Cytokine and Chemokine Testing

Toxicity of the study gel will be evaluated through colposcopic evaluations and by measuring markers of inflammation. Symptoms of genital irritation and product acceptability will be compared to colposcopic findings of epithelial disruption. In addition, cytokine and chemokine levels will be used as surrogate markers of inflammation.

Although an increase in proinflammatory cytokines has been observed in a small human study of N-9, [10] to date, no studies have directly compared genitourinary symptoms, colposcopic epithelial disruption, and proinflammatory cytokines and chemokines. It is anticipated that this study will aid future microbicide studies to more broadly assess toxicity by including cytokines and chemokines as markers of inflammation.

1.3.7 Hepatitis B and Tenofovir

Patients with CHBV have been successfully treated with antiretrovirals which lower Hepatitis B Virus (HBV) viral load and normalize liver enzyme levels. When treatment with antiretrovirals stops, HBV viral load and liver enzyme levels usually rebound.

HBV may develop resistance to antiretrovirals. Over time, the non-resistant viruses decline, but the evolution of resistant HBVs can lead to increased HBV viral load and liver enzyme levels.

1.3.7.1 Hepatitis B Resistance to Tenofovir

The ability of oral tenofovir to alter HBV replication or engender resistance in HBV co-infected patients undergoing oral tenofovir therapy for HIV infection has not been fully determined. However, we hypothesize that intravaginal administration of tenofovir as a microbicide should result in low serum levels. Studies of patients with HIV and HBV co-infection treated with oral adefovir (a nucleotide drug similar to oral tenofovir, inhibiting the HBV DNA polymerase)

have shown that emergence of resistance is a rare event, occurring in 1.6% of 124 CHBV patients at 96 weeks of therapy.[9]

1.3.7.2 Hepatitis B Virus Rebound

While the systemic absorption of tenofovir from the vaginal gel is small (approximately 1% of the oral therapeutic tenofovir dose), based on experience with oral adefovir, acute exacerbations of hepatitis have been reported in patients who have discontinued anti-hepatitis B therapy, including therapy with Hepsera [adefovir dipivoxil].[11,12]

Therefore, hepatic function should be monitored closely in patients who discontinue anti-hepatitis B therapy. Participants with detectable HBV antigenemia will be monitored at various points throughout the study through HBV viral load tests and liver function tests (LFT). They will return at 4, 8 and 12 weeks after study gel use (week 28, 32 and 36 visits) to be monitored for unexpected rebound of HBV levels. If participant agrees, blood specimens will be collected for future resistance testing, these specimens will be collected at baseline, and weeks 12, 24, 28, 32 and 36.

2 STUDY OBJECTIVES AND DESIGN

2.1 Primary Objective

The primary objective of this study is:

- To assess the local and systemic safety of tenofovir 1% gel for vaginal use in HIV uninfected women versus a placebo gel over 24 weeks of daily and coitally dependent use

2.2 Secondary Objective

The secondary objective of this study is:

- To assess the acceptability of, and adherence to tenofovir gel for vaginal use in HIV uninfected women versus placebo gel over 24 weeks of daily or coitally dependent use

2.3 Exploratory Objectives

The exploratory objectives of this study are:

- To measure vaginal flora characteristics, and to descriptively examine changes in these characteristics over the course of prolonged study gel use
- To assess the effects of study gel on cytokine and chemokine expression in cervical secretions
- To evaluate the association between cytokine and chemokine expression
- To correlate cytokine and chemokine expression with colposcopic evidence of inflammation and epithelial disruption

2.4 Study Design

Phase II four arm, three site, randomized, double blind, controlled trial comparing tenofovir 1% vaginal gel used once daily and tenofovir 1% vaginal gel used prior to intercourse to a placebo gel, with 24 weeks of product exposure and follow up. Participants in all four arms will receive condom counseling and free water or silicone base lubricated, non N-9 or spermicide containing, male, latex condoms on an ongoing basis. The study will be conducted at three sites: Pune, India, UAB and BLHC.

Arm	Description	N	Frequency
1	Tenofovir gel coitally dependent use	50	Up to two hours before each act of penile-vaginal sexual intercourse to a maximum of twice daily
2	Placebo gel coitally dependent use	50	Up to two hours before each act of penile-vaginal sexual intercourse to a maximum of twice daily
3	Tenofovir gel daily use	50	Once daily at bedtime or longest period of rest
4	Placebo gel daily use	50	Once daily at bedtime or longest period of rest

2.4.1 Study Visits and Evaluations

Study visits and evaluations are outlined in Section 5 and summarized in Appendix I. Participants will be encouraged (but not required) to bring their male partner(s) with them to any and all study visits, especially Screening and Enrollment visits.

After providing written informed consent, potential participants will be screened for eligibility. During the eligibility screening participants will be asked to respond to demographic questions, and will give contact information. They will undergo HIV and STI counseling and testing (pre-test counseling and preliminary testing at the first visit, test results and post-test counseling provided at a later screening visit). They will also undergo a targeted medical history and behavioral screening, urine pregnancy testing, dipstick urinalysis, hematology, LFTs and renal function parameters (LFT and RFP), and HBV testing; a targeted physical exam, and, pelvic exam with Pap smear. The eligibility screening may take place during one visit or may require more than one visit over more than one day. For participants who are presumptively eligible at screening, an Enrollment Visit will be scheduled to take place within 56 days of screening, and at least two days after the end of participant's given menstrual period. Once all screening procedures are complete, and the participant has been provided her test results and appropriate post-test counseling, the enrollment visit may commence that same day, or at a later date up to 56 days post-screening.

At the Final Screening/Enrollment Visit participants will give updated information on their medical and menstrual history; have HIV/STI counseling and testing and, will be questioned on vaginal product behaviors (for pre-use assessment). They will also undergo urine pregnancy testing and dipstick urinalysis. They will give blood for hematology, LFT and RFP, HSV-2 (for batched assay), and plasma and serum archive. They will undergo a pelvic exam with colposcopy and cervical and vaginal swab collection for Gram stain, and, cytokine and chemokine testing. Vaginal swabs for quantitative assessment of vaginal microflora will be collected only at the US sites since these specimens cannot survive storage for greater than 48 hours. Additionally, participants identified as having CHBV at screening will give blood for HBV viral load testing and, storage for possible future HBV resistance testing. Once all assessments are completed and final eligibility has been confirmed, participants will be randomized into either the once daily or the coitally dependent group, and will receive either tenofovir or placebo gel. They will be provided with:

- Supplies of tenofovir or placebo gel with applicators and, breathable, unscented, non-deodorant, locally available (if possible) panty liners and/or menstrual pads and water or silicone base lubricated, non N-9 or spermicide containing, male, latex condoms for participants' partners

- Instructions for product application

- Instructions to contact the site to report signs or symptoms which are of concern to the participants

Depending on their randomization group, participants will be instructed to insert one dose (the entire contents of one applicator) of study gel into the vagina up to two hours before each act of penile-vaginal sexual intercourse or once daily at bedtime or longest period of rest. Participants in the coitally dependent group will not use study gel more than twice per day.

Follow up visits will be scheduled every four weeks after the Enrollment Visit (based on a 28 day menstrual cycle). After 4, and 12 weeks of study gel use, participants will return for follow up visits. At these visits, participants will give updated information on their medical and menstrual history, and will be interviewed to assess adherence to the study gel use regimen, their sexual practices, study gel acceptability, and to ascertain whether any adverse experiences have occurred. They will receive HIV/STI risk reduction counseling (and post-test counseling, if indicated). They will undergo a urine pregnancy test and have a pelvic examination with colposcopy and cervical and vaginal swab collection for Gram stain, and, cytokine and chemokine testing. Collection of vaginal swabs for quantitative assessment of the vaginal microflora will occur only at the US sites. Participants will also give blood for hematology, PK, LFT and RFP testing.

After 8, 16, and 20 weeks of study gel use, participants will return for follow up visits. At these visits, participants will give updated information on medical and menstrual history, be interviewed to ascertain whether any adverse experiences have occurred, and have HIV/STI risk reduction counseling. They will also undergo urine pregnancy testing.

Additionally at the Week 20 Visit participants in the daily use arm will be asked to insert their daily dose of study gel at least two to six hours prior to their visit, and participants in the coitally dependant arm will be asked to insert one dose of study gel at least two to six hours prior to the visit. A blood draw will be taken for PK testing two to six hours post-dosing.

After 24 weeks of study gel use, participants will stop using study gel, and return to the site for a follow up visit. At this visit participants will give updated information on medical and menstrual history, and will be interviewed to assess adherence to the study gel use regimen, their sexual practices, and to ascertain whether any adverse experiences have occurred. They will receive HIV and STI counseling and testing. They will undergo a urine pregnancy test and dipstick urinalysis, a pelvic exam with colposcopy, and cervical and vaginal swab collection for Gram stain, and, cytokine and chemokine testing. Vaginal swabs for quantitative assessment of the vaginal microflora will occur only at the US sites. Participants will also give blood for hematology, LFT and RFP, and for the plasma and serum archive. During this visit, non-CHBV participants will complete a study burden assessment.

The week 24 visit will be the final visit for non-CHBV participants unless there is an unresolved AE. All unresolved AEs continuing at the end of study participation will be followed until resolution or the AE has stabilized (Refer to Section 6.3).

At follow up visits, participants will be asked about douching practices.

CHBV participants will be monitored for exacerbation of hepatitis B infection. HBV viral load will be measured at Enrollment and at weeks 12, 24, 28, 32 and 36. Blood will also be archived from these time points to allow future characterization of HBV resistance. During these visits, participants will give updated information on medical and menstrual history. RFP will also be evaluated at the weeks 28, 32 and 36 visits. On the final Week 36 Visit, a study burden assessment will be administered.

At any of the study follow up visits, or at any ad hoc visits initiated by participants between scheduled visits, abnormalities noted on pelvic exam or menstrual/medical history will be evaluated and followed according to Appendix II; continued/discontinued study gel use will be guided by Appendix II.

Participants who are found to have an STI or other reproductive tract infection (RTI) will be offered/referred for counseling, treatment, and follow up care in accordance with the Centers for Disease Control and Prevention (CDC) treatment guidelines (based on site specific capacity). Observed single-dose treatment will be provided whenever possible. Participants with STIs will be encouraged to refer their partners for testing and treatment in accordance with local laws if applicable.

3 STUDY POPULATION

A total of 200 sexually active, HIV uninfected women with a normal, lower genital tract (defined by the entry criteria in Sections 3.1 and 3.2 below) will be enrolled in this study. They will be recruited from staff (not supervised by the protocol team staff) and consumers from family planning, STI, postnatal clinics, colleges, and other venues. There are three sites, Pune, India; and, UAB and BLHC in USA. The Pune site will enroll approximately 100 participants, and, the UAB and BLHC sites will enroll 100 participants between the two sites, for a total of 200 participants among all three sites.

Participants will be selected for the study according to the criteria in Sections 3.1 and 3.2 (and the guidelines in Section 3.4). They will be recruited, screened, and enrolled as described in Section 3.3 (and assigned to a study treatment/product/intervention group as described in Section 7.4). Issues related to participant retention and withdrawals from the study are described in Sections 3.5 and 3.6, respectively.

3.1 Inclusion Criteria

Women must meet the following criteria (along with entry criteria to be confirmed at the enrollment visit) determined by participant self report at screening (unless otherwise stated) to be eligible for inclusion in the study:

- be between the ages of 18 and 50 at the time of enrollment as verified according to site standard operating procedure (SOP)
- be willing and able to provide written informed consent (as assessed by a site specific assessment of comprehension) at screening and enrollment
- be in general good health (as determined by the site clinician) at screening and enrollment
- be HIV uninfected (per HPTN HIV Antibody Testing Algorithm, Appendix III) at screening
- have a normal Pap test result or are able to document a normal Pap test result in the 90 days prior to screening

  Note: Pap smears will be reported as per the 2001 Bethesda System and will be presumed normal in the absence of intra-epithelial lesion or malignancy.

- be sexually active, defined as having had penile-vaginal intercourse at least once in the 30 days prior to screening
- be willing to use an effective method of contraception during the study, defined as either a hormonal based method (except vaginal rings); an intrauterine device (IUD) (inserted at least 30 days prior

to enrollment); female sterilization; or sexual activity with a partner who had a vasectomy

- be willing to undergo all study related assessments (clinical and laboratory), including speculum examination, colposcopy, urine testing, and blood draws
- be willing to adhere to follow up schedule as required by the protocol
- be willing to use tenofovir gel or placebo gel as required by the protocol
- agree to not participate in spermicide and/or vaginal microbicides study or any other device or drug study while enrolled in the study
- agree to use study provided water or silicone base lubricated, non N-9 or spermicide containing, male, latex condoms for each act of intercourse
- agree to use study provided panty liners and/or menstrual pads for protection from product leakage, while using the study gel if necessary

3.2 Exclusion Criteria

Women who meet the following criteria determined by participant self report at screening (unless otherwise stated) will be excluded from the study:

- are menopausal or post menopausal at enrollment

Note: Menopause is defined as the cessation of menses of 12 calendar months, unless on long acting progestins.

- have had a hysterectomy
- have a history of adverse reaction to products containing latex
- will use a diaphragm and/or spermicide for contraception
- are taking systemic tenofovir, adefovir or any chronic hepatitis B medications, or plan to while participating in this study
- have a history of adverse reaction to tenofovir and/or adefovir
- have a history of prior participation in this study as indicated by the study site's screening log and participant identification code list

- have a Grade 3 or higher laboratory liver, renal, or hematology abnormality as specified below in accordance with The DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table) Version 1.0, Dec 2004, with the exception of the creatinine level in order to exclude participants with potential early renal disease.

    - have an absolute lymphocyte count less than 500/mm^3
    - have an absolute neutrophil count less than 750/mm^3
    - have Hgb level less than 9 g/deciliter
    - have an decreased platelet count less than 50,000/mm^3
    - have a decreased white blood cell less than 1,500/mm^3
    - have aspartate aminotransferase (AST) level greater than 5.0 times upper limit of normal (x ULN)
    - have alanine transaminase (ALT) level greater than 5.0 x ULN
    - have total bilirubin level greater than 2.5 x ULN
    - have alkaline phosphatase (ALP) level greater than 5.0 x ULN
    - have a creatinine level greater than 1.25 X ULN

    Normal laboratory values are based on site specific local laboratory (LL) normal reference ranges.

    Note: CHBV will be defined as a positive hepatitis B surface Antigen (HBsAg) test. CHBV participants will not be excluded from the study. However their status will be captured through hepatitis B screening (at study entry and exit and where clinically indicated), HBsAg, LFTs, HBV viral load tests, and significant medical background history. Blood specimens will be collected for possible future testing for HBV resistance to tenofovir gel.

- have had a gynecological surgical procedure in the 90 days prior to enrollment

- are pregnant (based on urine pregnancy test at Screening and Enrollment)

- is within 90 days of last pregnancy outcome at enrollment

- have a pelvic exam finding by either naked eye and/or colposcopic inspection or palpation involving deep epithelial disruption, and/or generalized erythema, and/or severe edema as defined in Appendix II at screening or enrollment

Note: Otherwise eligible participants with exclusionary pelvic exam finding(s) may be enrolled and randomized after the

exclusionary finding(s) have resolved. If resolution is documented within 56 days of providing informed consent for screening, the participant may be enrolled without being rescreened.

- have an STI or RTI according to CDC guidelines, via laboratory tests or on examination, and requiring treatment at screening or enrollment including:
  - symptomatic BV
  - symptomatic candidiasis
  - trichomoniasis
  - chlamydia (CT)
  - gonorrhea (GC)
  - syphilis diagnosed by positive rapid plasma reagin (RPR) positive , confirmed by *Treponema pallidum* hemagglutination (TPHA)
  - Herpes simplex virus type 1 (HSV-1)and/or Herpes simplex virus type 2 (HSV-2) (active lesions)
  - chanchroid
  - pelvic inflammatory disease (PID)
  - cervical or vaginal warts

  Note: women with genital warts that are located exterior to the labia minora (i.e. labia majora, mons) will not be excluded.

  - genital sores or ulcers
  - vaginitis
  - cervicitis

  Note: women diagnosed with an STI during screening or in the process of enrollment will be referred/offered for treatment based on site-specific capability in accordance with CDC guidelines. These participants will be eligible for enrollment once they have completed treatment(s) and are asymptomatic for the STI(s). CDC guidelines are available at:
  http://www.cdc.gov/STD/treatment

  Note: Signs of asymptomatic BV may include the presence of white to grey homogeneous discharge, positive whiff test (amine odor) with addition of potassium hydroxide (KOH), pH greater than 4.5, presence of clue cells, a decrease in lactobacilli morphotypes, and increase in non-lactobacilli morphotypes. Women with clinical criteria or evidence of BV and with symptoms (symptomatic discharge, odor, itching) will be excluded. Women without symptoms, but with clinical or laboratory evidence of BV, are still eligible.

- have injected non-therapeutic drugs intravenously in the 12 calendar months prior to enrollment

- have participated in any other spermicide and/or vaginal microbicide study or any device or drug study 30 days prior to enrollment

- have a history of vaginal intercourse more than an average of two times per day in the two weeks prior to screening

- are breastfeeding at screening or enrollment

- have any other condition that, in the opinion of the site investigator, would preclude provision of informed consent, make participation in the study unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the study objectives

3.3 Recruitment Process

The study will be conducted at Pune, India, and, BLHC and UAB in the US.

Pune, India
Study participants will be recruited mainly from three communities which are part of NARI's community contact program. NARI is collaborating with 3 Non-Governmental Organizations (NGOs), specifically Deepagriha, John Paul Slum Development Program, and Jagruti. The peers working under these NGOs will be given study specific information for participant information. Peers regularly arrange community meetings with men and women which are attended by NARI community educators. Information about the study will be given during these group meetings, and willing and eligible participants will be referred to the study clinic.

Participants will also be enrolled from other sources such as NARI peripheral clinics, and other communities. Specific ideas about recruitment venues and strategies will be sought from local CABs.

IRB approved informational material, such as flyers and booklets, will be developed for community awareness.

UAB, USA
Women will be recruited from UAB campus staff (except staff supervised by HPTN 059 study staff), members of Community Based Organizations (CBOs), student populations from other area universities, colleges, clinics, and public gathering places that predominantly serve women.

Recruitment will also be conducted via outreach by project staff, and using IRB approved flyers on public kiosks, UAB campus newspaper ads and local radio ads if needed. Women who have responded to previous recruitment efforts or participated in

previous studies and meet eligibility requirements will be contacted by phone or by mail with their prior permission. Recruitment information may be sent to medical staff and case managers throughout the medical center as well other local hospitals and clinical practices.

Study staff will give talks on microbicides (e.g., medical grand rounds) and videos may be shown at CBO meetings to increase awareness about microbicide research. More specific recruitment flyers will be handed out at these talks. Specific ideas about recruitment venues and strategies will be sought from local Community Advisory Boards (CAB).

The study will also be listed with the US Department of Health and Human Services (DHHS) AIDS Clinical Trials Service, which is accessible via phone (1-800-TRIALS), and on the internet at www.actis.org. It will be listed with local websites when feasible.

BLHC, USA
Women will be recruited from staff (except staff supervised by HPTN 059 study staff), and members of community-based organizations, student populations from area universities, colleges, clinics, and public gathering places that predominantly serve women.

Recruitment will also be conducted via outreach by project staff, using IRB approved flyers on public kiosks and local newspaper ads if needed. Women who have responded to previous recruitment efforts or from previous studies who may meet eligibility requirements will be contacted by phone or by mail with their prior permission. Recruitment information may be sent to medical staff and case managers at local hospitals and clinical practices to give to provide to women who may be eligible.

Study staff will give talks on microbicides (e.g., medical grand rounds) and videos may be shown at Community Based Organizations (CBO) meetings to increase awareness about microbicide research and more specific recruitment flyers will be handed out at these talks. Specific ideas about recruitment venues and strategies will be sought from local Community Advisory Boards (CAB).

The study will also be listed with the US Department of Health and Human Services (DHHS) AIDS Clinical Trials Service, which is accessible via phone (1-800-TRIALS), and on the internet at www.actis.org. It will be listed with local websites when feasible.

3.4 Co-Enrollment Guidelines

Participants will be instructed not to participate in any other spermicide/vaginal microbicide study, or any device or drug study 30 days prior to enrollment and throughout study.

3.5 Participant Retention

With the consent of the participant, clinic staff will obtain contact information from people who would be expected to know the whereabouts of the participant enrolled in

this study. The need to attend all scheduled follow up visits must be emphasized to each study participant at every visit. If a participant misses a scheduled study visit, the study site staff will try to establish communication with the participant through all possible means (e.g., telephone, field contact, and writing), without breaching the participant's confidentiality. Study site staff is responsible for developing and implementing site-specific SOPs to achieve complete follow up.

Once participants are enrolled in this study, the study site staff will make every effort to ensure the participation of non-CHBV participants for 24 weeks of follow up, and the participation of CHBV participants for 36 weeks of follow up, in order to minimize possible bias associated with loss-to-follow-up. Each site will establish participant retention procedures to target an average retention rate of 95% at 24 weeks. Study site staff at each site is responsible for developing and implementing site-specific SOPs to target this goal. Suggestions for such procedures include:

- Thorough explanation of the study visit schedule and procedural requirements during the informed consent process, and re-emphasis at each study visit.

- Thorough explanation of the importance of all four study treatment groups to the overall success of the study.

- Collection of detailed locator information at the study Screening Visit, and active review and updating of this information at each subsequent visit.

- Use of mapping techniques to establish the location of participant residences and other locator venues.

- Use of appropriate and timely visit reminder mechanisms.

- Immediate and multifaceted follow up on missed visits.

- Mobilization of trained outreach workers or "tracers" to complete in-person contact with participants at their homes and/or other community locations.

- Regular communication with the study community at large to increase awareness about HIV/ Acquired Immunodeficiency Syndrome (AIDS) and explain the purpose of HIV prevention research and the importance of completing research study visits.

- Encouraging but not requiring partner involvement at all follow up visits.

3.6 Participant Withdrawal

Regardless of the participant retention methods just described, participants may voluntarily withdraw from the study for any reason at any time. The site investigator also may withdraw participants from the study in order to protect their safety and/or if they are unwilling or unable to comply with required study procedures after consultation with the Protocol Chair, DAIDS Medical Officer, Statistical and Data Management Center (SDMC) Protocol Statistician, and HPTN CORE Protocol Specialist.

Participants withdrawn from the study will be accounted for statistically as described in Section 7.6.1.

Unless they withdraw their consent, participants who are randomized into the study but voluntarily discontinue study gel use, those who use study gel but not as advised by study staff, those who discontinue study gel use as advised by the study staff, those who inject non-therapeutic drugs intravenously once enrolled, or those who become pregnant will not routinely be withdrawn from the study. Rather, every effort will be made to complete the regularly scheduled safety evaluations, as described in Section 5.9.

Participants who become pregnant during the study will discontinue product use while they are pregnant; however they will remain in follow up and may resume product use after birth or pregnancy termination, as evidenced by a negative pregnancy test and normal pelvic exam performed by the study staff.

Participants also may be withdrawn if the study sponsor, government or regulatory authorities, or site Institutional Review Board/Ethics Committee (IRB/EC) terminate the study prior to its planned end date.

Every reasonable effort will be made to complete a final evaluation (as described in Sections 5.5 and 5.6), of participants without chronic HBV infection (non-CHBV) who terminate from the study prior to week 24, and for CHBV participants who terminate from the study prior to week 36. Study staff will record the reason(s) for all withdrawals from the study in participants' study records.

3.7 Male Involvement

Participants will be encouraged (but not required) to bring their male partner(s) with them to any and all study visits, especially Screening and Enrollment visits. Male partners will be provided information on all study related visit procedures. Male partners may also be contacted at their residence by trained study staff to give study-specific information if the male partner of the participant is willing.

4 STUDY TREATMENT, PRODUCT, AND INTERVENTION

Tenofovir gel is an investigational drug and consideration should always be given to measures that minimize contact during handling, preparation and disposal procedures.

4.1 Drug Formulation

4.1.1 Tenofovir Gel

Tenofovir gel is a clear, transparent, viscous gel packaged in epoxy inner-lined aluminum tubes with a white polyethylene screw cap equipped with a puncture tip. Each tube contains six grams of 1% tenofovir gel (weight/weight) formulated in purified water with edetate disodium, citric acid, glycerin, methylparaben, propylparaben, hydroxyethylcellulose, and pH adjusted to four to five. The study gel is inserted with a polyethylene applicator capable of administering a four-gram (equal to four mL) dose of gel.

Tenofovir gel must be stored at controlled room temperature at all times. Controlled room temperature is defined as 25 °C (77°F). Excursions are permitted to 15°C to 30°C (59°F to 86°F).

4.1.2 Placebo

The placebo for this study is the carrier vehicle gel, which is identical to tenofovir gel without the tenofovir component. The placebo gel will be supplied in identically packaged tubes with applicators as the tenofovir gel.

Placebo gel must be stored at controlled room temperature at all times. Controlled room temperature is defined as 25 °C (77°F). Excursions are permitted to 15°C to 30°C (59°F to 86°F).

4.2 Usage Regimen

Study participants will receive supplies of tenofovir or placebo gel from the study site for use during the study period. All participants will be instructed to start using tenofovir or placebo gel at the time of enrollment and continue using the study gel for 24 weeks. Frequency of use will be determined once the participant has been randomized to a specific arm: coitally dependent or daily use.

Arm	Description	N	Frequency
1	Tenofovir gel coitally dependent use	50	Up to two hours before each act of penile-vaginal sexual intercourse to a maximum of twice daily
2	Placebo gel coitally dependent use	50	Up to two hours before each act of penile-vaginal sexual intercourse to a maximum of twice daily
3	Tenofovir gel daily use	50	Once daily at bedtime or longest period of rest
4	Placebo gel daily use	50	Once daily at bedtime or longest period of rest

Staff will review and provide instruction for applicator use as needed. Detailed written and oral instructions for the use of either tenofovir or placebo gel according to the protocol will be provided to the participant.

4.2.1 Arms 1 and 2

Participants will be instructed to insert one dose (the entire contents of one applicator) of study gel into the vagina up to two hours before each act of penile-vaginal sexual intercourse. The study gel can be inserted a maximum of twice per day.

4.2.2 Arms 3 and 4

Participants will be instructed to insert one dose (the entire contents of one applicator) of study gel into the vagina once daily (before bedtime or longest period of rest).

4.3 Study Product Packaging, Labeling, and Study Related Product Supply

The current Investigational new drug application (IND) for tenofovir gel is held by DAIDS. Tenofovir gel, placebo and applicators for this study will be provided by Gilead Sciences, Inc.

Study gel (active or placebo) will be packaged in cartons containing 10 gel tubes and 10 individually wrapped plastic applicators. These study gel cartons will be labeled and securely sealed with tamper-evident tape. A removable portion of the study gel carton will contain unblinded information regarding the identity of the study gel. The Study Pharmacist will remove all unblinded information and record it appropriately, according to instructions in the NIAID Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks (dated September 2002), and, as directed in writing by the HPTN 059 Protocol Pharmacist, prior to dispensation.

Study Pharmacists will order and receive study gel cartons from the National Institute of Allergy and Infectious Diseases (NIAID) Clinical Research Product Management Center (CRPMC).

Sites will be responsible for obtaining breathable, unscented, non-deodorant panty liners for distribution to the participants, and, water or silicone base lubricated, non N-9 spermicide containing, male, latex condoms, as described in Section 4.3.2, to distribute to participants throughout the study .

4.3.1 Tenofovir gel, Placebo gel and Applicators

Gilead will manufacture tenofovir gel and placebo gel under good manufacturing practice conditions. Both products will be provided in identically packaged tubes with applicators.

4.3.2 Condoms, Panty Liners and/or Menstrual Pads

Water or silicone base lubricated, non N-9 or spermicide containing, male, latex, condoms, and, breathable, unscented, non-deodorant locally available panty liners and/or menstrual pads will be provided by the site for all participants. Male condoms will be promoted for use for all sexual encounters during the study period.

A list of condoms that have been tested to be compatible with tenofovir and the placebo gel will be provided to the study sites.

Panty liners and/or menstrual pads will be provided by the site at enrollment and at each follow up visit during study gel use and upon request from the participant, by the study site coordinator or designee. These supplies are provided to participants to assist with product leakage during the study.

Participants will be asked to use study provided menstrual pads and/or panty liners if needed for hygienic purposes and to protect against product leakage. To avoid potential confounding of AE data (e.g., abrasions, changes in vaginal microflora, and allergic reactions to product components) that may be related to menstrual hygiene products (except tampons), participants will be asked to report if they have used any products other than the products provided by the study site staff.

Use of the participant's preferred brand of tampons is acceptable, and is not expected to confound AE data.

4.4 Product Accountability

The Study pharmacists must maintain complete records of all study gel cartons received from the CRPMC. Additional documentation will also be required by the Pharmacists for study gel re-supply, transfers, chain of custody, returns, destruction (if applicable) and other related issues as outlined in the NIAID Pharmacy Guidelines and instructions for DAIDS Clinical Trials Network (dated September 2002), and as directed in writing by the HPTN 059 Protocol Pharmacist.

4.5 Adherence

Data related to adherence will be collected through the administration of a structured interview including open-ended response options at the Week 4, 12 and 24 visits. The interviewer will ask questions about:

- Condom use
- Frequency of intercourse, product and condom use since the last visit.
- Detailed questions about last sexual act with study gel and last sexual act without study gel, including:
    - Use of product, and when product was used in relation to sexual intercourse, or period of longest rest
    - Use of condoms or other birth control methods
    - Type of partner

Douching history and any ongoing douching use will be captured on the Case Report Forms (CRF) at Weeks 4, 12 and 24.

4.5.1 Adherence Counseling

Adherence counseling will be provided to study participants assigned to the four study gel groups upon enrollment into the study, and as needed thereafter to help ensure high rates of study gel use. Counseling will be provided in accordance with standard study methods that will address such topics as client-centered strategies to remember to use the study gel for each episode of vaginal intercourse or daily (depending on group they are randomized to); to ensure the availability of the study gel both in the home and away from home; and to negotiate study gel use with "primary" and "non-primary"' partners. Counseling also will include reminders to contact study staff with questions about study gel use and requests for additional supplies. For participants who have adherence problems, every effort will be made to identify adherence strategies to increase their rates of study gel use throughout the course of the study.

Participants will be counseled to avoid douching or cleaning the vagina as this will alter exposure to study gel. They also will be instructed to:

- Only use the study gels vaginally
- Not douche or otherwise clean the vagina, or insert other vaginal products, within two hours before and two hours after using study gel (menstruating participants are allowed to use tampons as needed)
- Not use other participants' study gel
- Not distribute their study gel to other women

Note: Participant behaviors regarding condom and study gel use data will be collected via standardized interviewer-administered questionnaires developed by the Protocol Team in conjunction with study site staff and community representatives, to maximize the accuracy of self-reported data. In order to minimize "socially-desirable" reporting, these questionnaires will be administered prior to the delivery of HIV/STI risk reduction and adherence counseling.

4.5.2 Adherence Assessment

Data on adherence to the study gel use regimen will be collected at the Weeks 4, 12, and 24 visits via interviewer-administered questionnaires. These

questionnaires will ascertain participants' frequency of sexual intercourse, condom use, study gel use, and stated reasons for non-use. They will be administered prior to the delivery of HIV/STI risk reduction and adherence counseling. The HPTN Study Monitoring Committee (SMC) will monitor adherence rates over time, and adherence counseling methods will be updated if needed to address lower-than expected rates.

4.6 Acceptability Assessment

A vaginal product behavior assessment will be administered at the Enrollment Visit. Product acceptability will be assessed at Weeks 4, 12 and 24.

The acceptability instrument will collect information on the following categories:

- Participant's reactions to the study gel – likes and dislikes
- Participant's perception of her primary partner's reactions to the study gel – likes and dislikes
- Participant's perception of her non-primary partner (if applicable) reactions to the study gel – likes and dislikes
- Study gel impact on sexual encounter
- Ease or difficulty of use

The final acceptability assessment will be conducted at the week 24 Visit for all participants, or, at trial discontinuation (which ever comes first). The final assessment will include all the questions on the standard instrument, with some additional questions to probe further on issues that emerge as salient during the course of the trial.

4.7 Concomitant Medications

Any concomitant medications will be permitted for the participant with the exception of those not permitted under criteria for inclusion and exclusion. All concomitant medications will be reported on the study participants' clinical records and recorded on the CRFs for all medications received as of the first Screening visit, and throughout the study. In addition to prescribed and over-the-counter medications, all vitamins, herbal remedies, and other traditional preparations will be recorded on the CRFs as well. Alcohol and recreational or street drug use will be recorded in clinical progress notes if needed for interpretation/documentation of observed participant health status. Medication used for the treatment of AEs that occur during study participation also will be recorded on applicable CRFs.

To protect the integrity of the lower genital tract and reduce the possibility of AEs due to agents other than the study gel and applicator, any spermicides, diaphragms, or contraceptive vaginal rings should not be used during this study. Participants who report current use of these contraceptives during screening or while enrolled in the study will be referred to family planning services for counseling and, provision of alternative contraceptives. Participants will not be terminated from the study if they report current use of these contraceptives methods or devices while enrolled in the study. Participants

will be encouraged to avoid use of vaginally-applied or inserted medications/preparations two hours before and two hours after having vaginal intercourse.

4.8 Toxicity Management

In response to AEs reported by study participants and/or observed upon exam by study staff, the study site investigator or designee will recommend either continuation or holding study gel use consistent with the criteria in Appendix II. Study gel use also will be held or discontinued in the event of a expedited adverse event (EAE) that is judged by the site Investigator or designee to be definitely, probably, possibly, or probably not related to the study gel or applicator.

Unless the participant withdraws her consent, she will remain in the study to complete the safety evaluations (unless clinically contraindicated) according to Appendix I, and/or as specified in Appendix II.

4.9 Procedures to be Followed in the Event of Pregnancy

All participants will be instructed to report pregnancies to site investigator or to the study staff who will in turn report to the site investigator; the site investigator will inform the Protocol Safety Review Team (PSRT - the Protocol Chair and Co-Chairs, study site Investigators, NIAID Medical/Program Officer, a manufacturer representative CORE Protocol Specialist, SDMC Biostatistician, and SDMC Project Manager or their designees). The site Investigator will counsel the participant and discuss possible risks if the pregnancy is continued according to site-specific SOPs.

Sites will provide water or silicone base lubricated, non N-9 or spermicide containing, male, latex condoms, and, facilitate participants' access to all contraceptive methods. However, in the event of pregnancy, sites will counsel participants and will facilitate access to services, according to the site-specific SOPs.

Participants who become pregnant during the course of the study will discontinue study gel use while they are pregnant, but will not routinely be withdrawn from the study. Rather, if the participant does not withdraw her consent, every effort will be made to complete the safety evaluations according to Appendix I, and/or as specified in Appendix II, and will follow the modified study procedures described in Section 5.9.2, until their study exit date or their pregnancy outcome is ascertained, whichever is longer.

Participants who become pregnant may resume product use after giving birth or pregnancy termination, as evidenced by a negative pregnancy test and normal pelvic exam performed by study staff.

Refer to Section 5.10 on Final Contact procedures for participants who become pregnant during the study.

Refer to Section 6.4.1 regarding the reporting of pregnancy outcomes.

5 STUDY PROCEDURES

An overview of the study visit and evaluations schedule is presented in Appendix I. Presented below is additional information on visit-specific study procedures. A detailed instruction guide to standardize all study procedures across sites will be provided in the study-specific procedures manual.

5.1 Screening Visit (up to Day –56)

After providing written informed consent, potential participants may be screened for eligibility over two or more visits if necessary, and eligibility must be confirmed at the enrollment visit. All evaluations will be completed in a step-wise manner for potential participants who meet the study eligibility criteria. For participants who do not meet the eligibility criteria, screening will be discontinued when ineligibility is determined.

For participants who are found to be presumptively eligible based on the evaluations listed below at these visits, final eligibility will be confirmed at Enrollment Visit scheduled to take place within 56 days of the initial Screening Visit.

5.1.1 Clinical Procedures

- assign participant ID
- explain study requirements to the participant
- administer comprehension test according to site SOPs
- obtain written informed consent(s)
- collect demographic and locator information
- obtain behavioral eligibility information
- provide HIV/STI pretest, and, risk reduction counseling
- collect medical and menstrual history
- provide counseling on contraceptive options
- perform targeted physical exam
- collect urine for pregnancy testing; GC and CT testing; dipstick urinalysis for protein, glucose and blood; urine microscopy and culture if dipstick is positive for leukocyte esterase or nitrates
- perform pelvic examination including:
    - naked eye exam of external genitalia
    - speculum exam of vagina and cervix
    - collection of pH sample from the vaginal wall
    - collection of swab specimen from the lateral vaginal wall for:
        - two dried smears (Gram stain assessment at the HPTN CL)
        - wet mount for candidiasis, trichomoniasis, and clue cells

- collect genital ulcer swab for multiplex polymerase chain reaction (PCR) (at the HPTN CL) if ulcer or other anogenital finding thought to be herpetic identified
- collection of ecto-and endocervical specimen for Pap smear (unless documentation of a normal Pap smear test result in the 90 days prior to screening is available and provided)

- when clinically indicated:
  - offer/refer for STI treatment
- collect blood for HBsAg test, HIV serology, syphilis serology, HSV-2 serology, complete blood count (CBC), LFT, [ALP, AST, gamma glutamyl transferase (GGT), ALT, total bilirubin], RFP, [blood urea nitrogen, creatinine)]
- provide test results when available, with associated post-test counseling

5.1.2 Laboratory Procedures

- perform urine pregnancy test
- perform urine nucleic acid amplification testing (NAAT) for GC and CT
- record results of dipstick urinalysis for protein, glucose and blood (perform microscopy and culture if dipstick is positive for leukocyte esterase or nitrates)
- perform enzyme immunoassay/western blot (EIA/WB) test for HIV
- perform syphilis, HBsAg
- prepare specimen for HSV-2 serology
- perform CBC, LFT and RFP

5.2 Final Screening/Enrollment Visit and Baseline Evaluations (Day 0)

Participants who are found to be presumptively eligible during the screening process will complete an Enrollment Visit post menses, and within 56 days after the initial Screening Visit. Participants who do not complete an Enrollment Visit within 56 days of screening must repeat the entire screening process.

All participants will receive their screening test results at their Enrollment Visit (if not previously received). For those whose test results meet the study eligibility criteria, the procedures below will be undertaken in a stepwise manner to confirm eligibility. As was the case at the Screening Visit, procedures will be discontinued if ineligibility is determined at this visit.

5.2.1 Clinical Procedures

- administer comprehension test according to site SOPs
- obtain written informed consent(s)

  Note: Consent for storage of specimens for future research may be obtained prior to or after enrollment, but must be obtained prior to collection of specimens

- update locator information

To determine enrollment eligibility:

- update medical and menstrual history
- collect urine for pregnancy testing; dipstick urinalysis for protein, glucose and blood; urine microscopy and culture if dipstick is positive for leukocyte esterase or nitrates
- perform pelvic exam including:
    - naked eye exam of external genitalia
    - speculum exam of vagina and cervix
    - collection of pH sample from the vaginal wall
    - collection of swab specimen from the lateral vaginal wall for wet mount for candidiasis, trichomoniasis, and clue cells
    - colposcopic examination

  Additionally for baseline evaluations:
    - collection of two cervical swabs for cytokine and chemokine testing (for analysis at HPTN CL)
    - collection of one cervical swab for one dried smear for detection of neutrophils (Gram stain assessment at the HPTN CL)
    - collection of vaginal swab for quantitative vaginal culture (for assessment at the HPTN CL from US sites only)
    - collection of swab specimen from the lateral vaginal wall for two dried smears (Gram stain assessment at the HPTN CL)

Baseline evaluations:

- provide HIV/STI pre-test, and, risk reduction counseling
- provide screening test results (if not previously provided), and associated post-test counseling
- collect urine for GC and CT testing
- collect blood for HBsAg, HIV serology, HSV-2 serology, syphilis serology, plasma and serum archive, CBC, LFT and RFP collect blood specimen for HBV viral load (CHBV participants only, for analysis at the HPTN CL)

- collect blood for storage of specimen for possible future HBV resistance testing *if participants have provided written informed consent to have specimens stored for future research* (CHBV participants only)
- administer vaginal product behavior assessment
- provide study gel and instructions for use (with panty liners and/or menstrual pads, and condoms)
- when clinically indicated:
    - offer/refer for STI treatment
- provide test results when available, with associated post-test counseling

5.2.2 Laboratory Procedures

- perform urine pregnancy test
- perform urine NAAT for GC and CT
- record results of dipstick urinalysis for protein, glucose and blood (perform microscopy and culture if dipstick is positive for leukocyte esterase or nitrates)
- prepare quantitative vaginal specimen for shipment to HPTN CL (US sites only)
- prepare HBV viral load for analysis at the HPTN CL (if applicable)
- perform EIA/WB test for HIV and syphilis serology
- prepare blood specimens for HSV-2 serology, and, plasma and serum archive
- perform CBC, LFT, RFP and HBsAg
- prepare specimen to archive for possible future HBV resistance testing (if participant has consented to specimen storage)

Note for all follow up visits: All follow up visits should be scheduled, ideally, on dates (within the visit window) when the participant is not on her menses. If a study visit does occur during the participant's menses, all visit procedures except the pelvic exam, colposcopy, and associated specimen collections (if clinically indicated), should be performed at that time. If indicated, the pelvic exam, colposcopy, and associated specimen collections required for the given visit will be rescheduled for a date as soon as practical (preferably within the visit windows) after the end of participant's menses.

5.3 Weeks 4, 12 Visits
(Week 4: target day 28, allowable Day 21 – 35)
(Week 12: target day 84, allowable Day 77 – 91)

5.3.1 Clinical Procedures

- update locator information
- review study requirements with participant
- administer adherence and acceptability assessments

- provide HIV/STI pre-test, and, risk reduction counseling
- provide test results from previous visit (if not previously provided) with associated post-test counseling
- update medical and menstrual history
- collect urine for pregnancy testing
- collect blood for CBC, LFT and RFP
- collect blood for PK (for analysis at the HPTN CL)
- collect blood for HBV for possible future resistance testing (CHBV participants at week 12 only for analysis at the HPTN CL)
- collect blood for HBV viral load (CHBV participants at week 12 only)
- perform pelvic exam including:
    - naked eye exam of external genitalia
    - speculum exam of vagina and cervix
    - colposcopic examination
    - collection of pH sample from the vaginal wall
    - collection of two cervical swabs for cytokine and chemokine testing (for analysis at HPTN CL)
    - collection of one cervical swab for one dried smear for detection of neutrophils (Gram stain assessment at the HPTN CL)
    - collection of vaginal swab for quantitative vaginal culture (for assessment at the HPTN CL for US sites only)
    - collection of swab specimen from the lateral vaginal wall for two dried smears (Gram stain assessment at the HPTN CL)
    - collection of swab specimen from the lateral vaginal wall for wet mount for candidiasis, trichomoniasis, and clue cells
    - collection of swab specimen from the lateral vaginal wall for two dried smears (Gram stain assessment at the HPTN CL)
    - collect genital ulcer swab for multiplex PCR (at the HPTN CL) if ulcer or other anogenital finding thought to be herpetic identified
- provide study gel and instructions for use (with panty liners and/or menstrual pads, and condoms)
- when clinically indicated only (not to be done routinely, specific elements and tests on lists below can be conducted based on clinical judgment. Other tests can be done as needed):
    - collect urine for GC, CT, dipstick urinalysis for protein, glucose, and blood; urine microscopy and culture if dipstick is positive for leukocyte esterase or nitrates
    - collect blood for HIV serology, syphilis serology, HSV-2 serology
    - collect blood for plasma and serum archive
    - offer/refer for STI treatment

- provide test results when available, with associated post-test counseling

5.3.2 Laboratory Procedures

- perform urine pregnancy test
- perform CBC, LFT and RFP
- prepare specimen for PK analysis (at the HPTN CL)
- prepare HBV viral load for analysis (at the HPTN CL at Week 12 only for CHVB participants)
- prepare specimen to archive for possible future HBV resistance testing at Week 12 only (if participant has consented to specimen storage)
- when clinically indicated only, perform the following procedures:
    - perform EIA/WB test for HIV
    - perform syphilis and HBsAg
    - prepare specimen for HSV-2 serology
    - perform HBV viral load (if applicable)

5.4 Weeks 8, 16, 20 Visits
(Week 8: target day 56, allowable Day 49 - 63)
(Week 16: target day 112, allowable Day 105 – 119)
(Week 20: targeted day 140, allowable Day 133 – 147)

5.4.1 Clinical Procedures

- update locator information
- review study requirements with participant
- provide HIV/STI risk reduction counseling
- provide test results from previous visit (if not previously provided), with associated post-test counseling
- update medical and menstrual history
- collect urine for pregnancy testing
- provide study gel and instructions for use (with panty liners and/or menstrual pads, and condoms)
- when clinically indicated only (not to be done routinely, specific elements and tests on lists below can be conducted based on clinical judgment. Other tests can be done as needed):
    - provide HIV/STI pre-test counseling
    - collect urine for dipstick urinalysis for protein, glucose and blood; and, urine microscopy and culture if dipstick is positive for leukocyte esterase or nitrates
    - collect urine for GC and CT
    - collect blood for HIV serology, HBsAg, syphilis serology, HSV-2 serology, CBC, LFT and RFP
    - collect blood for plasma and serum archive
    - perform pelvic exam including:

        - naked eye exam of external genitalia
        - speculum exam of vagina and cervix
        - collection of pH sample from the vaginal wall
        - collect genital ulcer swab for multiplex PCR (at the HPTN CL) if ulcer or other anogenital finding thought to be herpetic identified
        - colposcopic examination
        - collection of swab specimen from the lateral vaginal wall for:
            - two dried smears (Gram stain assessment at the HPTN CL)
            - wet mount for candidiasis, trichomoniasis, and clue cells
    - offer/refer for STI treatment
- provide test results when available, with associated post-test counseling

Additionally at the Week 20 Visit only:

- collect blood for PK (for analysis at the HPTN CL)

  Note: Participants will be instructed to apply the study gel between two and six hours prior to the Week 20 Visit

5.4.2 Laboratory Procedures

- perform urine pregnancy test
- prepare Week 20 blood specimen for PK analysis (at the HPTN CL)
- when clinically indicated only, perform the following procedures:
    - perform urine NAAT for GC and CT
    - record results of dipstick urinalysis for protein, glucose and blood (perform microscopy and culture if dipstick is positive for leukocyte esterase or nitrates)
    - perform EIA/WB test for HIV
    - perform syphilis and HBsAg
    - prepare specimen for HSV-2 serology
    - perform CBC, LFT and RFP

5.5 Week 24/Early Termination Visit (target day 168, allowable Day 161 – 175)

5.5.1 Clinical Procedures

- update locator information
- review study requirements with participant
- administer adherence and acceptability assessment
- provide HIV/STI pre-test, and, risk reduction counseling

- provide test results from previous visit when available with associated post-test counseling (if not previously provided)
- update medical and menstrual history
- collect urine for pregnancy testing; GC; CT; dipstick urinalysis for protein, glucose and blood; urine microscopy and culture if dipstick is positive for leukocyte esterase or nitrates
- collect blood for HIV serology, HBsAg, syphilis serology, HSV-2 serology, plasma and serum archive, CBC, LFT and RFP
- collect blood for HBV for possible future resistance testing (CHBV participants only)
- collect blood specimen for HBV viral load (CHBV participants only for analysis at the HPTN CL)
- perform pelvic exam including:
    - naked eye exam of external genitalia
    - speculum exam of vagina and cervix
    - colposcopic examination
    - collection of pH sample from the vaginal wall
    - collection of cervical swab for cytokine and chemokine testing (for analysis at HPTN CL)
    - collection of one cervical swab for one dried smear for detection of neutrophils (Gram stain assessment at the HPTN CL)
    - collection of vaginal swab for quantitative vaginal culture (for assessment at the HPTN CL for US sites only)
    - collection of swab specimen from the lateral vaginal wall for two dried smears (Gram stain assessment at the HPTN CL)
    - collection of swab specimen from the lateral vaginal wall for wet mount for BV, candidiasis, trichomoniasis and clue cells
    - collect genital ulcer swab for multiplex PCR (at the HPTN CL), if ulcer or other anogenital finding thought to be herpetic identified
- when clinically indicated only:
    - offer/refer for STI treatment
- provide test results when available, with associated post-test counseling

5.5.1.1 Clinical Procedures (Non-CHBV participants only)

Non-CHBV participants will have all procedures listed above and:

- administer study burden assessment

5.5.2 Laboratory Procedures

- perform urine pregnancy test
- perform urine NAAT for GC and CT
- record results of dipstick urinalysis protein, glucose and blood (perform microscopy and culture if dipstick is positive for leukocyte esterase or nitrates)
- perform EIA/WB test for HIV
- perform syphilis and HBsAg
- prepare specimen for HSV-2 serology
- prepare specimens for blood for plasma and serum archive
- perform CBC, LFT and RFP
- prepare HBV viral load for analysis (at the HPTN CL CHBV participants only)
- prepare specimen to archive for possible future HBV resistance testing (if participant has consented to specimen storage)

5.6 Weeks 28, 32 and 36 Visits (After completion of study gel use - CHBV Participants Only) (target day 196, allowable Day 189 – 203) (target day 224, allowable Day 217 – 231) (target day 252, allowable Day 245 – 259)

5.6.1 Clinical Procedures

- update locator information
- review study requirements with participant
- update medical and menstrual history
- provide HIV/STI pre-test, and risk reduction counseling
- provide test results from previous visit (if not previously provided) when available with associated post-test counseling
- collect blood for HBV viral load and resistance testing
- collect blood for HBV serum archive
- collect blood for LFT
- administer study burden assessment (Week 36 visit only)

5.6.2 Laboratory Procedures

- perform LFT
- prepare HBV viral load (for analysis at the HPTN CL)
- prepare specimen to archive for possible future HBV resistance testing

5.7 Interim Contacts and Visits

Interim contacts and visits (those between regularly scheduled follow up visits) may be performed at participant request or as deemed necessary by the Investigator at any time during the study. Participants will have a urine pregnancy test at each interim visit. All interim contacts and visits will be documented in participants' study records and on applicable case report forms.

Some interim visits may occur for administrative reasons. For example, the participant may have questions for study staff or require additional study supplies. Other interim contacts and visits may occur in response to AEs experienced by study participants. When interim contacts or visits are completed in response to participant reports of AEs, study staff will assess the reported event clinically and provide or refer the participant to appropriate medical care; all AEs associated with genital symptoms will be evaluated according to the pelvic exam procedures described for the regularly scheduled follow up visits, and diagnosis and follow up of any observed abnormalities will proceed according to Appendix II.

5.8 Colposcopic Images

Records of colposcopic images are not required for enrollment and follow up visit examinations. The colposcopist will document findings in the participant's chart notes and on the study case report forms. When clinically appropriate, the clinician may choose to retain images in order to complement documentation of baseline findings, abnormal findings or injury.

5.9 Follow up Procedures for Participants Who Discontinue Study Product

Participants who discontinue study product will be encouraged to remain in the study if they are willing, for safety evaluations according to the study follow up schedule with the exceptions described in the following Sections. PK sampling and analysis will not be completed for these participants.

5.9.1 Participants Who Seroconvert to HIV

Study staff will capture seroconversions on study CRFs. All protocol-specified procedures will continue except:

- HIV serology
- PK assessments
- Provision of study gel
- Counseling for HIV/STI risk reduction. Counseling will be modified to address primary and secondary HIV/STI prevention for infected women

5.9.2 Participants Who Become Pregnant

All protocol-specified procedures will continue except:

- Provision of study gel
- Bimanual pelvic exam (unless clinically indicated)
- PK assessments

5.9.3 Participants Who Become Infected With Hepatitis B

Participants who are incidentally found to be acutely infected with hepatitis B during the course of the study will not be withdrawn from the study. All protocol specified procedures will continue and the infection will be managed in accordance with current clinical practice at each site. Hepatitis B symptoms will be managed in accordance with conventional clinical practice.

If the participant provides written informed consent, she may be followed according to protocol evaluations for CHBV participants.

5.9.4 Participants Who Voluntarily Discontinue Study Gel:

All protocol-specified study procedures will continue except:

- Provision of study gel

5.9.5 Participants Who Discontinue Study gel Use Permanently (as advised by study staff):

All protocol-specified study procedures will continue except:

- Provision of study gel
- PK assessments

5.10 Final Contact

The week 24 follow up visit for all participants will include laboratory testing for HIV and other infections; and, the week 36 visit for CHBV participants will include laboratory tests for hepatitis B surface antigen testing and liver and renal function testing. Since the results may not be available at these final visits for participants, a final contact (in person or by telephone [except for positive HIV test results]) may be required to provide the final study test results, post-test counseling, and treatment from these visits. In addition, for participants who become pregnant prior to the study end date, an additional contact may be required to ascertain the participant's pregnancy outcome. Study sites may complete the in-person contacts at the study site or at community based locations, depending on site capacities and site and participant preferences. All final contacts must be documented in participant study records.

Final acceptability and adherence instruments will be administered at the Week 24 visit or study exit visit (which ever comes first). These instruments will be the same as those

used during the trial, but will include some additional questions, to probe about overall adherence and acceptability.

5.11 Study Burden Assessment

Participant's perceptions of the burden of study participation will be assessed at study exit. These data will inform on use-adherence, future acceptability, and ethics considerations for future studies. To improve participant's ability to speak freely, questions will be asked by a staff member who has not had previous contact with the participant (if possible).

6 SAFETY MONITORING AND ADVERSE EVENT REPORTING

6.1 Safety Monitoring

A sub-group of the Protocol Team, including the Protocol Chair, Medical Officer, and Protocol Statistician, will serve as the PSRT. Close cooperation among the PSRT and other study team members will be necessary in order to monitor participant safety and respond to occurrences of toxicity in a timely manner.

6.2 Clinical Data Safety Review

Participant safety is of paramount importance to the HPTN. A multi-tiered safety review process will be followed for the duration of this study. The review process, which is both timely and extensive in scope, includes review of medical history information, clinical and laboratory AEs and concomitant medications. The study site investigators are responsible for the initial evaluation and reporting of safety information at the participant level, and for alerting the PSRT if unexpected concerns arise. Participant safety is also monitored at the Network level through a series of routine reviews conducted by the SDMC Clinical Affairs staff, a PSRT and the sponsor. Additional special reviews may also be conducted at each of these levels as dictated by the occurrence of certain events.

HPTN SDMC Clinical Affairs staff will review incoming safety data on an ongoing basis. Values identified during review that are considered questionable, inconsistent, or unexplained will be queried for verification. AE reports submitted in an expedited manner to the DAIDS Safety Office will be forwarded upon receipt to the DAIDS Medical Officer and SDMC Clinical Affairs staff for review.

At the beginning of the study, the PSRT will aim to meet via conference call every two weeks during the period of study implementation, to review clinical and laboratory data reports (blinded by study treatment) generated by the HPTN SDMC. The content, format and frequency of the clinical data reports will be agreed upon by the PSRT and the SDMC in advance of study implementation. In addition to the routine safety data reviews, the PSRT will convene on an ad hoc basis to make decisions regarding the handling of any significant safety concerns. If necessary experts external to the HPTN representing expertise in the fields of microbicides, biostatistics, HIV transmission and medical ethics may be invited to join the PSRT safety review. A decision to 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.

Decisions regarding permanent discontinuation of study gel in individual participants and in the study overall will be made by the PSRT based on careful review of all relevant data and may involve sponsor consultation with the US Food and Drug Administration (FDA).

In the unlikely event that the protocol team has serious safety concerns that lead to a decision to permanently discontinue study gel for all participants and stop accrual into the study, the protocol team will request an unblinded review of the data by the NIAID Data and Safety Monitoring Board (DSMB) before recommending that the study be

stopped. Members of the NIAID DSMB will be independent investigators with no financial interest in the outcomes of this study. If at any time, a decision is made to discontinue study gel in all participants, DAIDS will notify the US FDA and the Protocol Chairs will notify the responsible IRBs/ECs expeditiously.

Clinical data safety review will be followed according to Section 14.2 of the HPTN Manual of Operations (MOP). The MOP can be accessed on the HPTN website at:

http://www.hptn.org/web%20documents/HPTNMOP/HPTNMOPTOC.pdf

6.3 Adverse Event Reporting Requirements

An AE is defined as any untoward medical occurrence in a clinical research participant administered an investigational product and which does not necessarily have a causal relationship with the investigational product. As such, an AE can be an unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of an investigational product, whether or not considered related to the product. This definition will be applied to all four treatment arms. The term “investigational product” for this study refers to both the tenofovir gel and the placebo gel, as well as the study gel delivery applicators.

Study participants will be provided a 24-hour telephone number and instructed to contact the study clinician to report any AEs they may experience, except for life-threatening events, for which they will be instructed to seek immediate emergency care. Where feasible and medically appropriate, participants will be encouraged to seek medical care where the study clinician is based, and to request that the clinician be paged or otherwise contacted upon their arrival. With appropriate permission of the participant, whenever possible records from all non-study medical providers related to AEs will be obtained and required data elements will be recorded on study case report forms. All participants reporting an AE will be followed clinically, until the AE resolves (returns to baseline) or stabilizes.

For participants who develop any colposcopic abnormality, they will be followed until resolution of the AE. Sites selected for this study will have the capacity and expertise to perform colposcopies independent from the study and will be able to provide follow up care for participants after the study, when necessary.

Participants will be instructed to report problems experienced by male partners to the study clinician, who may suggest follow up care or a referral for such care.

Study site staff will document on study CRFs all AEs reported by or observed in enrolled study participants during the 24 weeks of study gel use, regardless of severity and presumed relationship to study gel or applicators. Study site staff will document on study CRFs all AEs reported by or observed in CHBV participants during their additional 12 weeks of follow up after study gel use. All AEs, except vulvovaginitis and cervicitis will be graded using the DAIDS AE Grading Table Version 1.0, Dec 2004, (also referred to as the “Toxicity Table”). Vulvovaginitis and cervicitis will be graded as follows:

Table 2: Severity Grading for Vulvovaginitis and Cervicitis

	Vulvovaginitis	Cervicitis
Grade 1	Vulvar and/or vaginal discomfort (including itching or burning), pelvic exam findings indicative of inflammation, and/or other exam findings* (including findings involving epithelial disruption) that do not require medical therapy and that cause no or minimal interference with usual social and functional activities	Cervical inflammation or other findings on exam (including erythema, mucopurulent discharge, and/or friability) that do not require medical therapy and that cause no or minimal interference with usual social and functional activities
Grade 2	Vulvar and/or vaginal discomfort (including itching or burning), pelvic exam findings indicative of inflammation, and/or other exam findings* (including findings involving epithelial disruption) that require minimal medical therapy (such as a course of topical or oral antibiotics or antifungals) or cause greater than minimal interference with usual social and functional activities	Cervical inflammation or other findings on exam (including erythema, mucopurulent discharge, and/or friability) that require minimal medical therapy (such as a course of oral antibiotics) or that cause greater than minimal interference with usual social and functional activities
Grade 3	Vulvar and/or vaginal discomfort (including itching or burning), pelvic exam findings indicative of inflammation, and/or other exam findings* (including findings involving epithelial disruption) that result in inability to perform usual social and functional activities and/or require significant medical intervention such as a surgical procedure or hospitalization	Cervicitis or other findings on exam (including erythema, mucopurulent discharge, and/or friability) that require significant medical intervention (such as intravenous antibiotics) or that cause inability to perform usual social and functional activities
Grade 4	Life threatening — vulvovaginitis with perforation	Life threatening

* Findings include erythema, edema, grossly white finding, petechiae, ecchymosis, peeling, ulceration, abrasion, laceration.

The investigator or designee will assess the relationship of all AEs to the study gel based on the Manual for Expedited Reporting of Adverse Events to DAIDS, dated May 6, 2004, the Investigator's Brochure, and his/her clinical judgment.

6.4 Expedited Adverse Event Reporting Requirements

The EAE reporting requirements and definitions for this study and the methods for expedited reporting of AEs to the DAIDS Regulatory Compliance Center (RCC) Safety Office (RCCSafetyOffice@tech-res.com) are defined in "The Manual for Expedited Reporting of Adverse Events to DAIDS" (DAIDS EAE Manual) **dated May 6, 2004**. The DAIDS EAE Manual is available on the RCC website: http://rcc.tech-res-intl.com.

AEs reported on an expedited basis must be documented on the DAIDS Expedited Adverse Event Reporting Form (EAE Reporting Form) available on the RCC website: http://rcc.tech-res-intl.com.

6.4.1 EAE Reporting Requirements for this Study

EAE Reporting Level

This study uses the Intensive Level of expedited AE reporting as defined in the DAIDS EAE Manual.

Study Agents for Expedited Reporting to DAIDS

The study agent that must be considered in determining relationships of AEs requiring expedited reporting to DAIDS are tenofovir 1% gel/placebo, and the study agent delivery applicators.

Grading Severity of Events

The DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 1.0, December, 2004, must be used and is available on the RCC website at http://rcc.tech-res-intl.com/.

EAE Reporting Periods

AEs must be reported on an expedited basis at the Intensive Level during the Protocol-defined EAE Reporting Period, which is:

The entire study duration for an individual participant (from study enrollment until study completion or discontinuation of the participant from study participation for any reason); and for a period of 12 weeks after study gel use. Thereafter, pregnancy outcomes that meet criteria for expedited AE reporting (e.g., fetal losses) occurring among participants known to be pregnant at Week 24 will be reported.

After the end of the Protocol-defined EAE Reporting Period stated above, sites must report serious, unexpected, clinical suspected adverse drug reactions if the study site staff becomes aware of the event on a passive basis, i.e., from publicly available information.

Information on all AEs experienced by study participants will be included in reports to the FDA and other applicable government and regulatory authorities. Site staff will report information on AEs to the IRB/ECs in accordance with all applicable regulations and site-specific IRB/EC requirements.

6.5 Study Monitoring Committee Review

The HPTN SDMC will prepare study progress reports and reports of AEs experienced by study participants (blinded to treatment assignment) for review by the HPTN SMC. The SMC will conduct interim reviews of study progress (blinded to treatment assignment), including rates of participant accrual, retention, rates of adherence to study gel use, and product safety. These reviews will take place approximately every 90 days, or as needed. At the time of these reviews, or at any other time, the SMC may recommend that the study proceed as designed, proceed with design modifications, or be discontinued.

7 STATISTICAL CONSIDERATIONS

7.1 REVIEW OF STUDY DESIGN

The primary aim of this Phase II study is to assess the incidence of vulvar, vaginal and cervical epithelial disruption, erythema and inflammation. These signs as well as symptoms including intermenstrual bleeding, dysuria, and irritation will be collected by symptom review and visual examination of the reproductive tract during a speculum examination in all women during 24 weeks of follow up. Participants who have signs of epithelial disruption detected by naked eye and/or colposcopic examination will be followed according to Appendix II. Because genital infection can cause formation of genital ulcers, inflammation and enhance cervical friability, testing for a range of infections will be performed concurrently with colposcopic examination if clinically indicated. HIV uninfected women participating in one of four treatment arms, following a coitally dependent or once daily regimen for 24 weeks, will be evaluated to assess the safety and effect of tenofovir 1% gel over the treatment period.

7.2 Study Endpoints

7.2.1 Primary Endpoints: Safety/Toxicity

The safety/toxicity primary endpoints associated with the primary objective of this study are as follows:

- macroscopic evidence of damage (judged not to be due to pathogen or iatrogenic trauma) to the cervical epithelium, and, vulvar and/or vaginal epithelium, including ulceration and other lesions, severe erythema, and/or severe edema, judged as definitely, probably, possibly, or probably not related to the study gel or applicator

- laboratory evidence of Grade 3 or higher toxicity for hematology, liver or renal function as defined by the DAIDS AE Grading Table Version 1.0, December 2004, which cannot be directly attributed to another cause after consultation with the protocol chairs, the study site investigator, and the DAIDS Medical Officer, and judged as definitely, probably, possibly, or probably not related to the study gel or applicator

7.2.2 Secondary Endpoints: Adherence and Acceptability Endpoints

The secondary objectives of this study are as follows:

- Adherence to the study gel regimen will be assessed by an interview-administered questionnaire at the weeks 4, 12 and 24 visits. Summary measures of study gel and condom use over time will be compared between study arms (refer to Section 4.5)

- Acceptability of the study gel will be evaluated by an interview administered questionnaire at enrollment (for vaginal product behavior assessment) and weeks 4, 12 and 24. Reasons for voluntary discontinuation and non-compliance related to study gel use will be recorded and compared between study arms (refer to Section 4.6)

The expanded safety data collected by colposcopy examination and vaginal flora and cytokine and chemokine specimens are part of the exploratory objectives of the study.

7.3 Accrual, Follow up, and Sample Size

Per entry criteria described in Sections 3.1 and 3.2, the recruitment target will be:

- 200 HIV uninfected women whose self reported sexual activity is at least once in the 30 days prior to screening, but not more than twice per day in the 14 days prior to screening

Approximately 100 women will be recruited at the Pune, India site and approximately 100 women will be recruited between the two US sites. Each study site will plan to complete their enrollment over the course of a six (6) calendar month accrual period, according to the following schedules of monthly enrollment minimums. The Pune site can exceed their monthly minimum but they cannot exceed their total target. In addition, each US site will be allowed to exceed their target enrollment of 50 women as long as the total number of women recruited by both US sites does not exceed 100 women. Therefore, if enrollment at one of the US sites is below the minimum recruitment slot would be available to be picked up by the other US site. The enrollment targets for the sites are as follows:

Accrual Month	**Pune, India**	**BLHC + UAB = US Sites**
Study Month 1	8	7 + 7= 14 women
Study Month 2	14	7 + 7 = 14 women
Study Month 3	18	10 + 10 = 20 women
Study Month 4	20	10 + 10 = 20 women
Study Month 5	20	10 + 10 = 20 women
Study Month 6	20	6 + 6 = 12 women
TOTAL	**100 Women**	**50 + 50 = 100 Women**

Each enrolled woman will be followed for 24 weeks. CHBV participants will return to site at 4, 8 and 12 weeks after completion of study gel use (Weeks 28, 32, and 36 Visits). Therefore, the entire study should be completed within 15 calendar months.

Each site will target retention of 95% of enrolled participants over the 24 week follow up period.

7.4 Random Assignment

Women will be randomized to one of the four arms. The randomization scheme will be generated and maintained by the HPTN SDMC.

The SDMC will provide each study site with one set of randomization envelopes to be stored and used in the study clinic. Clinic staff will assign these envelopes in sequential order, by envelope number, to eligible participants.

Assignment of the randomization envelope is considered the effective act of participant enrollment/randomization. Clinic staff will prepare a written prescription, contained within the envelope that, among other things, documents the randomization envelope number and dosing frequency to which the participant was assigned. Clinic staff will store assigned randomization envelopes and their contents in participants' study charts.

7.5 Blinding

Study staff, with the exception of Study Pharmacy Staff, and, participants will be blinded to the random assignments of all study participants. Both study gels will be supplied in identical, single-use tubes, and single use applicators packaged in individual wrappers.

Blinding will be maintained (with the exception of Study Pharmacy Staff) 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 4.8, 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 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.

7.6 Data Monitoring and Analysis

7.6.1 Safety Analysis

The primary aim of the study is to assess the local and systemic toxicity of two frequencies of use, daily and coitally dependent, application of tenofovir 1% gel versus a placebo gel among HIV uninfected women. The placebo gel provides information regarding signs, symptoms and/or morbidity that may be attributed to normal variation and/or the study procedures or use of applicator rather than the investigational product being studied. Primary data analyses will tabulate the number of primary endpoints observed during the study, by frequency of use and product assignment within frequency of use. All participants who enroll in the study will be included in each tabulation. Individual participants will contribute once to the calculation of event rates.

The proposed total sample size is N=200, 4 arms of 50 women. For a given arm, if the true rate of a given toxicity endpoint is 5%, 50 women per arm provide 83% power to exclude toxicity endpoint rates greater than 16%, where the safety and toxicity endpoint for a woman is defined as:

(1) having at least one grade 3 or higher adverse experience during follow up judged by the investigator to be definitely, probably, possibly, or probably not related to the study gel or applicator, or;

(2) having at least one macroscopic finding or other clinical evidence of damage during follow up (judged not to be due to pathogen or iatrogenic trauma) to the vulvar and/or vaginal epithelium and/or cervical mucosa including ulceration and other lesions, severe erythema, and/or severe edema judged definitely, probably, possibly, or probably not related to the study gel or applicator

For a given frequency of use, safety and toxicity rates of placebo and active gels will be formally compared. Fifty (50) women per arm will assure with 74% power that a 95% confidence interval for the difference between the placebo and active gel toxicity rates has an upper limit no more than 10% when the true toxicity rates for placebo and active gel are both 5%.

Upon enrolling in the study, female participants will be assigned at random to use either the active gel or the placebo gel. Randomization will be stratified by site to ensure equal balanced assignment to each product at each site. Women lost to attrition and/or off study gel will not be replaced; however every effort will be made to complete their regularly scheduled safety evaluations. Two primary analysis datasets will be used for these analyses. The first dataset will be consistent with the intention-to-treat principle in order to preserve the initial benefit of randomization. A randomized woman will be excluded from this dataset only under the following circumstances:

1. If she does not satisfy a major entry criterion (as defined in the statistical analysis plan), or;
2. If study gel was never distributed to her and no data were collected post-randomization

The second dataset will be a subset of the full analysis dataset and will include women that are compliant with the protocol. A randomized woman will be excluded from this dataset only under the following circumstances:

1. If she does not satisfy certain entry criteria (as defined in Sections 3.1 and 3.2), or;
2. If study gel was never distributed to her and no data were collected post-randomization, or;
3. If she has failed to adhere to the study gel regimen (non adherence defined in statistical analysis plan), or;
4. If she was off study gel, because of pregnancy or other reasons (e.g., IV drug use during follow up), for more than a certain proportion of total follow up time (proportion defined in statistical analysis plan)

Events reported during study gel use will be analyzed separately from events reported during the 12 weeks post-study gel use.

Women off study gel and/or non-adherent that are included in the full analysis dataset will potentially lower the rate of safety endpoints in the active gel arms. Therefore, the 'per protocol' dataset will be used to explore the sensitivity of the conclusions obtained with the analyses based on the full analysis dataset.

7.6.2 Analysis of PK Data

Blood levels of tenofovir will be evaluated after vaginal administration. Specimens from participants receiving placebo gel will not be assayed. A single tenofovir blood level will be drawn at the Week 4, 12 and 20 visits. For the Weeks 4 and 12 visit specimens, the participants will not be instructed to insert the study gel prior to these visits, and the analysis will be performed in an exploratory way by investigation plasma concentrations of tenofovir during terminal elimination (Blood draws are estimated to occur 12-16 hours post-dose, depending on an individual participant's study gel use). For the Week 20 visit specimen, the analysis will be performed in an exploratory way, by investigating plasma concentrations of tenofovir during the peak absorption period following dosing, however at this visit all participants *will* be instructed to insert the study gel prior to the visit, so that blood will be drawn approximately two to six hours post-dose, the period coinciding with peak concentrations in previous studies [HPTN 050]. Tenofovir blood levels will be further investigated by correlating serum tenofovir levels with HSV-2 serostatus, the presence or absence of genital tract inflammation, and frequency of sexual activity.

7.6.3 Analyses of Adherence, Study Gel and Condom Use and Sexual Behavior

The proportion of women with no coital activity in the last seven days will be computed. These monthly rates will be presented in tables by (1) study arm and (2) study arm by site. Appropriate statistical tests will be used to compare study arms within frequency of use.

Also, for each woman with at least one sexual vaginal act during the 24 weeks of study gel use, the four following proportions will be computed:

- Number of acts protected by condom only divided by the total number of coital acts
- Number of acts protected by study gel only divided by the total number of coital acts
- Number of acts protected by study gel and condoms divided by the total number of coital acts
- Number of acts unprotected by study gel and unprotected by condoms divided by the total number of coital acts

Appropriate summary measures of these four proportions will be presented in tables by (1) study arm and (2) study arm by site. Appropriate statistical tests will be used to compare study arms within frequency of use.

Finally, the proportion of women off study gel at each study month will be computed. These monthly rates will be presented by (1) study arm and (2) by study arm and site. In addition, a table describing the reasons for discontinuation of study gel will be presented by (1) study arm and (2) by study site.

7.6.4 Analysis of Acceptability Data

Assessment of the acceptability of the study gels will be done at enrollment (to assess pre-use vaginal product behavior) and weeks 4, 12 and 24. For these visits, the primary measure of acceptability will be defined as the proportion of women indicating that they would use the study gel that they are currently using if it was found to prevent HIV. This measure of acceptability will be presented, for Weeks 4, 12 and 24, in a table by (1) study arm and (2) by study arm by site. Formal statistical comparisons between study arms within frequency of use will be performed only for the study exit visit.

8 HUMAN SUBJECTS CONSIDERATIONS

8.1 Ethical Review

This protocol and the template informed consent form(s) contained in Appendix IV — and any subsequent modifications — will be reviewed and approved by the HPTN Protocol Review Committee and DAIDS Prevention Science Review Committee with respect to scientific content and compliance with applicable research and human subjects regulations.

The protocol, site-specific informed consent form, participant education, outreach and recruitment materials, and other requested documents — and any subsequent modifications — also will be reviewed and approved by the ethical review bodies responsible for oversight of research conducted at the study site.

Subsequent to initial review and approval, the responsible IRBs/EC will review the protocol at least annually. The site Investigator will make safety and progress reports to the IRBs/ECs at least annually, and within 90 days of 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. Study sites will submit documentation of continuing review to the DAIDS Protocol Registration Office, via the HPTN CORE, in accordance with the current DAIDS Protocol Registration Policy and Procedure Manual.

Participants who seroconvert during the course of this study will have the option of returning to the clinic for the scheduled visits and continue to receive counseling and support services from site staff when and where available, but will be off study gel.

This study will identify persons who are infected with HIV, either as part of the study screening process or during follow up of enrolled participants. Study staff will provide participants with their HIV test results in the context of post-test counseling. They also will refer persons found to be HIV infected to available sources of medical and psychosocial care and support, as well as to any available research studies for HIV infected persons. Persons found to be HIV infected will be encouraged to refer their partners for testing and treatment in accordance with local laws if applicable.

For any participants who become pregnant during follow up, site staff will offer counseling on options available to the participant, in accordance with site-specific SOPs.

Condom use will be promoted and encouraged to all participants throughout this study.

For participants who develop any colposcopic abnormality, they will be followed until resolution of the AE. Sites selected for this study will have the capacity and expertise to perform colposcopies independent from the study and will be able to provide follow up care for participants after the study, when necessary.

8.1.1 Prisoner Participation

HPTN 059 does not meet the criteria for prisoner participation per US 45 Code of Federal Regulations (CFR) 46.306 (a)(2)(D). HPTN 059 is not suitable for further reviews by local IRBs for the inclusion of prisoners.

8.2 Informed Consent

Written informed consent will be obtained from each study participant prior to the initiation of any study procedures. Each study site is responsible for developing study informed consent forms and a test of the participant's comprehension of the study for local use, based on the informed consent form samples in Appendix IV, which describes the purpose of the study, the procedures to be followed, and the risks and benefits of participation, in accordance with all applicable US regulations and local guidelines. The study site also is responsible for translating the template form into local languages, and verifying the accuracy of the translation by performing an independent back-translation.

Literate participants will document their provision of informed consent by signing their informed consent forms. Non-literate participants will be asked to document their informed consent by marking their informed consent forms (e.g., with an X, thumbprint, or other mark) in the presence of a literate third party witness. Further details regarding DAIDS requirements for documenting the informed consent process with both literate and non-literate participants are provided in the DAIDS SOP for Source Documentation. Any other site-specific IRB/EC requirements for obtaining informed consent from non-literate persons also will be followed.

Participants will be provided with a copy of their informed consent form if they are willing to receive it. Study staff will document the informed consent process as described in the Study-Specific Procedures Manual.

Both sites will have a CAB, and obtain input and feedback from the CAB. Protocol team members will work with study staff and community representatives to develop locally-appropriate information materials about the study and a standardized approach to the informed consent process to be implemented at all study sites.

These materials will include information to be made available to male partners. Although each woman will be encouraged to inform male partners about her participation in the study, partner consent, assent or approval will not be required.

8.3 Risks

Participants may experience discomfort when having pelvic exams and/or phlebotomy for this study. During phlebotomy, they also may feel dizzy or faint, or develop a bruise, swelling or infection where the needle is inserted. Participants also may become embarrassed, worried, or anxious while waiting for their HIV and STI test result. Trained counselors will be available to help participants deal with these feelings.

Although study sites will make every effort to protect participant privacy and confidentiality, it is possible that participants' involvement in the study could become known to others, and that social harms may result (i.e., because participants could become known as HIV 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.

Administration of tenofovir gel intravaginally at 0.3% and 1% concentrations in the HPTN 050 Phase I study resulted in minimal local irritation and little or no systemic adverse effects were identified. Although 92% of participants reported at least 1 AE, 87% of those reported AEs were mild, and 70% of the AEs were limited to the genitourinary tract. Four severe AEs were reported, with only one, lower abdominal pain, thought to be product-related[6]. Therefore the risks associated with tenofovir gel are believed to be less than those identified for systemic use.

In the HPTN 050 Phase I study of tenofovir gel, serum PK analysis in a subset of participants demonstrated that there is no clinically significant systemic toxicity. Fourteen of 24 women with PK results had low, but detectable, serum tenofovir levels.[6] (Refer to Section 1.3.1)

Given that Phase I data demonstrates measurable plasma concentrations of tenofovir in some participants, participants with CHBV might be at risk for development of tenofovir resistant HBV.[6] Participants with CHBV will be eligible for enrollment; however they will be monitored through HBV viral load, and LFTs.

It is not known what effect tenofovir gel could have on the HIV virus or HIV disease progression in HIV infected participants or their partners. There is a theoretical risk that tenofovir absorbed systemically from tenofovir gel could result in mutations of the HIV virus in participants who become infected with HIV during the study, or their partner, if the partner is infected with HIV. Limited resistance data from the HPTN 050 study show that no new resistance mutations evolved in plasma or cervicovaginal lavage specimens after 14 days of tenofovir gel use. No participant had high level tenofovir mutations (e.g., K65R).[6]

Some of the possible side effects of the study gel are dryness, itching, burning, or pain in the genital area. The following side effects have been associated with the use of oral tenofovir: [4, 13]

- Upset stomach, vomiting, gas, loose or watery stools
- Dizziness
- Abdominal pain
- Lack of energy
- Kidney damage or failure
- Inflammation or swelling and possible damage to the pancreas
- Shortness of breath
- Rash
- Low phosphate

- Increase of LFTs in children
- Allergic reaction, which may include fever, rash, upset stomach, vomiting, loose or watery stools, abdominal pain, achiness, shortness of breath or a general feeling of illness
- Changes in bone growth and strength were seen in study animals given tenofovir. It is unknown if long term use of tenofovir gel will cause bone abnormalities in adults. Bone thinning has been seen in adults and children taking oral tenofovir

Tenofovir and its oral form administered in systemic dose toxicology studies to rats, dogs and monkeys at exposures (based on areas under the plasma concentration curve (AUC)) greater than or equal to six fold those observed in humans caused bone toxicity. In monkeys the bone toxicity was diagnosed as osteomalacia. Osteomalacia observed in monkeys appeared to be reversible upon dose reduction or discontinuation of tenofovir. In rats and dogs, the bone toxicity manifested as reduced bone mineral density (BMD). The mechanism(s) underlying bone toxicity is unknown. However, systemic absorption of tenofovir from the vaginal gel appears to be minimal, (approximately 1% of the therapeutic oral dose) and the perceived risk of an effect on BMD is low.

There have been other side effects in patients taking the oral form of tenofovir (TDF). However, these side effects may have been due to other medicines that patients were taking or to the illness itself.[14, 15]

Although the oral form of tenofovir is labeled as a Pregnancy Category B agent, it is not definitively known if tenofovir gel has any effect on pregnancy, whether it has any effect on the fetus, or if it is secreted in breast milk. Participants will be counseled on the importance of not becoming pregnant during the study. If the participant does become pregnant during the study, study gel use will be stopped and study staff will discuss the choices available to the participant. They will continue with their follow up visits through their originally scheduled study exit date or until their pregnancy outcome is ascertained, whichever is longer.

This and future studies of tenofovir gel are needed to more fully assess the risk profile of topical administration since it is possible that tenofovir gel could cause any of the effects listed above or other adverse effects not reported previously, including effects leading to death or permanent disability. The latter outcomes are unlikely, but may occur due to low levels of systemic drug absorption after topical exposure and to the rarity of severe allergies.

8.4 Benefits

There may be no direct benefits to participants in this study. However, 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 a safe and effective microbicide that prevents sexual transmission of HIV.

In addition, participants will receive HIV/STI counseling and testing as part of the study screening process. Participants will also have pelvic exams and colposcopies. Participants will also have blood and urine tests for a number of STIs, and will be provided with STI treatment if applicable. (Refer to Appendix I, Schedule of Study Visits and Evaluations)

8.5 Incentives

Pending IRB approval, participants will be compensated for their time and effort in this study, and/or be reimbursed for travel to study visits and time away from work. Site-specific reimbursement amounts will be specified in the study informed consent forms.

8.6 Participant Confidentiality

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. All laboratory specimens, colposcopic photographs, reports, study data collection, process, and administrative forms will be identified by coded number only to maintain participant confidentiality. All records that contain names or other personal identifiers, such as locator forms and informed consent forms, will be stored separately from study records identified by code number. All local databases will be secured with password-protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link participants ID numbers to identifying information will be stored in a separate, locked file in an area with limited access

Participants' study information will not be released without the written permission of the participant, except as necessary for monitoring by NIAID and/or its contractors (e.g., the DAIDS monitoring contractor), Gilead Sciences, representatives of the HPTN CORE, the HPTN CL and/or SDMC, the FDA, and other regulatory authorities. In addition, for the participant's from India, study related information may be released to the Drug Controller General of India and the Indian Council of Medical Research if necessary.

A Federal Certificate of Confidentiality will be sought for this study. The Certificate applies in the US sites only and will protect study staff from being compelled to disclose study related information by any Federal, State or local civil, criminal, administrative, legislative, or other proceedings.

8.7 Communicable Disease reporting

Study staff will comply with all applicable local requirements to report communicable diseases including HIV identified among study participants and their partners (if obligated by local law) to local health authorities. Participants will be made aware of all reporting requirements during the study informed consent process. For participants from India, study related information may be released to the Drug Controller General of India and the Indian Council of Medical Research if necessary.

8.8 Access to HIV-Related Care

8.8.1 HIV Counseling

HIV pre-test, risk reduction, and post-test 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. Counseling will be provided in accordance with a standard study counseling manual, and will emphasize the unknown efficacy of the candidate microbicides in preventing HIV infection.

In accordance with the policies of the US National Institutes of Health, participants must receive their HIV test results in order to take part in this study.

Water or silicone based, non N-9 or spermicide containing, male, latex condoms will be provided to participants for use throughout the duration of their participation.

8.8.2 Care for Participants Identified as HIV-Infected

This study will identify persons who are infected with HIV, either as part of the study screening process or during follow up of enrolled participants. Study staff will provide participants with their HIV test results in the context of post-test counseling. They also will refer persons found to be HIV infected to available sources of medical and psychosocial care and support, as well as to any available research studies for HIV infected persons. Persons found to be HIV infected will be encouraged to refer their partners for testing and treatment in accordance with local laws if applicable.

For any participants found to be newly HIV infected who also become pregnant during follow up, every effort will be made to facilitate access to single-dose nevirapine (and/or other interventions) to reduce the probability of HIV transmission to the participant's infant.

Participants seroconverting for antibodies to HIV will be counseled appropriately. They will be encouraged to return for follow up visits, but will be taken off study gel (on study/off product). The medical care and referral of the participants who seroconvert will vary by site and will depend on both the local standard of care and the guidelines to be set by the National Institutes of Health.

8.9 Study Discontinuation

This study may be discontinued at any time by NIAID, the HPTN, the product manufacturers, the US FDA, other government or regulatory authorities, or site IRB/ECs.

Monitoring of AEs/EAEs will be ongoing through regular SMC reviews (safety monitoring). If needed, the PSRT will convene ad hoc meetings in the event of an

abnormal number of reported AEs/EAEs judged definitely, probably, possibly, or probably not related to study gel or applicator, or any other conditions deemed as an emergency event by team members.

Decisions regarding discontinuation of study gel due to safety concerns are described in Section 6.2.

9 LABORATORY SPECIMENS AND BIOHAZARD CONTAINMENT

Each study site will adhere to standards of good laboratory practice, the HPTN CL Manual; and site-specific SOPs for proper collection, processing, labeling, transport, and storage of specimens to the LL. Specimen collection, testing, and storage at the LL will be documented using the HPTN Laboratory Data Management System (LDMS).

9.1 Local Laboratory Specimens

As described in Section 5, the following types of specimens will be assayed at the LL:

- blood for CBC, LFT and RFP
- blood for HIV, syphilis serology, and, HBsAg
- urine for pregnancy testing, GC and CT urine NAAT, and, dipstick urinalysis (microscopy and culture if indicated)
- vaginal smears for wet mount for BV, candidiasis, trichomoniasis and clue cells
- ecto-and endocervical cells for Pap smear
- blood for HSV-2 (India only)

9.2 Central Laboratory Specimens

As described in Section 5, the following types of specimens will be assayed at the HPTN CL:

- vaginal smears for Gram stain
- genital ulcer swab for multiplex PCR
- plasma for quality assurance HIV testing
- blood for plasma and serum archive (or LL if possible)
- HBV viral load testing
- cervical swab for cytokine and chemokine testing
- cervical swab for Gram stain
- vaginal swab for quantitative culture (US sites only)
- blood for PK analysis
- blood for storage for possible future HBV resistance testing
- blood for HSV-2 (U.S. sites only)

9.2.1 Shipping to HPTN CL

All specimens will be shipped in accordance with IATA specimen shipping regulations. All shipments will be documented using the HPTN LDMS.

9.3 Quality Control and Quality Assurance Procedures

The HPTN CL has established a proficiency testing program at each study site. HPTN CL staff also will conduct periodic visits to each site to assess the implementation of on-site laboratory quality control procedures, including proper maintenance of laboratory testing equipment, use of appropriate reagents, etc. HPTN CL staff will follow up directly with site staff to resolve any quality control or quality assurance problems identified through proficiency testing and/or on-site assessments.

Throughout the course of the study, the SDMC will select a random sample of stored specimens to test for quality assurance (QA) purposes. The total number of specimens undergoing QA testing will be about 50 samples or 10% of all specimens collected, which ever is greater.

The SDMC will inform site staff of the samples selected for the quality assurance testing, and site staff will ship the selected specimens to the HPTN CL. All specimens from participants who seroconvert will be collected. The HPTN CL will test the specimens for HIV antibody and compare the results of their tests with the results obtained by the local labs. The HPTN CL staff will follow up directly with site staff to resolve any quality assurance problems identified through the process.

9.4 Specimen Storage and Possible Future Research Testing

Study staff will store serum and plasma collected from each study participant at the time of study entry, seroconversion (if applicable), and week 24 visit. All such specimens will be subject to possible safety and quality assurance testing during and after the study as described in Section 9.3 above, and will be destroyed at the end of the study after all protocol required and quality assurance testing has been conducted. In addition, CHBV participants will be asked to provide written informed consent for additional plasma specimens to be collected during the study and stored after the end of the study for possible future research testing. Any residual specimens of participants who do not consent to long-term storage and for future resistance testing will be destroyed at the end of the study, after all protocol required and quality assurance testing has been conducted.

9.5 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 currently recommended by the CDC. All infectious specimens will be transported in accordance with United States regulations (42 CFR 72).

10 ADMINISTRATIVE PROCEDURES

10.1 Study activation

Following ethical review and approval, study sites will submit required administrative documentation – as listed in the study-specific procedures manual – to the HPTN CORE. CORE staff will work with study site staff and complete DAIDS protocol registration in accordance with the current DAIDS Protocol Registration Policy and Procedure Manual. Included in this step will be CORE and DAIDS review of each site-specific study informed consent form.

The DAIDS RCC Protocol Registration Office will review all site-specific informed consent forms and approve them for use according to DAIDS policies. The study cannot be initiated at a site until the site is fully registered with the DAIDS RCC Protocol registration office (through HPTN CORE) and has received written notification of protocol activation by CORE.

Pending successful registration and submission of all required documents, CORE staff will "activate" the site to begin study operations. Study implementation may not be initiated until a study activation notice is provided to the site.

10.2 Study Coordination

DAIDS holds the IND application for this study (#55,690). Copies of all regulatory documents submitted to this IND by DAIDS will be forwarded to Gilead Sciences, Inc. for cross-referencing with the company's other INDs for tenofovir gel. Assignment of all sponsor responsibilities for this study will be specified in a Clinical Trials Agreement executed by DAIDS and Gilead Sciences, Inc.

Study site staff will be provided with the DAIDS SOPs for Source Documentation and Essential Documents; the Manual for Expedited Reporting of Adverse Events to DAIDS; and the DAIDS AE Grading Table. Training and written instructions outlining management and reporting, dispensing study gels and documenting product accountability, and other study operations will be provided.

Study case report forms will be developed by the study team and HPTN SDMC. Data will be transferred to the HPTN SDMC, entered, and cleaned using the SDMC DataFax data management system. Quality control reports and queries routinely will be generated and distributed to the study sites for verification and resolution.

Close coordination between the study site Investigator, NIAID Medical Officer, Protocol Specialist, Biostatistician, CORE Protocol Specialist, SDMC Protocol Operations Coordinator, Data Managers, and other protocol team members will be necessary to track study progress, respond to queries about proper study implementation, and address other issues in a timely manner. Rates of accrual, adherence, follow up, and AE incidence will be monitored closely by the team as well as the HPTN SMC. The PSRT will address issues related to study eligibility and AE management and reporting

as needed to assure consistent case management, documentation, and information-sharing across sites.

10.3 Study Monitoring

On-site study monitoring will be performed in accordance with DAIDS policies. Study monitors will visit the site to:

- verify compliance with human subjects and other research regulations and guidelines
- assess adherence to the study protocol, study-specific procedures manual, and local counseling practices
- confirm the quality and accuracy of information collected at the study site and entered into the study database

Site investigators will allow study monitors to inspect study facilities and documentation (e.g., informed consent forms, clinic and laboratory records, other source documents, case report forms), as well as observe the performance of study procedures. Investigators also will allow inspection of all study-related documentation by authorized representatives of the HPTN CORE, SDMC, HPTN CL, NIAID, Gilead Sciences, Inc., FDA, and US and in-country government and regulatory authorities. A site visit log will be maintained at the study site to document all visits.

10.4 Protocol Compliance

The study will be conducted in full compliance with the protocol. The protocol will not be amended without prior written approval by the Protocol Chair and NIAID Medical Officer. All protocol amendments must be submitted to and approved by the relevant IRB(s)/EC(s) and the RCC prior to implementing the amendment.

10.5 Investigator's Records

The Investigator will maintain, and store in a secure manner, complete, accurate and current study records throughout the study. In accordance with US regulations, the Investigator will retain all study records for at least two years following the date of marketing approval for the study gel for the indication in which it was studied. If no marketing application is filed, or if the application is not approved, the records must be retained for two years after the FDA is notified that the IND is discontinued. Study records include administrative documentation — including site registration documents and all reports and correspondence relating to the study — as well as documentation related to each participant screened and/or enrolled in the study — including informed consent forms, locator forms, case report forms, notations of all contacts with the participant, and all other source documents.

10.6 Use of Information and Publications

Publication of the results of this study will be governed by DAIDS and HPTN policies. Any presentation, abstract, or manuscript will be submitted by the Investigator to the HPTN Manuscript Review Committee, DAIDS, and Gilead Sciences Inc., for review prior to submission.

11 REFERENCES

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APPENDIX I: SCHEDULE OF STUDY VISITS AND EVALUATIONS

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EVALUATIONS	Screening (up to –56 days)	Final Screening	Enrollment Visit (Day 0)	Weeks 4, 12,	Weeks 8, 16, 20	Week 24or Early Termination	Weeks 28. 32. 36 (CHBV participants)
Obtain informed consent (s)	X		X				
Obtain demographic information	X						
Obtain/update locator information	X	X	X	X	X	X	●
Obtain behavioral eligibility information	X						
Provide HIV/STI pre-test counseling	X	▲	X	▲	▲	X	
Provide HIV/STI risk reduction counseling	X	X	X	X	X	X	X
Provide HIV/STI post-test counseling	X	▲	X	▲	▲	X	▲
Obtain/update medical and menstrual history	X	X	X	X	X	X	●
Perform targeted physical exam	X						
PERFORM PELVIC EXAM:							
naked eye exam of external genitalia	X		X	X	▲	X	
speculum exam of vagina and cervix	X		X	X	▲	X	
colposcopic exam			X	X	▲	X	
vaginal pH - LL	X		X	X	▲	X	
wet mount for BV, candidiasis, trichomoniasis - LL	X		X	X	▲	X	
*cervical swab for cytokine and chemokine testing - CL			X	X		X	
*cervical swab for Gram stain - CL			X	X		X	
vaginal swab for quantitative culture (US sites only) - CL			X	X		X	
*vaginal swab for Gram stain assessment - CL	X		X	X		X	
*genital ulcer swab for multiplex PCR - CL	▲		▲	▲	▲	▲	
ecto- and endocervical cells for Pap smear [a] - LL	X						
PERFORM LABORATORY EVALUATIONS:							
urine pregnancy test - LL	X	X	X	X	X	X	
urine NAAT for GC and CT - LL	X		X	▲	▲	X	
dipstick urinalysis-LL	X		X	▲	▲	X	
urine microscopy and culture - LL	▲		▲	▲	▲	▲	
HIV serology (EIA/WB when indicated) - LL	X	▲	X	▲	▲	X	
HBsAg - LL	X		X	▲	▲	X	
HBV viral load - CL			●	●[b]		●	●
*PK sampling - CL				X	X[c]		
*HBV serum archive (if participant provides consent) - LL			●	●[b]		●	●
syphilis serology - LL	X		X	▲	▲	X	
*HSV-2 serology – LL or CL	X		X	▲	▲	X	
*plasma and serum archive - LL			X	▲	▲	X	
CBC - LL	X		X	X	▲	X	
LFT and RFP - LL	X		X	X	▲	X	●[d]

Continued on next page…

APPENDIX I: SCHEDULE OF STUDY VISITS AND EVALUATIONS continued

EVALUATIONS	Screening (up to – 56 days)	Final Screening	Final Screening/ Enrollment (Day 0)	Weeks 4, 12,	Weeks 8, 16, 20	Week 24 or Early Termination	Weeks 28. 32. 36 (CHBV participants)
Provide test results [e]	X	X	X	X	X	X	X
Offer/refer for STI treatment	▲	▲	▲	▲	▲	▲	
Obtain random assignment			X				
Provide study gel and instructions for use (with panty liners and/or menstrual pads, and condoms)			X	X	X		
Administer behavioral and adherence assessment			X[f]	X		X	
Administer acceptability assessment				X		X	
Administer study burden assessment						X[g]	●[h]

* - Items marked with * indicates those specimens will be batched for shipment
X - Protocol specific evaluation for all participants
▲- If clinically indicated
● - CHBV participants only

a - Unless documentation of a normal Pap test result in the 90 days prior to screening
b - Assessment will be performed at Week 12 only
c - Assessment will be performed at Week 20 only
d - RFP only
e - When available
f - Behavioral assessment done only at the Enrollment Visit
g - Non-CHBV participants only
h - Assessment will be performed at Week 36 only

LL – at the Local Lab
CL – at the HPTN CL for assay

APPENDIX II: OUTCOMES DIAGNOSTICS, AND FOLLOW UP EVALUATIONS

APPENDIX II: OUTCOMES, DIAGNOSTICS, AND FOLLOW UP EVALUATIONS

Condition	Study gel Use	Evaluation	Follow up and Treatment Action
Deep Epithelial Disruption (Ulceration)	Hold study Gel (until evaluated)	Swab for herpes simplex culture. Perform syphilis serology (Herpes serology optional)	Re-evaluate in 48 - 72 hours and reinstate study gel use if resolved. If the ulcer has become worse or not healed in 48 - 72 hours consider a biopsy. Ask participant to return in 7–10 days for follow up syphilis serology. If there is reoccurrence and there is no other aetiology, then consider permanent discontinuation.
Superficial Epithelial Disruption (Abrasion/Peeling)	Continue	Naked eye evaluation and/or colposcopy	Re-evaluate by speculum examination in 48 - 72 hours. If condition is significantly worse, hold study gel use. Otherwise continue study gel use.
Localized erythema or edema: area of less than 50% of vulvar surface or combined vaginal and cervical surface	Continue	Naked eye evaluation and/or colposcopy	If asymptomatic, re-evaluate at next regularly scheduled visit. If symptomatic, re-evaluate by speculum examination in 5 - 7 days. If worsened significantly, hold study gel use, until further evaluation is scheduled. Otherwise, continue study gel use.
Generalized erythema or severe edema: area of more than 50% of vulvar surface or combined vaginal and cervical surface affected by erythema	Hold study Gel (until evaluated)	Naked eye evaluation and/or colposcopy	Re-evaluate in 48 - 72 hours and reinstate study gel use if resolved. If there is reoccurrence and there is no other aetiology, then consider permanent discontinuation.
Vaginitis	Hold study Gel (until evaluated, except for asymptomatic candida vaginitis)	Perform wet mount for candida vaginitis, trichomoniasis, and BV	Provide treatment and reevaluate in 48 - 72 hours. If resolved reinstate study gel use.

APPENDIX II: OUTCOMES, DIAGNOSTICS, AND FOLLOW UP EVALUATIONS continued

Condition	Study gel Use	Evaluation	Follow up and Treatment Action
Intermenstrual Bleeding/Spotting	Hold study Gel (until evaluated)	Naked eye evaluation and/or colposcopy	If determined to be endometrial bleeding with no other source, continue study gel use. Re-evaluate in 48 - 72 hours if the participant reports the bleeding/spotting has not resolved.
Suspected cervicitis (findings on exam such as discharge from the cervical os)	Continue (at clinician's discretion)	Evaluate for *N. gonorrhoeae and C. trachomatis*.	Re-evaluate in 48 - 72 hours. If condition is worse, hold study gel use until further evaluation is scheduled
Petechial hemorrhage	Continue	Naked eye evaluation and/or colposcopy.	Re-evaluate by speculum examination in 48 - 72 hours. If condition is significantly worse, hold study gel use, until further evaluation is scheduled. Otherwise continue study gel use
Ecchymosis	Continue	Naked eye evaluation and/or colposcopy.	Re-evaluate by speculum examination in 48 - 72 hours. If the condition is significantly worse, hold study gel use until further evaluation is scheduled. Otherwise continue study gel use.
EAE that is judged by the site investigator or designee to be definitely, probably, possibly, or probably not related to the study gel or applicator	For Grades 1, 2, and 3 - Hold study Gel (until evaluated) For Grade 4 – Permanent Discontinuation	Evaluate as according to current clinical practice at the site Not applicable	Provide treatment as clinically indicated, when resolved reinstate study gel use at clinician's discretion Not applicable

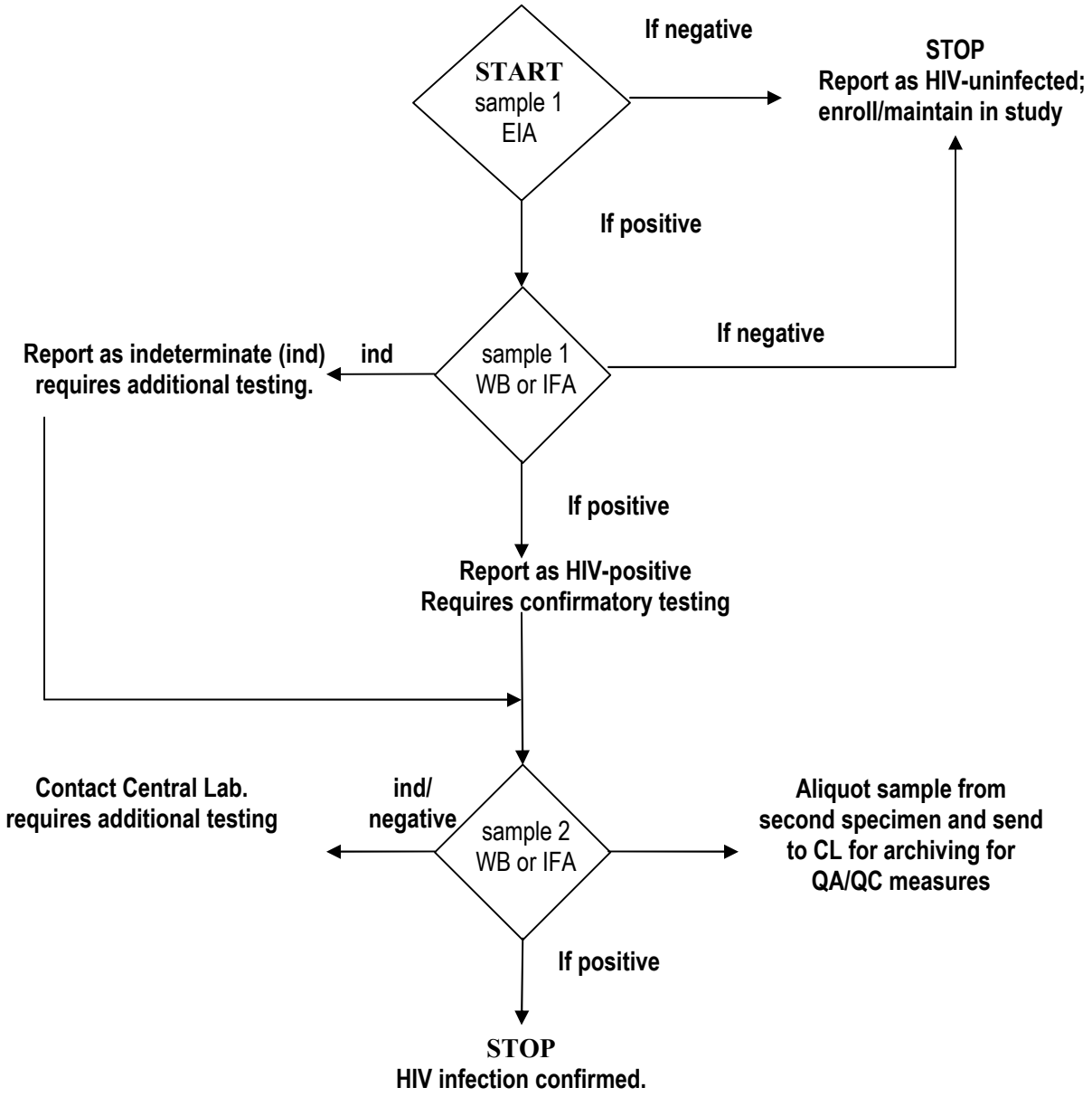
- For trichomoniasis or symptomatic BV, treat or refer for treatment. If resolved, restart study gel use. If observed at Week 24 visit, treat and follow up to document resolution

- For symptomatic candida vaginitis: manage with oral medication and re-evaluate in 3 - 5 days. If resolved, restart study gel use. If observed at Week 24 visit, treat and follow up to document resolution

- For asymptomatic candida vaginitis:
    - If a participant has asymptomatic candida vaginitis she should continue study gel use and be re-evaluated in 7 days
    - If at the Week 24 Visit there are signs and symptoms compatible with vaginitis, treat and follow up to document resolution

APPENDIX III: HPTN HIV ANTIBODY ALGORITHM

APPENDIX III:



Note: HIV positive results will only be reported to participants once the result is confirmed by Western Blot Testing. Once a participant's HIV status is confirmed, sites will follow site specific SOPs for notification to local agencies.