

Section 2. Protocol

This section contains a complete reference copy of the MTN-008 protocol. At the time of this printing, protocol Version 1.0, dated 29 March 2010, reflects current protocol specifications.

To ensure that this manual continues to reflect current protocol specifications in the future:

- Upon receipt of any protocol clarification memos, add a copy of the memo to this section.
- Upon receipt of any letters of amendment, add a copy of the letter of amendment to this section.
- Upon receipt of any full protocol amendments, replace the contents of this section with the amended protocol.

Further information on the content and required handling of protocol clarification memos, letters of amendment, and full amendments is available in Section 9.2 of the MTN Manual of Operations.

SSP Section 2, Version 1.0 24 March 2011

MTN-008

Expanded Safety Investigation of Tenofovir 1% Gel in Pregnancy and Lactation

Microbicide Trials Network

Sponsored by:

**Division of AIDS, US National Institute of Allergy and Infectious Diseases
US *Eunice Kennedy Shriver* National Institute of Child Health and Human
Development
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MTN-008

Expanded Safety Investigation of Tenofovir 1% Gel in Pregnancy and Lactation

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LIST OF ABBREVIATIONS AND ACRONYMS

3TC	lamivudine
ABC	abacavir
AE	adverse events
AIDS	acquired immunodeficiency syndrome
ALT	alanine transaminase
APR	Antiretroviral Pregnancy Registry
APV	amprenavir
ART	antiretroviral therapy
AST	aspartate aminotransferase
AUC	area under the curve
BV	bacterial vaginosis
c/m	cord/maternal blood
CBC	complete blood count
CFR	Code of Federal Regulations
C_{max}	maximum concentration
CORE	Coordinating and Operations Center
C-PMPA	radiolabeled tenofovir
CPQA	Clinical Pharmacology Quality Assurance
CRS	clinical research site
CTA	clinical trial agreement
DAERS	DAIDS Adverse Event Reporting System
DAIDS	Division of AIDS
ddC	zalcitabine
ddl	didanosine
dL	deciliter
DLV	delavirdine
DNA	deoxyribonucleic acid
EC_{50}	50% effective concentration
EFV	efavirenz
FDA	(US) Food and Drug Administration
FHCRC	Fred Hutchinson Cancer Research Center
FHI	Family Health International
FTC	emtricitabine
g	gram
GCP	Good Clinical Practices
HBsAg	hepatitis B surface antigen
HEC	hydroxyethylcellulose
HIV	human immunodeficiency virus
HIV-1	human immunodeficiency virus - Type 1
HPTN	HIV Prevention Trials Network
ICH	International Conference on Harmonisation
IMPAACT	International Maternal Pediatric Adolescent AIDS Clinical Trials Group
IND	investigational new drug
LDMS	Laboratory Data Management System
LLOQ	lower limit of quantitation
mg	milligram
mL	milliliter
MTN	Microbicide Trials Network
NFV	nelfinavir
NIAID	National Institute of Allergy and Infectious Diseases
NICHHD	National Institute of Child Health and Human Development
NIH	National Institutes of Health
NL	Network Laboratory

NNRTI	non nucleoside reverse transcriptase inhibitor
NRTI	nucleoside reverse transcriptase inhibitor
NVP	nevirapine
OBARS	UAB Obstetrical Automated Record System
PACTG	Pediatric AIDS Clinical Trials Group
PD	pharmacodynamics
pH	measure of the acidity or basicity of a solution
PI	Principal Investigator
PK	pharmacokinetics
PMPA	tenofovir 1% gel
PMTCT	preventing mother-to-child transmission
PoR	Pharmacist of Record
PSRT	Protocol Safety Review Team
PTID	participant identification number
RCC (DAIDS)	Regulatory Compliance Center
RMP	Rectal Microbicide Program
RT	reverse transcriptase
RTI	reproductive tract infection
SAE	serious adverse event
SCHARP	Statistical Center for HIV/AIDS Research and Prevention
SDMC	Statistical Data and Management Center
SIV	simian immunodeficiency virus
SMC	Study Monitoring Committee
SOP	standard operating procedures
SQV	saquinavir
ss	steady state
STEP	an experimental HIV vaccine clinical trial
STI	sexually transmitted infection
TDF	tenofovir disoproxil fumarate
T _{max}	time after administration of a drug when the maximum plasma concentration is reached
UAB	University of Alabama at Birmingham
ULN	upper limit of normal
USA	United States of America
UTI	urinary tract infection
w/v	weight per volume
ZDV	zidovudine

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Expanded Safety Investigation of Tenofovir 1% Gel in Pregnancy and Lactation

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MTN-008

Expanded Safety Investigation of Tenofovir 1% Gel in Pregnancy and Lactation

INVESTIGATOR SIGNATURE FORM

**Version 1.0
March 29, 2010**

A Study of the Microbicide Trials Network

Sponsored by:

Division of AIDS, US National Institute of Allergy and Infectious Diseases
US *Eunice Kennedy Shriver* National Institute of Child Health and Human Development
US National Institutes of Health

Study Product Provided by:

CONRAD

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), CONRAD, or the Microbicide Trials Network (MTN) 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 Food and Drug Administration (FDA) is notified that the Investigational New Drug application (IND) is discontinued. Publication of the results of this study will be governed by MTN policies. Any presentation, abstract, or manuscript will be submitted to the MTN Manuscript Review Committee, DAIDS, NICHD, and CONRAD 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

MTN-008

Expanded Safety Investigation of Tenofovir 1% Gel in Pregnancy and Lactation

PROTOCOL SUMMARY

- Short Title:** Tenofovir Gel in Pregnancy and Lactation
- Clinical Phase:** Expanded Phase 1
- IND Sponsor:** Division of AIDS, NIAID, US NIH
- Protocol Chair:** Richard Beigi, MD, MSc
- Sample Size:** Pregnancy Cohort – approximately 90 mother-infant pairs
Lactation Cohort – approximately 15 mother-infant pairs
- Study Population:**
- Pregnancy Cohort**
- Healthy women, 3rd trimester gestation, HIV-uninfected, pregnant, 18 – 40 years old, without evidence of maternal or fetal complications in the current pregnancy
 - Group 1: approximately 45 participants between 37 0/7 and 39 1/7 weeks gestation (inclusive) on Study Day 0
 - Group 2: approximately 45 participants between 34 0/7 and 36 6/7 weeks gestation (inclusive) on Study Day 0
 - Infants of women in the Pregnancy Cohort
- Lactation Cohort**
- Healthy women, 18 – 40 years old, currently breastfeeding an infant between 4 – 26 weeks old (inclusive)
 - Breastfeeding infants of women in the Lactation Cohort, 4 – 26 weeks old (inclusive)
- Study Sites:** Alabama Microbicide CRS
Pitt CRS
Other sites as approved by the MTN Executive Committee
- Study Design:** Expanded Phase 1 double-blinded, placebo-controlled, randomized safety and pharmacokinetic (PK) trial
- Accrual into Pregnancy Cohort Group 2 will be contingent upon reassuring safety data obtained from Group 1, according to assessment by the MTN Study Monitoring Committee (further described in Section 10)
 - Accrual into the Lactation Cohort may occur in parallel and will not include participants who take part in the Pregnancy Cohort. The Lactation Cohort is not placebo-controlled

Study Duration:**Pregnancy Cohort**

Mother: Approximately 3-10 weeks per adult participant for scheduled procedures, depending on gestational age at study entry and timing of delivery

Infant: Birth through approximately 2 weeks of life for scheduled procedures

Lactation Cohort

Mother: Approximately 3 weeks per adult participant for scheduled procedures

Infant: Approximately 3 weeks for scheduled procedures

Approximately 16 total months for planned accrual and study duration for Pregnancy and Lactation Cohorts

Study Products:

Tenofovir 1% gel

Hydroxyethylcellulose (HEC) placebo gel (Pregnancy Cohort only)

Study Regimen:**Pregnancy Cohort**

The first dose of study product (tenofovir 1% gel or HEC placebo gel) will be administered at the study site at the Enrollment Visit (Day 0). The second through sixth doses of study product will be administered at home. The seventh and final dose of study product will be administered at the study site at the Day 6 Visit.

Lactation Cohort

The first dose of study product (tenofovir 1% gel) will be administered at the study site at the Enrollment Visit (Day 0). The second through sixth doses of study product will be administered at home. The seventh and final dose of study product will be administered at the study site at the Day 6 Visit.

≤ 4 Weeks Days Day 0 Day 1 Day 3 Day 6 Day 14 2 Weeks (+/- 7 Days)

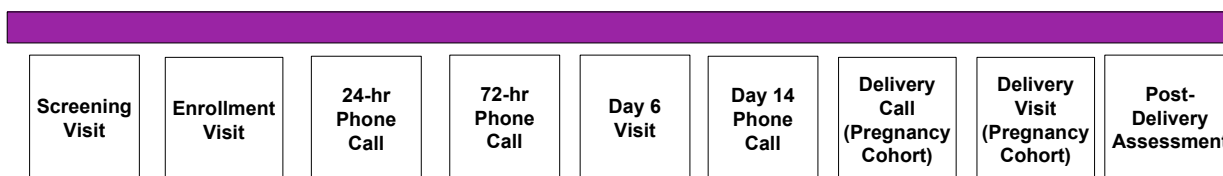


Figure 1: Study Visit Schedule

Primary Objectives:

1. To assess safety and tolerability of tenofovir 1% gel used daily for 7 days in pregnancy and lactation
2. To assess the pharmacokinetics of tenofovir 1% gel used daily for 7 days in pregnancy and lactation

Primary Endpoints:

1. Pregnancy Safety and Tolerability – Maternal Outcomes
 - Grade 2 or higher adverse events (AEs) in the following categories
 - Specific laboratory abnormalities
 - Alanine transaminase (ALT)
 - Aspartate aminotransferase (AST)
 - Creatinine
 - Specific genital/pelvic signs/symptoms
 - Dyspareunia
 - Pain (vulvar, vaginal, and/or pelvic)
 - Tenderness (vulvar, vaginal, and/or pelvic)
 - Itching (vulvar and/or vaginal)
 - Edema (vulvar, vaginal, and/or cervical)
 - Erythema (vulvar, vaginal, and/or cervical)
 - Lesions (vulvar, vaginal, and/or cervical)
 - Vulvar rash
 - Vaginal dryness
 - Dysuria
 - Vulvovaginitis
 - Cervicitis
 - Specific pregnancy complications
 - Postpartum hemorrhage
 - Postpartum endometritis
 - Chorioamnionitis
 - Third trimester bleeding
 - Preterm premature rupture of membranes (prior to onset of labor)
 - Term premature rupture of membranes (prior to onset of labor)

- Spontaneous preterm delivery
 - For adverse events not included above, Grade 3 or higher adverse events judged by the investigator to be related to the study gel or applicator
- 2. Pregnancy Safety and Tolerability – Infant Outcomes
 - Infant in the Pregnancy Cohort diagnosed (and confirmed on review of medical records) with any of the following during the 7 days following delivery
 - Intensive care admission greater than 24 hours. *Note: all known intensive care unit admissions will be reviewed for potential toxicities related to study product exposure*
 - Sepsis
- 3. Lactation Safety and Tolerability – Maternal Outcomes
 - Grade 2 or higher AEs in the following categories
 - Specific laboratory abnormalities
 - ALT
 - AST
 - Creatinine
 - Specific genital/pelvic signs/symptoms
 - Dyspareunia
 - Pain (vulvar, vaginal, and/or pelvic)
 - Tenderness (vulvar, vaginal, and/or pelvic)
 - Itching (vulvar and/or vaginal)
 - Edema (vulvar, vaginal, and/or cervical)
 - Erythema (vulvar, vaginal, and/or cervical)
 - Lesions (vulvar, vaginal, and/or cervical)
 - Vulvar rash
 - Vaginal dryness
 - Dysuria
 - Vulvovaginitis
 - Cervicitis
- 4. Lactation Safety and Tolerability – Infant Outcomes
 - Infant in the Lactation Cohort with the following during the period of study participation, confirmed on review of medical records
 - Inpatient admission (confirmed on review of medical records) with diagnosis of AE judged to be related to study product
- 5. Tenofovir levels
 - Maternal blood of participants in the Pregnancy and Lactation Cohorts
 - Breast milk of participants in the Lactation Cohort

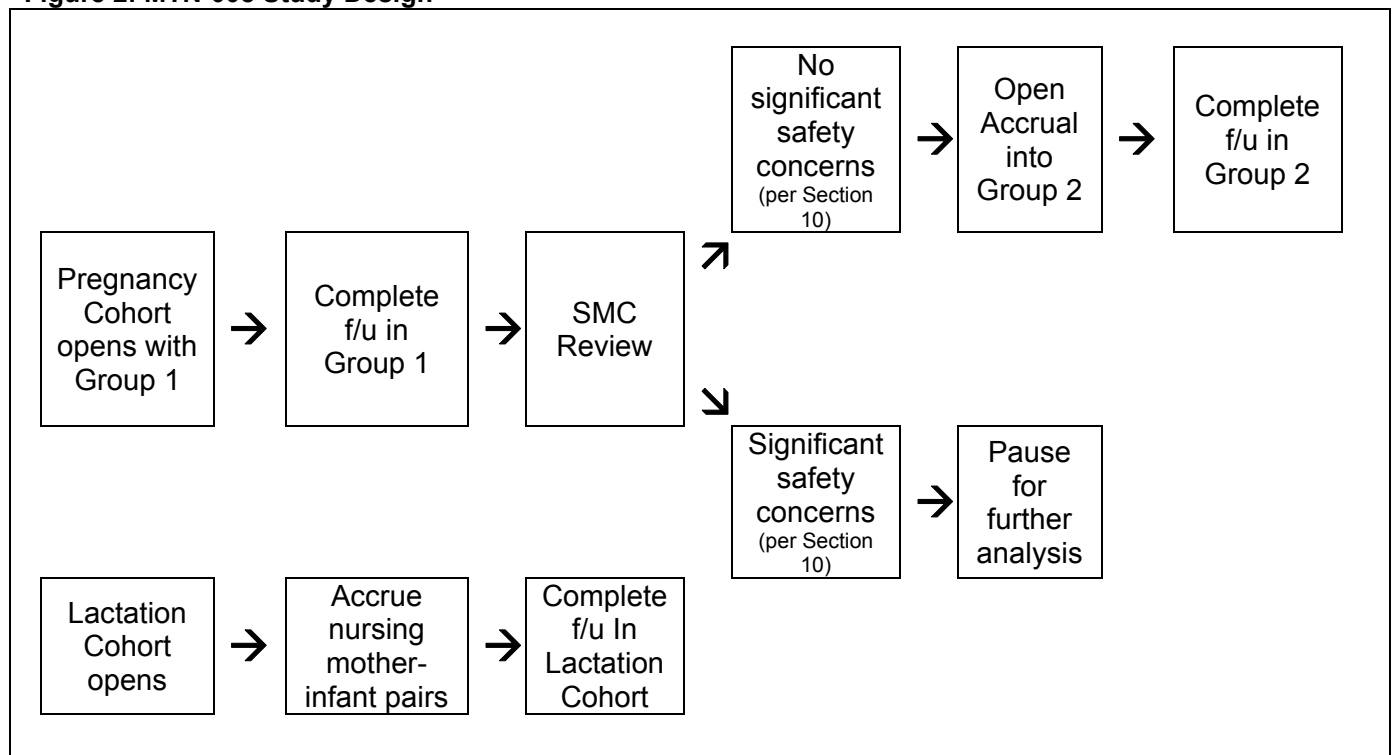
Secondary Objectives:

1. To assess for the presence of tenofovir in blood among infants of participants in the Pregnancy and Lactation Cohorts
2. To assess the impact of tenofovir gel exposure on the presence of select organisms associated with neonatal sepsis among participants in the Pregnancy Cohort, (e.g., Group B β -hemolytic *Streptococcus*, *E. coli*)
3. To assess the adherence to daily use of tenofovir 1% gel for 7 days and its acceptability among pregnant and lactating women

Exploratory Objectives:

1. To measure vaginal flora characteristics and to descriptively examine changes in these characteristics over the course of daily tenofovir 1% gel use among pregnant and lactating women
2. To assess the effects of tenofovir 1% gel on biomarker expression in vaginal and cervical secretions of pregnant and lactating women

Figure 2: MTN-008 Study Design



KEY ROLES

1.1 Protocol Identification

Protocol Title: Expanded Safety Investigation of Tenofovir 1% Gel in Pregnancy and Lactation

Protocol Number: MTN-008

Short Title: Tenofovir Gel in Pregnancy and Lactation

Date: March 29, 2010

1.2 Sponsor Identification

Sponsor: DAIDS/NIAID/NIH
6700 B Rockledge Drive
Bethesda, MD 20892 USA

Sponsor: US National Institute of Child Health and Human Development (NICHD)
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Network Laboratory

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Pharmacology: MTN NL Pharmacology CORE
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Data Center

Data Center: Statistical Center for HIV/AIDS Research & Prevention
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INTRODUCTION

HIV/AIDS Prevention Methods

More than twenty five years into the Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS) epidemic, the search for safe and effective methods of HIV prevention continues. As HIV incidence continues to rise in many areas of the world, the overwhelming majority of new HIV infections are occurring via heterosexual transmission, particularly among women in sub-Saharan Africa. Increasing availability of antiretroviral therapy (ART) for infected persons will not address HIV incidence, because the pace of new infections exceeds that of treatment initiation by a ratio of 6:1.¹ Although male circumcision appears to provide considerable protection against HIV infection in men² and when adopted widely, should reduce risk of HIV transmission at the population level, a major benefit to vulnerable women will not begin to accrue for several years, even with widespread uptake of this procedure.³

A series of reports has recently emphasized the daunting challenge of preventing HIV acquisition in women at high risk. The cervical diaphragm was shown to be of no benefit in a large randomized controlled trial.⁴ Trials of cellulose sulfate, a candidate microbicide, were halted when preliminary evidence in one study suggested a trend towards increased risk of HIV infection among women who received the product.⁵ The STEP study, a trial of a promising HIV vaccine based on an adenovirus delivery system, was halted in 2007 after the first interim analysis met

the futility criteria. Additionally, the interim analysis of the STEP trial indicated that the risk of HIV infection was higher in vaccine recipients, among the subset of participants with pre-existing high titers of antibodies to adenovirus type 5, particularly among men who were not circumcised.⁶ A Phase 3 trial of the candidate microbicide Carraguard[®] failed to demonstrate efficacy in preventing male-to-female HIV transmission, although it was shown to be safe for vaginal use.⁷

HIV Prevention Trials Network (HPTN) 035, a Phase 2/2b, four-arm, multi-site, randomized controlled trial evaluated the safety and effectiveness of two candidate vaginal microbicides, BufferGel and 0.5% PRO 2000/5 Gel (P). In this trial, PRO 2000 was found to be 30% effective, though the finding was not statistically significant. HPTN-035 was the first human clinical study to suggest that a microbicide may prevent male-to-female sexual transmission of HIV infection.^{8,9}

MDP 301 is a recently completed Phase 3 placebo-controlled study of 0.5% PRO 2000 Gel. Unfortunately, MDP 301 results did not demonstrate any protection against HIV.¹⁰ Thus, continued investigation of promising chemoprophylactic strategies, including their use among special populations remains of vital importance for the field of HIV prevention.

Tenofovir Gel and the MTN Research Agenda

Tenofovir 1% vaginal gel (tenofovir gel) has been chosen as a high-priority microbicide candidate for a number of reasons, including its activity in target cells for HIV infection (Langerhans cells, monocyte/macrophages, and T cells) of the vagina and cervix and the low frequency of local and systemic toxicity observed in clinical studies. Importantly, animal studies have demonstrated that tenofovir gel prevents the establishment of systemic infection in macaques when administered prior to or following intravaginal challenge with simian immunodeficiency virus (SIV) and that it inhibits vaginal transmission of SIV in macaques.¹¹ Data from HPTN 059 show that women using daily tenofovir gel had no difference in adverse events (AEs) or adverse changes in vaginal flora compared to those in the placebo gel arm. A Phase 2 study among sexually active women (MTN-001) has been designed to compare the safety and pharmacokinetics (PK) of oral versus vaginal tenofovir. Thus far, vaginal administration of tenofovir gel has been shown to be safe and well-tolerated among reproductive age women.²

To pursue a comprehensive research agenda, the MTN strives to include in its clinical trials portfolio a diverse set of at-risk populations, including pregnant and lactating women. MTN-008 will build on the preliminary safety data obtained in the first Phase 1 study of tenofovir 1% gel among healthy term gravidas undergoing cesarean section, MTN-002. The proposed study of repeat dosing in third trimester pregnancy and during lactation is the next step for the microbicide field in the evaluation of maternal-child safety and PK.

Candidate Microbicides: Pregnancy and Lactation

The study of candidate microbicides in pregnancy is compelling for many practical and scientific reasons. Microbicides are intended to be used among sexually active women and men to reduce the risk of transmission of HIV and other STIs. Pregnancy (both intended and unintended) is common in sexually active reproductive age women, and among pregnant and post-partum women, sexual activity is common, including sexual activity with multiple partners. Previous investigations suggest that pregnancy may represent a time of potentially heightened risk for the sexual acquisition of HIV.^{12, 13}

If microbicides become widely available, pregnant women will likely use them regardless of the availability of information regarding their safety in pregnancy. In the absence of sufficient safety data, recommendations to perform a pregnancy test prior to each use would provide a logistical barrier to widespread implementation. On a global level, pregnancy represents a time of maximal importance for HIV prevention given the documented increased risk for maternal-to-child perinatal HIV transmission with the high systemic HIV viral load characteristic of acute HIV infection. In theory, anti-HIV microbicides might be used to augment the prevention of maternal-to-child transmission of HIV; therefore, precise pharmacokinetic information and investigation are needed.

Pregnancy is common among sexually active women of childbearing age. The pregnancy rate among young sexually active women not using contraception in the United States is approximately 100/1,000 women/year, with the peak being in the 20-29 year old age range.¹ Pregnancy is estimated to occur in approximately 85% of non-contracepting sexually-active females trying to conceive per year. This rate varies across areas where HIV is common. When studying the same population of women using multiple forms of contraception, including perfect and non-perfect use, the pregnancy rate approximates 0.5-15%/year.^{14,15} Thus, pregnancy could reasonably be expected to be common among female microbicide users.

Among pregnant and post-partum women, numerous investigators have demonstrated that sexual activity is common, and sometimes includes multiple partners, which increases the risk of HIV and sexually transmitted infection (STI) acquisition. Solberg et al., using puerperal recollection of sexual activity during the previous pregnancy, demonstrated that although the majority of women reported decreased coital frequency as pregnancy progressed, the large majority continued sexual activity, with 90% reporting sexual activity in the first trimester and greater than 50% reporting sexual activity in the 3rd trimester.¹⁶ Klebanoff et al. found similar results using the Collaborative Perinatal Project dataset, demonstrating that 90% were sexually active in the first trimester and greater than 25% were still engaging in coitus at 38-39 gestational weeks.¹⁷ The Vaginal Infections and Prematurity Study Group data demonstrated that greater than 14% of women had more than one sexual partner in the previous year, and 4% stated they had more than one sexual partner during the incident pregnancy.¹⁸ Lastly, Rowland et al. using post-partum survey data demonstrated that within six weeks postpartum, nearly 50% of women

had resumed sexual activity.¹⁹ These studies highlight the fact that pregnancy is a time of frequent sexual activity with an ongoing risk for HIV acquisition.

Some studies have suggested an increased risk of HIV seroconversion in pregnancy, although none were designed to specifically address that question.^{13,20} The issue of susceptibility to HIV during pregnancy has recently been more directly addressed by Gray et al.¹² as part of the Rakai Community Cohort Study, and investigated HIV acquisition over a five-year period (1994-1999). The study identified 2,625 women who began a pregnancy with negative HIV serology, reported sexual activity, and had follow-up postpartum serology available. This was compared to 24,258 non-pregnant/non-lactating sexually-active women with complete serology. Using multivariate modeling, the risk ratio for HIV acquisition during pregnancy was 2.15 (95%CI 1.39-3.37) compared to non-pregnant/non-lactating women. This analysis controlled for numerous behavioral characteristics, including sexual activity of the male partners that suggests the pregnancy itself was physiologically responsible for the increased risk of HIV seroconversion. In contrast, a more recent prospective cohort study by Morrison et al. suggested that pregnancy may not heighten the risk of HIV acquisition.^{1, 21} While the susceptibility to sexual transmission of HIV inherent in pregnancy is still controversial, new HIV infections during pregnancy carry a large public health burden given the high viral loads that accompany new HIV infection and the documented importance of viral load on maternal-to-child transmission.² These findings compel the medical community to improve HIV prevention strategies in this potentially vulnerable physiologic time period of pregnancy to decrease the overall burden of new HIV infections.

In addition to the above reasons for investigation in pregnancy, a potential role exists for microbicides in decreasing maternal-to-child intrapartum transmission. If microbicides do decrease genital tract viral load late in pregnancy, this method could be used in regions of the world where oral and intravenous medications are logistically difficult to use. Potentially, the use of microbicides could augment the current armamentarium of drugs used in pregnancy, and may provide a local method to decrease perinatal HIV transmission without exposing fetuses to systemic levels of medications. Continued investigation is necessary to examine this potential strategy.

An Institute of Medicine report on the methodological challenges in HIV prevention trials included among its key recommendations the need to evaluate the potential effects products may have on pregnant women and their fetuses.²² This report and a recent NICHD-sponsored meeting highlight the increasing international attention to the complicated issue of pregnancy in HIV prevention trials, and compel the MTN to continue to move forward with carefully designed trials addressing microbicide use among pregnant women. Data presented at an NICHD-sponsored workshop (“Pregnancy and Contraception in Microbicide Development”) demonstrated this challenge. The documented pregnancy rates from recently completed and ongoing HIV prevention trials range from 3.6-28.7 per 100 woman-years of follow-up (2.9-21.5% of enrolled participants). Typically, participants are removed from the study

treatment as soon as pregnancy is documented. This practice complicates the planning, cost, logistics and statistical analysis of microbicide studies. The recent workshop provided a forum to discuss strategies to deal with pregnancy-related issues in HIV prevention research. Representatives from the FDA participated in this workshop. Notably, FDA representatives indicated that “there is a unique opportunity to systematically and rigorously collect safety, pharmacokinetic, and activity data on the use of microbicides in pregnancy”.

There is ample evidence for the health benefits to infants who are exclusively breastfed during the first six months of life.²³ Breastfeeding is commonly practiced among women living in countries with a high prevalence of HIV infection, with the breastfeeding period commonly extending for one to two years or more.²⁴ Women with multiple children may spend many years of their lives in serial periods of pregnancy and breastfeeding, sometimes with overlap between the two states. These women constitute a unique population at risk for HIV infection, and because of the potential for new infections to occur during the perinatal and breastfeeding periods, one in which HIV prevention is of vital importance. However, in studies of investigational agents for the prevention of HIV infection in adults, breastfeeding women are commonly excluded due to the lack of sufficient safety and pharmacokinetic data. This restriction impacts not only the availability of this important data, but also the power of a prevention trial, as woman-years of follow-up are lost due to time off of study product. Thus, it is critical that the prevention field rapidly accumulate these data.

Due to the favorable safety profile of the oral formulation of tenofovir in pregnancy and lactation, the absence of significant safety findings in the first study of tenofovir gel in pregnancy, as well as advanced stage of evaluation in the prevention field, the MTN has proposed to move forward with the study of tenofovir 1% gel in pregnancy and lactation.

Tenofovir 1% Gel

2.4.1 Description

Tenofovir 1% gel contains 1 gram (g)/100 milliliter (mL) of PMPA (9-R-2-phosphonomethoxypropyl adenine monohydrate), an acyclic nucleotide analogue with activity *in vitro* against retroviruses, including HIV-1 and HIV-2, as well as hepadnaviruses.¹¹ Further information is available in the current version of the tenofovir gel investigator’s brochure.

2.4.2 Mechanism of Action

Tenofovir disoproxil fumarate is an acyclic nucleotide analogue of adenosine monophosphate.¹² Tenofovir disoproxil fumarate requires initial conversion to tenofovir via diester hydrolysis and subsequent phosphorylations by cellular enzymes to form tenofovir diphosphate. Tenofovir diphosphate inhibits the activity of HIV-1 reverse transcriptase (RT) by competing with the natural substrate

deoxyadenosine 5'-triphosphate and, after incorporation into deoxyribonucleic acid (DNA), by DNA chain termination. Tenofovir diphosphate is a weak inhibitor of mammalian DNA polymerases α , β , and mitochondrial DNA polymerase γ .

2.4.3 Strength of Study Product

The strength of the tenofovir gel will be the strength (1%) previously tested in CONRAD A04-095 (IND 73,382) and A04-099 (IND 73,382), HPTN 050 (IND 55,690), HPTN 059 (IND 55,690), MTN-001 (IND 55,690), and MTN-002 (IND 55,690), and planned or in use in MTN-003 (IND 55,690), Rectal Microbicide Program (RMP)-02/MTN-006 (IND 73,382) and MTN-007 (IND 73,382). Due to the density of the study gel, the 4 mL application in this study contains approximately 44 milligrams (mg) of tenofovir.

2.5 HEC Placebo Gel

2.5.1 Description

HEC placebo gel or the “universal” placebo gel is a vaginal product that contains hydroxyethylcellulose as the gel thickener, purified water, sodium chloride, sorbic acid and sodium hydroxide.²⁵ The gel is isotonic and formulated at a pH of 4.4 to avoid disrupting the normal vaginal pH and has minimal buffering capacity in order to avoid the inactivation of sexually transmitted pathogens. Hydroxyethylcellulose, the gelling agent, is used to approximate the viscosity of other microbicide gel candidates.

Table 1: HEC Placebo Gel Formulation

Ingredient	Amount (w/w)
Purified Water, USP	96.3
Hydroxyethylcellulose, NF	2.7
Sodium Chloride, USP	0.85
Sorbic Acid, NF	0.1
Sodium Hydroxide, NF	qs pH 4.4

2.5.2 Mechanism of Action

The placebo gel is designed to be inactive in the vagina.²⁵ The gel is isotonic and formulated at a pH of 4.4 to avoid disrupting the normal vaginal pH and has minimal buffering capacity to avoid the inactivation of sexually transmitted pathogens.

2.5.3 Strength of Study Product

There is no active ingredient in the HEC placebo gel. 2.7% w/w HEC placebo gel will be used in this study.

2.6 *In vitro* Studies

2.6.1 *In vitro* Studies of Tenofovir

Anti-HIV-1 Activity

The *in vitro* antiviral activity of tenofovir against laboratory and clinical isolates of HIV-1 was assessed in lymphoblastoid cell lines, primary monocyte/macrophage cells and peripheral blood lymphocytes. The EC₅₀ (50% effective concentration) values for tenofovir were in the range of 0.04 µM - 8.5 µM. In drug combination studies of tenofovir with nucleoside reverse transcriptase inhibitors (NRTI) (abacavir [ABC], didanosine [ddI], lamivudine [3TC], stavudine [d4T], zalcitabine [ddC], and zidovudine [ZDV]), non nucleoside reverse transcriptase inhibitors (NNRTIs) (delavirdine [DLV], efavirenz [EFV], and nevirapine [NVP]) and protease inhibitors (amprenavir [APV], indinavir [IDV], nelfinavir [NFV], ritonavir [RTV], and saquinavir [SQV]), additive synergistic effects were observed. Tenofovir displayed antiviral activity *in vitro* against HIV-1 clades A, B, C, D, E, F, G, and O (EC₅₀ values 0.5 µM - 2.2 µM) and showed strain-specific activity against HIV-2 (EC₅₀ values ranged from 1.6 to 5.5 µM).

Resistance

HIV-1 isolates with reduced susceptibility to tenofovir have been selected in cell culture.²⁶ These viruses expressed a K65R mutation in RT and showed a 2 – 4 fold reduction in susceptibility to tenofovir.

In Study 903 of treatment-naïve patients (VIREAD + lamivudine + efavirenz versus stavudine + lamivudine + efavirenz), genotypic analyses of isolates from patients with virologic failure through Week 144 showed development of efavirenz and lamivudine resistance-associated substitutions to occur most frequently and with no difference between treatment arms. The K65R substitution occurred in 8/47 (17%) analyzed patient isolates on the VIREAD arm and in 2/49 (4%) analyzed patient isolates on the stavudine arm. Of 8 patients whose virus developed K65R in the VIREAD arm through 144 weeks, 7 of these occurred in the first 48 weeks of treatment and one at Week 96. Other substitutions resulting in resistance to VIREAD were not identified in this study.

In Study 934 of treatment-naïve patients (VIREAD + EMTRIVA + efavirenz versus zidovudine (AZT)/lamivudine (3TC) + efavirenz), genotypic analysis performed on HIV-1 isolates from all confirmed virologic failure patients with >400 copies/mL of HIV-1 RNA at Week 144 or early discontinuation showed development of efavirenz resistance-associated substitutions occurred most frequently and was similar between the two treatment arms. The M184V substitution, associated with resistance to EMTRIVA and lamivudine, was observed in 2/19 analyzed patient isolates in the VIREAD + EMTRIVA group and in 10/29 analyzed patient isolates in the zidovudine/lamivudine group. Through 144 weeks of Study 934, no patients have developed a detectable K65R substitution in their HIV-1 as analyzed through standard genotypic analysis.

Cross-resistance

Cross-resistance among certain RT inhibitors has been recognized.²⁶ The K65R substitution selected *in vitro* by tenofovir is also observed in some HIV-1 isolates from subjects treated with abacavir, didanosine, or zalcitabine. HIV-1 isolates with this mutation also showed reduced susceptibility to emtricitabine (FTC) and lamivudine. Therefore, cross-resistance among these drugs may occur in patients whose virus harbors the K65R substitution.

2.6.2 *In vitro* Studies of HEC Placebo Gel

Anti-HSV Activity

CF-1 mice (n=10 per group) pretreated with medroxyprogesterone acetate were administered 0.02 mL of HEC placebo gel or phosphate-buffered saline (PBS) vaginally, followed by a 0.01 mL of HSV-2 viral inoculum of 10 ID₅₀ 0.3 minutes later.²⁵ On day 3, vaginal lavage was cultured on human foreskin fibroblasts, and mice were considered infected if a cytopathic effect was observed after 3 days of incubation. Infection rate following pretreatment with HEC placebo gel (90%) was not significantly different from pretreatment with PBS (80%) or from mice given no treatment (80%). HEC placebo gel did not enhance susceptibility of mice to HSV-2 when administered 12 hours before vaginal challenge.²⁵

Anti-HIV-1 Activity

In vitro analyses of anti-HIV activity were also performed on HEC placebo gel following a viral binding assay that consisted of a 2-hour incubation of test compound, HIV-1_{IIIB}, and MT-2 cells.²⁵ Cell culture followed by further assessments performed after this incubation period showed no significant antiviral or cytotoxic activity. The HEC placebo gel had negligible effect on virus-induced cytopathic effect at a 1:5 dilution, the highest concentration tested. Additional *in vitro* studies on potential HIV-1 infection of neoplastic T cell lines concluded that the HEC placebo gel had little or no effect on the infection and replication of HIV in human target cells, or the specific replication steps of virus attachment or cell-to-cell fusion.²⁵

The effect of the HEC placebo gel on vaginal transmission of SHIV_{162p3} (10³ TCID₅₀) to rhesus monkeys (n=5, n=3, respectively) was determined in two separate studies.²⁷ Macaques pretreated with medroxyprogesterone acetate were vaginally administered 1 mL of the HEC placebo gel formulation 15 minutes prior to challenge with 0.5 mL SHIV_{162p3}. Investigators monitored total RNA load in the animal plasma for a total of 8 weeks by means of a standard quantitative RT-PCR. The first study utilized the HEC placebo gel formulation at pH 6.5; the second study utilized a formulation at pH 4.4. In both studies, all monkeys were infected, as determined by the presence of viral RNA in circulating blood, regardless of the pH of the formulation.

Cytotoxicity

Dilutions of the HEC placebo gel in culture medium exhibited negligible toxicity to human vaginal epithelial cells (standard MTT assay), even at the lowest dilution tested (1:2).²⁵ Exposure of human vaginal epithelial cells to the HEC placebo gel resulted in minimal IL-1 α induction, even at the lowest dilutions tested (lowest dilution, 1:2).²⁷

Spermatozoa Motility

Analyses of pH (HEC placebo gel mixed with human seminal plasma, 8.03 \pm 0.26) found that the HEC formulation did not show significant buffering capacity and could not acidify the alkaline pH of seminal plasma, a favorable result in a placebo formulation.²⁷ *In vitro* assessments of spermicidal activity utilizing human semen from healthy donors showed that the HEC placebo gel had no significant deleterious effects on sperm motility, even after a 60-minute incubation.

2.6.3 Condom Compatibility Studies of Tenofovir 1% Gel

The compatibility of tenofovir 1% gel was also tested with three types of lubricated male latex condoms.¹¹ A matched placebo gel and HEC placebo gel were used as comparator gels. The condoms tested were representatives of leading brands on the US market (Trojan[®] and Durex[®]) with either silicone or aqueous lubricant. The airburst test was used to evaluate changes in film integrity (strength) and test specimens were measured before and after treatment with the gels to assess changes in strength properties following the application of the three gel preparations. All three gels were shown to be compatible with the above condoms. The compatibility of tenofovir 1% gel with Alatech[™] Healthcare male latex silicone-lubricated condoms was also evaluated, with matched placebo gel used as a comparator. The two application treatments of tenofovir 1% gel and matched placebo gel increased airburst volumes by 5 – 6 L compared with the baseline. With an increase in volumes there was a decrease in airburst pressures by 0.2 kPa. This implies a physical change to a more elastic condom. This slight change in physical properties suggests an interaction of the tenofovir 1% gel with the silicone lubricant, but does not indicate that the condoms are unsuitable for use in clinical studies.

2.7 Animal Studies

2.7.1 Animal Studies of Tenofovir 1% Gel

Pharmacokinetics

Single-dose PK of vaginally-administered tenofovir gel in female rabbits has been previously examined (0.5 mL, 1% weight/volume (w/v) tenofovir, 5 mg/animal, 50 μ Ci/kg).ⁱⁱ Plasma radioactivity concentrations were highest at the first sample time point (0.5 hr) and below the level of quantitation at 24 hours. PK parameters including the proportion of dose absorbed systemically could not be estimated, due to very low plasma concentrations.

In a tissue distribution study using the same radiolabeled tenofovir (C-PMPA) 1% vaginal gel formulation, dose and strength as the above study, eighteen female rabbits were administered an intravaginal dose using a gavage needle.¹¹ An additional eighteen rabbits received an intravaginal dose of 3% w/v tenofovir (15 mg per animal). Analysis of vaginal tissue sections found no clear relationship between tissue concentration and dose, with no consistent pattern of distribution. Very little radioactivity was recovered in non-vaginal tissues. Concentrations in blood (0.002 to 0.047 µg-eq/g of tissue) exemplified the variability of distribution of the product although the effect of oral absorption due to grooming behaviors of the animals may have impacted these results.

The PK, excretion and tissue distribution of ¹⁴C-PMPA were evaluated in rats following intravaginal administration of an earlier formulation of tenofovir gel containing propylene glycol.¹¹ Four female rats received a single intravaginal dose administered as an aqueous gel containing 20 mg tenofovir/g. Plasma concentrations of total radioactivity were highly variable; this was attributed to inconsistent retention of the formulation within the vagina, or possibly oral absorption related to grooming. The apparent maximum concentration (C_{max}) for tenofovir occurred at the earliest time point (15 minutes), suggesting that absorption from the vagina was relatively rapid. Thereafter, plasma concentrations declined with an approximate half-life of 1.6 hours. The bioavailability of intravaginal tenofovir was estimated by comparison of the observed area under the curve ($AUC_{(0-24)}$) with historical AUC data for an intravenous dose of 10 mg/kg tenofovir in rats (9.71 µg h/mL). The observed systemic bioavailability of intravaginal tenofovir was 7.9%.

In the excretion and distribution study, two groups of four additional rats received a single intravaginal dose of ¹⁴C-PMPA (approximately 10 mg/kg, 100 µCi/kg) administered as aqueous gel containing 20 mg tenofovir/g. This study found that much of the dose was lost from the vaginal orifice by leakage. Vaginal tissue contained 0.1% of the dose and less than 0.01% of the dose was recovered in the ovaries and uterus.

The PK of C-PMPA was evaluated via plasma and vaginal biopsies collected from four rhesus monkeys following single-dose intravaginal tenofovir 1% vaginal gel.¹¹ Radioactivity was detected starting at 15 minutes post-application, with peak concentration of tenofovir in vaginal tissue at 8 hours and remaining high at 12 hours. No significant radioactivity was detected in whole blood or plasma.

Toxicology

The preclinical toxicity of tenofovir gel has been evaluated in 14-day rat and 10-day rabbit vaginal irritation and toxicity studies.^{11,28} Daily intravaginal administration of tenofovir gel produced no vaginal irritation in rats (≤10% tenofovir) and minimal to mild vaginal irritation in rabbits (3% or 10% tenofovir).

14-Day Vaginal Irritation and Toxicity Study of Tenofovir Gel in Rats

Ten female Sprague Dawley rats/group received either 0% (vehicle control), 1%, 3%, or 10% tenofovir gel (2.5% HEC formulation) by intravaginal administration (0.5 mL/dose) once daily for 14 days. There were no mortalities, and no tenofovir-related clinical signs of toxicity or changes in body weight, food consumption, or absolute/relative kidney weights. Individual and mean vaginal (gross) irritation scores for all tenofovir-dosed animals sacrificed at Day 15 were graded as 0 (no erythema or edema); microscopic irritation scores for the vagina, cervix, ovaries, uterine horns, and vulva were graded as 0 (normal histology). No tenofovir-related histopathological effects on the vagina, cervix, ovaries, uterine horns, vulva, or kidneys were observed.

10-Day Vaginal Irritation Study of Tenofovir Gel in Rabbits

The potential irritant effects of tenofovir were evaluated in vaginal tissues of female New Zealand White rabbits using three different gel formulations (2.5% HEC or 1.0 – 2.0% Carbopol[®] 1342).²⁹ This study consisted of eleven treatment groups (five rabbits/group) that received either; a sham treatment or Conceptrol[®] (positive control); 0%, 0.3%, 1.0%, 3.0%, or 10.0% tenofovir formulated in the HEC placebo gel preparation; or 0% or 3.0% tenofovir formulated in a 1.0% or 2.0% Carbopol[®] 1342 gel preparation. With the exception of the sham dose group, all rabbits received dose formulation (1.0 mL/dose) daily applied topically to the mucosal surface of the vaginal vault for 10 consecutive days. No mortalities, tenofovir-related clinical signs of toxicity, or body weight changes were observed in this study. Group composite vaginal irritation scores for the 10% tenofovir topical gel (HEC formulation), 0% tenofovir (1.0% Carbopol[®] 1342 formulation), and Conceptrol[®] (positive control) dose groups were each rated as “mild.” Composite vaginal irritation scores rated “minimal” were observed for all other tenofovir, vehicle or sham treatment groups, regardless of formulation. No unacceptable level of mucosal irritation was observed in any treatment group based on protocol-derived criteria for this animal model. Generalized erosion and/or ulceration were observed only in animals receiving Conceptrol[®] positive control (2 of 5) or 10% tenofovir gel (2 of 5).

2.7.2 Animal Studies of Tenofovir in Lactation

A pilot study evaluating the PK of tenofovir in breast milk was conducted by Van Rompay and colleagues in two healthy, lactating rhesus macaques.³⁰ Both macaques, whose infants were weaned prior to the PK study, had been lactating for more than 10 weeks. The macaques received a single subcutaneous dose of tenofovir (30 mg/kg) and blood samples were collected pre-dosing as well as 0.5, 1, 2, 4, 8, and 24 hours post-dosing. Tenofovir was detected in the milk of both animals. However, the peak concentrations (0.808 µg/mL and 0.610 µg/mL) were very low at approximately 2 to 4% of the concentrations detected in serum (18.3 µg/mL and 30.2 µg/mL). These data suggest that lactating women using tenofovir vaginal gel should have undetectable or very low levels of tenofovir in their breast milk, and thus maternal gel use will likely be of no clinical significance to nursing infants. Additional studies of tenofovir conducted in rhesus macaques further

demonstrate the safety of prolonged administration of tenofovir in newborn and infant macaques.^{31, 32}

2.7.3 Animal Studies of HEC Placebo Gel

Toxicology

Intravenous Administration

Up to 55 intravenous injections of HEC were given to dogs (dose and number not specified) without causing injury other than that typical of the other water-soluble cellulose ethers.²⁵ Only transitory changes in the blood picture and the deposition of the material on the intima of the blood vessels were noted. Groups of rats maintained for two years on diets containing HEC (n not specified, up to 5%) did not exhibit any adverse effects.²⁵ HEC has also been administered to rats in single oral doses as high as 23,000 mg/kg without observed toxic effects (n not specified).

Local Tolerance

A 10-day rabbit vaginal irritation study (10 per arm, 2 arms, HEC placebo gel vs. 0.9% saline control) found that the HEC placebo gel was not irritating to the vaginal mucosa of rabbits when dosed daily for 10 days.^{25, 27} One animal in the HEC placebo gel group had an instance of vaginal redness (compared to four animals in the saline group), which did not persist and was not evident at the end of the study. Diarrhea, few feces, and soiling of the anogenital area were noted in that animal. Body weight changes were noted to be normal. In 9 of 10 animals, necropsy results were normal. Anogenital soiling was observed in the animal that exhibited erythema during the in-life phase of the study. Histopathologic changes observed were similar to those seen in the control group and likely attributable to those that occur because of the repeated insertion of a catheter, rather than due to any effect of the test samples.

Developmental Toxicology

Intraperitoneal administration of unformulated HEC to pregnant mice in a 1% and 4% concentration caused an increase in resorption, but no detectable increase in birth defects.³³ While no epidemiological studies of congenital anomalies in infants born to women exposed to HEC during pregnancy have been reported, the Teratogen Information System (TERIS) considers the magnitude of teratogenic risk to a child born after exposure during gestation to be none.

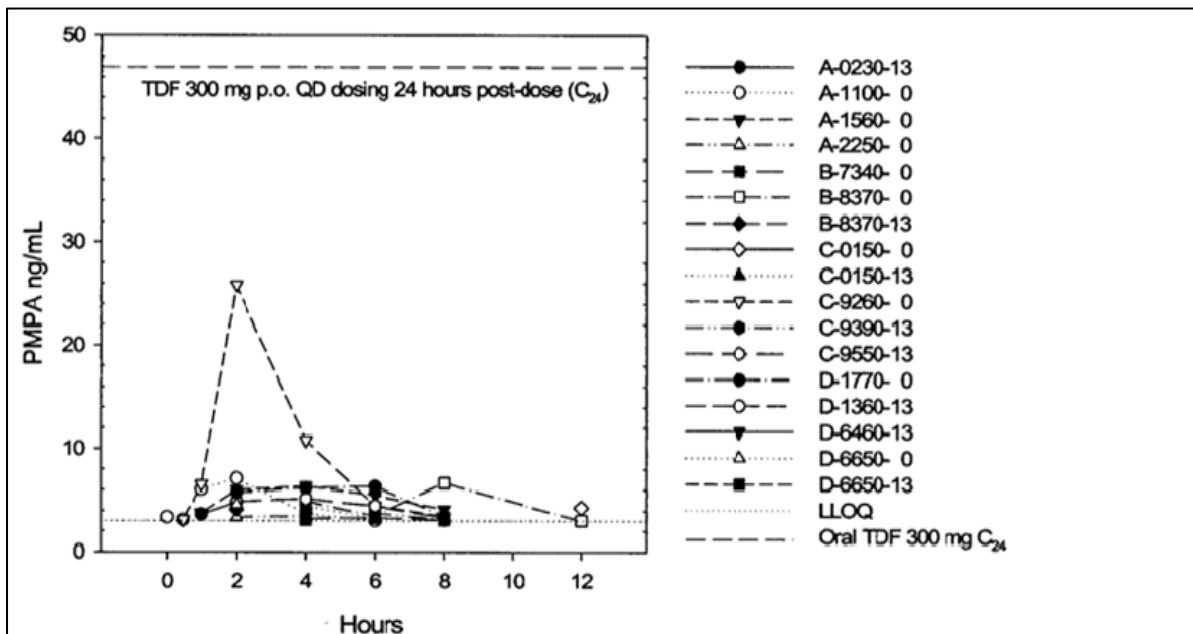
2.7.4 Clinical Studies of Tenofovir 1% Gel

Pharmacokinetics

Tenofovir (0.3% and 1%) gel was tested in 84 women in the HPTN 050 Phase 1 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. In addition to safety and acceptability, HPTN 050 also addressed PK of vaginally administered tenofovir gel.

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 C_{max} ranged from 3.0 - 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 two 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 C_{max} was 7.1 ng/mL. Considering all women in the PK cohort, the median tenofovir C_{max} was 3.4 ng/mL (interquartile range: below lower limit of quantitation (LLOQ) [3.0 ng/mL] to 4.7 ng/mL). The median C_{max} for all subjects (3.4 ng/mL) corresponds to approximately 1% of the maximum (C_{max} , steady-state (ss)) and 7% of the minimum (C24 single dose) blood concentrations at steady-state with 300 mg daily oral tenofovir dosing.³⁴

Figure 3 below demonstrates tenofovir blood concentration following vaginal administration of tenofovir 1% gel in HPTN 050. All levels for all women with measurable tenofovir levels in the blood are shown (14 of 25; 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 a dashed line.



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

Figure 3: HPTN 050

Similar PK data exist for HPTN 059. Among 285 participant visits in which PK blood draws were performed, tenofovir was detected among 50% and 59% of participants in the coitally-dependent and daily use regimens, respectively.³⁵

Tenofovir was evaluated as a candidate microbicide in CONRAD A04-095. The study assessed the concentration of tenofovir in 49 women who underwent a single-dose phase followed by a two-week phase. The participants administered study product either once or twice daily while in the two-week phase. Participants were asked to apply an intravaginal dose (4g) of tenofovir gel and provide blood samples at 0.5, 1, 2, 4, 6, 8, and 24 hr(s). Following the blood samples, participants were randomized to one of seven time-points [0.5, 1, 2, 4, 6, 8, and 24 hr(s)], for vaginal fluid collection and vaginal biopsies. Tenofovir was measured in blood plasma, fluid, and biopsies.

Data from a subset (N=21) of participants who completed the single-dose phase are presented here. The mean concentration in vaginal tissue at 0.5, 1, 2, 4, 6, 8 and 24 hr(s) were 275×10^3 , 450×10^3 , 186×10^3 , 89×10^3 , 69×10^3 , 24×10^3 and 15×10^3 ng/g of tissue, respectively, (LLOQ=1ng/mL) with a peak at 1-4 hrs. However, most blood plasma tenofovir concentrations were below 5 ng/mL. Although higher values (up to 19.5 ng/mL) were detected in four participants, these levels were not sustained. Vaginal fluid concentrations were high; $1.5\text{-}5.0 \times 10^6$ ng/mL through 8 hrs and $4.5\text{-}47.1 \times 10^4$ ng/mL at 24 hrs. Tissue elimination appeared to follow a multi-compartment model. Total tenofovir was detectable in vaginal tissue and fluid up to 24 hrs post single-dose exposure.³⁶

In MTN-002, the first microbicide trial to be conducted during pregnancy, 16 women received a single vaginal dose of tenofovir 1% gel prior to elective cesarean section. Tenofovir levels were measured in blood, amniotic fluid, cord blood, endometrial tissue, and placental tissue. Plasma tenofovir levels were compared to historical controls. No significant safety events were seen as the final safety review has now been performed for this unblinded study. Results are anticipated in May 2010.

Safety

In HPTN 050, the tenofovir 1% gel formulation was well-tolerated in both HIV-uninfected and -infected women.³⁷ Ninety-two percent reported at least one AE. The majority of these events were mild (87%) and limited to the genitourinary tract (77%). The five most frequently reported mild genital AEs were pruritus (n = 18), erythema (n = 14), petechiae/ecchymosis (n = 14), vaginal discharge (n = 13), and burning (n = 10). Four severe AEs were reported, but only one (lower abdominal pain) was thought to be product-related. Product concentration, sexual activity and HIV status were not associated with a specific AE pattern. No clinically significant systemic toxicity was observed. No serious adverse events (SAEs) were reported.

Among 84 female participants in HPTN 050, 76 had bacterial vaginosis (BV) evaluation (using Nugent score criteria) at both enrollment and Day 14. Of these, 30 women had asymptomatic BV at baseline; 15 of these were found to be BV negative

on Day 14. Among 46 women without BV at baseline, one 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 ($p = 0.0005$), suggesting that the gel did not increase women's risk of developing BV.³⁷

In a male tolerance study (CONRAD A04-099/IND 73,382), tenofovir 1% gel was well-tolerated in men following seven days of once-daily penile exposure.³⁸ There were few genital findings observed after product use and all findings were classified as mild, small in size and requiring no treatment. Reported symptoms were mild, of short duration and resolved by the final visit. There were no noticeable differences between signs and symptoms of genital irritation in the circumcised compared to uncircumcised group. Tenofovir gel applied to the penis for seven days was well-tolerated locally and systemically, and it is unlikely that male partners exposed to small amounts of tenofovir gel will experience significant genital or systemic toxicity.

A Phase 2 study of tenofovir 1% gel (HPTN 059) assessed safety and acceptability of, and adherence to, a regimen of tenofovir gel for vaginal use in HIV-uninfected women versus a placebo gel. Exploratory objectives included measurement of vaginal flora characteristics, assessment of the effects of gel on genital cytokine and chemokine expression, and the evaluation of cytokine and chemokine expression to correlate expression with evidence of inflammation, epithelial disruption and genital symptoms. The study was a four-arm, three-site, randomized, controlled trial comparing gel used once daily and gel used prior to intercourse, to placebo gel, with 6 months gel exposure and follow-up. The study was conducted among 200 women in Pune, India; Birmingham, Alabama, USA; and New York, New York, USA. Participants were sexually active, HIV-uninfected women aged 18 to 50, but not menopausal or post-menopausal. Participants received single-use unit dose tubes and single-use applicators.

No statistically significant differences were seen between those receiving active and placebo gels in complete blood count (CBC), liver function tests, or renal function tests. Among women using study gel, no participants had pelvic exam findings involving generalized erythema or severe edema or deep epithelial disruption at any follow-up visit during the study. At the Week 24 Visit, no participants had exam findings suggestive of vaginitis, cervicitis, superficial disruption, disrupted blood vessels, or intermenstrual bleeding. Adherence to study gel was high, and was supported by PK data. 79% of women reporting gel use in past 12 hours had low but detectable plasma tenofovir supporting self-reported adherence data. Daily and coital use was highly acceptable to women. These data suggest a favorable safety and acceptability profile of tenofovir gel.³⁵

Resistance

In HPTN 050, no new resistance mutations evolved in plasma or cervicovaginal lavage after 14 days of tenofovir gel use. Three women had plasma mutations associated with low-level tenofovir resistance identified at Days 0 and 14 (M41L, L210M, \pm T215I/Y).

2.7.5 Clinical Studies of HEC Placebo Gel

Unformulated HEC is known to be a non-irritating substance in humans (skin sensitization is unusual), with doses less than 2 gm/kg by ingestion not expected to be toxic.³³ No inhalation studies have been conducted, but exposure of humans to the dust in manufacturing operations over many years has not led to any known adverse effects. The HEC placebo gel formulation was developed and adopted for use in the HPTN 035 microbicide study, the Phase 2/2b Safety and Effectiveness Study of the Vaginal Microbicides Buffer Gel and 0.5% PRO2000/5 Gel (P) for the Prevention of HIV Infection in Women.

A randomized, closed label, Phase 1 study of daily vaginal HEC placebo gel exposure was conducted in 2003.³⁹ In this trial, 30 women were randomized to twice-daily vaginal applications of 3.5 mL of HEC placebo gel or polystyrene sulfonate (PSS) vehicle. The primary objective of this study was to assess and compare the effects of the test articles on symptoms and signs of irritation of the external genitalia, cervix, and vagina as seen on naked eye exam after 7 and 14 days of use including disruption of the epithelium and blood vessels as seen on colposcopy after 14 days of use. Secondary objectives included: an assessment and comparison of differences in vaginal health by evaluating the results of wet mounts, pH, and Gram-stained vaginal smears (Nugent score and neutrophil counts) after 7 and 14 days of use and vaginal cultures after 14 days of use; and an assessment of acceptability of the study products after 14 days of use among participants.

Results of this trial indicated that both gels appear safe for use twice a day for 14 days in sexually abstinent women. Two out of 14 women (14.3%) randomized to the HEC placebo gel reported at least one symptom of mild severity of genital irritation, which included genital burning, soreness and pelvic pain. Three out of 14 women (21.4%) had colposcopic findings that included erythema, petechiae and peeling. No deep genital disruption was observed in either product group. Minimal changes in wet mounts, pH, Nugent scores, neutrophils, and vaginal flora were observed in both product groups.

A pilot study to optimize trial procedures for a proposed Microbicides Development Programme placebo controlled trial utilized the universal placebo gel as the study gel. Final analysis of results has not been completed but there were no serious adverse product related events reported.²⁵

2.8 Other Clinical Studies of Tenofovir for HIV Prevention

Several other studies of tenofovir for HIV prevention are ongoing or in development. These include studies in Table 2.

Table 2: Studies of Tenofovir 1% Gel

Location	Sponsor	Population	Design
South Africa	CAPRISA	Sexually-active women	Phase 2B, two-arm, randomized placebo controlled, coitally dependent
South Africa, Uganda, USA	DAIDS/MTN-001/IND 55,690	Sexually-active women	Phase 2 Adherence and Pharmacokinetics Study of Oral and Vaginal Preparations of Tenofovir
South Africa, Uganda, Zambia, Zimbabwe	DAIDS/MTN-003/IND 55,690	Sexually-active women	Phase 2B Safety and Effectiveness Study of Tenofovir 1% Gel, TDF Tablet and FTC/TDF Tablet
USA	CONRAD RMP-02/MTN-006/IND 73,382	Sexually-abstinent (for active phases of study and for 5 days following biopsy collection) women and men	Phase 1 Rectal PK and Acceptability
USA	CONRAD/MTN-007/IND 73,382	Receptive anal intercourse (RAI) abstinent (for duration of study) women and men	Phase 1 Rectal Safety and Acceptability

2.9 Studies of Tenofovir in Pregnancy and Lactation

Oral tenofovir is being evaluated for use in prevention of perinatal maternal-to-child transmission of HIV-1. Data on the first cohort of 15 women enrolled to Pediatric AIDS Clinical Trials Group (PACTG)/International Maternal Pediatric Adolescent AIDS Clinical Trials Group (IMPAACT) Protocol 394 have been presented.⁴⁰ Women were given an oral dose of 600 mg of tenofovir either at the onset of labor or four hours before scheduled cesarean delivery, and PK and safety in the mother and infant were evaluated. No significant AEs in the women or infants were attributed to tenofovir. The maternal tenofovir concentrations were similar to those seen after chronic dosing with 300 mg daily in non-pregnant individuals, despite the dose of 600 mg. Median cord blood levels were 76 ng/mL (range 0-309 ng/mL) and the median cord /maternal blood (C/M) ratio was 0.69. All levels were below the level of quantitation (25 ng/mL) in the infants at 12, 24, and 36 hours of age. An updated “second phase” of the study is continuing with a dose of 900 mg to the mother at onset of labor or before cesarean delivery. Preliminary results from this higher dose investigation continue to demonstrate safety and an acceptable absorption profile among the women receiving this higher dose. In addition, the tenofovir levels from the cord blood obtained at the time of delivery suggest a nearly identical C/M ratio of 0.66.⁴⁰

HPTN 057 is a Phase 1 safety and PK study of tenofovir disoproxil fumarate (TDF) conducted in HIV-1infected pregnant women and their infants in Malawi and Brazil.⁴⁰ A total of 53 mother-infant pairs divided into two cohorts have been evaluated in this study. In Cohort 1, only the mother received TDF (600 mg). In Cohort 2, the infant received 4 mg/kg TDF as oral suspension. An investigation in a third cohort where both the mother and infant receive TDF is currently underway.

Table 3: HPTN 057

HPTN 057	Dosing Regimen		PK Sampling	
	Maternal	Infant	Maternal	Infant
Cohort 1 (N=30)	TDF 600 mg at onset of labor or 4 h prior to scheduled C-section	No study drug	Pre-dose & 1, 2, 4, 8, 12, 18-24, and 36-48 h after dose	Cord blood & 4, 12, 18-24, 36-48 h after birth
Cohort 2 (N=20)	No study drug	TDF oral susp. 4 mg/kg at birth, Day 3, and Day 5 First dose as soon as possible, within 12 h of birth	None	Day 0: Cord blood, pre-dose & 2, 10, 18-24 h after dose Days 3 and 5: pre-dose & 2, 10, 18-24, 36-48 h after dose
Cohort 3 (N=30)	TDF 600 mg at onset of labor or 4 h prior to scheduled C-section*	TDF oral susp. 4 mg/kg at birth, Day 3 and Day 5 First dose as soon as possible, within 12 h of birth**	<i>Trial currently underway</i>	

*dose may be adjusted to 900 mg based on results of Cohort 1

**dose may be adjusted to 6 mg/kg based on results of Cohort 2

In Cohort 1, mothers received TDF 600 mg at the onset of labor or 4 hours prior to cesarean delivery. Infants received TDF as soon as possible after birth, and on Days 3 and 5 of life. Maternal PK sampling for TDF plasma concentration occurred pre-dosing and at 1, 2, 4, 8, 12, 18-24, and 36-48 hours post-dosing. Cord blood was collected once, as well as infant blood for PK at 4, 12, 18-24, and 36-48 hours after birth. The mothers in this cohort delivered at median of 2.9 hours after dosing. Amniotic fluid samples were also collected from women who delivered via cesarean section at a median of 4.1 hours after dosing (n=5). Data from Cohort 1 are presented in Tables 4 and 5 below.

Table 4: Median (range) of Maternal Tenofovir Levels in HPTN 057 (Cohort 1)

	Pregnant women, 600 mg (n=30)	Nonpregnant adults, 600 mg ³⁴
T_{max} (hr)	1.0 (1.0-8.0)	1.5
C_{max} (ng/mL)	448 (110-928)	573
AUC (ng*hr/mL)	4221 (2767-24459)	4389
T_{1/2} (hrs)	19.5 (11.1-32.8)	11.9

Table 5: Tenofovir Concentrations in Breast Milk in HPTN 057 (Cohort 1)

Day Collected	#	Number with Detectable Tenofovir (conc [ng/mL])
1	1	1 (17.8)
2	3	2 (106,6.3)
4-6	21	1 (15.7)
41-44	13	0
79-89	21	0

Results from Cohort 1 also show that maternal tenofovir delivery concentration was 122 (bdl-527) ng/mL, amniotic fluid concentration (n=5) was 259 (142-725) ng/mL, and cord blood tenofovir concentration was 76 (bdl-191) ng/mL. Tenofovir concentrations after the single oral dose given in Cohort 1 were above LLOQ in 27/30 mothers at delivery. The ratio of cord blood to maternal delivery concentrations of tenofovir was 0.61 (0.06-1.64) and the ratio of amniotic fluid to maternal tenofovir delivery concentration was 3.01 (1.56-4.90).

These data demonstrate that tenofovir accumulates in amniotic fluid and crosses into cord blood fairly rapidly. Approximately one-third of the infants had cord blood concentrations below the target tenofovir concentration of >50 ng/mL. These infants were born either <1.5 or >7 hours after maternal dosing.

Mirochnick examined HPTN 057 PK of tenofovir disoproxil fumarate (TDF) after single-dose administration to HIV-1 infected mothers; mothers and infants were followed for safety and tolerance. Of the 53 mother-infant pairs enrolled, breast milk was collected from 25 breastfeeding mothers who received a single 600 mg dose of TDF tablets at onset of labor or 4 hrs prior to cesarean section. Tenofovir was detectable in 4/25 (16%) breast milk samples collected during the infants' first week of life with concentration of 13 (6-18) ng/mL. It is unclear from the limited data set the extent of infant tenofovir exposure during breastfeeding with chronic maternal tenofovir dosing.

In Cohort 2, newborns received TDF 4 mg/kg as soon as possible after birth and on Days 3 and 5. PK sampling was done on Day 0 (cord blood, pre-dose and 2, 10 and 18-24 hours post-dose) and on Days 3 and 5 (at pre-dose, 2, 10, 18-24 and 36-48 hours post-dose). Results from Cohort 2 show that infant plasma tenofovir concentrations were greater at Day 0 than on Days 3 or 5 (Table 6). The infant dosing schedule, however, did not maintain infant plasma tenofovir concentrations above 50 ng/mL, during the first week of life.

Table 6: Median (range) Tenofovir Concentrations in Infants (Cohort 2)

Day of Dose	0 n=23	3 n=21	5 n=21	Adults 300 mg qd ³⁴
T _{max} (hr)	2.0 (1.6-10.0)	2.1 (1.9-43.9)	2.0 (1.8-18.0)	2.0
C _{max} (ng/mL)	200 (66-428)	78 (27-363)	87 (22-252)	375
AUC (ng*hr/mL)	4013 (2003-8874)	2365 (728-8000)	1631 (884-4317)	3179
T _{1/2} (hrs)	21.6 (16.0-124.5)	19.5 (6.8-44.0)	18.1 (5.2-61.3)	11.7
Cl/F (mL/kg/hr)	69 (134-1808)	1375 (566-3425)	1713 (451-3562)	584

Additional safety data regarding the use of tenofovir in pregnancy are available from the Antiretroviral Pregnancy Registry (APR). Through January 31, 2009, a total of 1,063 cases of tenofovir exposure during pregnancy have been reported. Of these 1,063 exposures 678 occurred during the first trimester and 385 occurred later in pregnancy. There has been no increase in birth defects or unusual pattern of defects detected, first trimester: 16 of 678 (2.4%); later in pregnancy: 6 of 385 (1.6%).⁴¹ These numbers fall below the accepted background risk of 3% for major malformations and thus add to the growing body of evidence suggesting that tenofovir exposure during pregnancy is safe. The first microbicide study with

planned dosing in pregnant women (MTN-002) is currently enrolling participants. MTN-002 is a recently completed Phase 1 pharmacokinetic and placental transfer evaluation among term pregnant women undergoing planned cesarean delivery after receiving a single-dose of tenofovir 1% gel pre-operatively. The final safety review of this unblinded study showed no significant safety events.

Study Hypotheses and Rationale for Study Design

Study Hypotheses

We hypothesize that:

- Repeated exposure via daily use of tenofovir 1% vaginal gel by pregnant and lactating women for 7 days will be well-tolerated and not associated with significant toxicity
- Blood tenofovir concentrations will be detectable in approximately 80% of women in the Pregnancy Cohort who receive study gel
- Among women in the Pregnancy Cohort, only women who deliver during their study product dosing period will have detectable levels of tenofovir in cord blood and/or neonatal blood
- In the Lactation Cohort, tenofovir will only be detectable at low levels in breast milk, and when detectable, only in a minority of participants
- Blood tenofovir levels of women in the Lactation Cohort will be similar to those in the Pregnancy Cohort
- Tenofovir will not be detectable in the blood of nursing infants in the Lactation Cohort

2.10.2 Rationale

Tenofovir gel was chosen as the first microbicide to be tested in pregnancy for several reasons. Oral tenofovir disoproxil fumarate is classified by the FDA as a pregnancy category B drug. Tenofovir gel is the agent with the largest body of safety data among the topical antiretroviral agents and is currently being evaluated in Phase 2, 2B, and 3 trials. Parallel evaluations in pregnant women and demonstrations of safety could support continued study product use during pregnancy for women enrolled in effectiveness trials. This approach has been recently suggested by the FDA as a promising mechanism to address the issues of pregnancy-related dropout in HIV prevention trials, as well as the current lack of sufficient safety data on microbicide use in pregnancy. This protocol builds on the safety data obtained in MTN-002 (the first microbicide study to purposefully enroll pregnant women).

One component of the overall evaluation of the safety of tenofovir 1% gel among pregnant women in MTN-008 is the evaluation of the potential effects of study gel use on pregnancy outcomes. To compare women in MTN-008 to other pregnant women not using tenofovir 1% gel, a combination of placebo and historical controls will be used. Each of the groups in the Pregnancy Cohort will include 15 placebo controls (HEC) that will be used as the primary comparator for safety in the Pregnancy Cohort. However, historical control rates (local and national data) will be used for some pregnancy-related AEs with relatively low rates, as these may not be observed among the placebo controls.

The historical controls in MTN-008 are derived from local databases in Pittsburgh (MOMI database) and Birmingham (University of Alabama's Obstetrical Automated Record System or OBARS). Pregnancy-related outcomes from local Magee-Womens Hospital (tertiary hospital) delivery data from 2006 through 2008 (MOMI Database Magee-Womens Hospital, personal communication, David Crowe) are largely consistent with published rates of pregnancy outcomes.^{42, 43} The MOMI database is a local database encompassing the last 15 years of local maternity and newborn data at Magee-Womens Hospital. Magee-Womens Hospital performs approximately 9,500 deliveries per year. OBARS is a perinatal database in Birmingham that contains 1,000 coded antepartum, intrapartum, and postpartum items per patient, with data from 2007 through 2009. Given this large volume, these databases provide considerable data on pregnancy outcomes for background data for the population represented in this study.

For the smaller Lactation Cohort, the primary and secondary endpoints of laboratory and genital AEs will be compared to historical control data from HPTN 059 as participants from HTPN 059 were non-lactating, healthy, reproductive age women who used tenofovir 1% gel daily.

Inclusion of Pregnancy and Lactation Cohorts in a Single Study

MTN-008 will include cohorts of both pregnant and lactating women. The inclusion of both cohorts in a single study is supported by several arguments. A single study provides an efficient approach to the evaluation of the target study population, scientific objectives, cost and time-sensitive relevance for ongoing and future trials of HIV prevention agents.

Study Population

The two study populations have significant overlap; many pregnant women at risk for sexual transmission of HIV go on to become lactating women at risk of sexual transmission of HIV, essentially immediately following childbirth. The perinatal and postpartum/breastfeeding periods are part of a continuum within a single population that represents a knowledge gap in the microbicide field. A woman's combined time spent on pregnancy and breastfeeding approaches three to four years for a single infant in many countries where HIV is prevalent.

According to the FDA Guidance on Clinical Lactation Studies, it is efficient to nest clinical lactation studies within a larger clinical study on safety or efficacy outcomes or in combination with the postpartum assessment of the effects of pregnancy on the PK and/or PD of a drug. In this case, the clinical lactation study is combined with the latter.

Cost Efficiency

The inclusion of both cohorts in a single study design maximizes the use of resources, primarily via reducing time that would have been spent in protocol development, review, regulatory approval, site training, etc. for separate protocols. The design of MTN-008 is such that both cohorts will have roughly the same study procedures and visit schedule, and will require the involvement of study staff knowledgeable in the area of maternal-child health. If written as separate studies, the two protocols would require separate review by the same maternal-child health subcommittee of the site Institutional Review Board (IRB); however, this review would be done at separate times, adding unnecessarily to the time needed for regulatory approval.

Time-sensitive Relevance to Maximizing Power in HIV Prevention Trials

The inclusion of both cohorts in a single trial will allow the MTN to address very efficiently several outstanding key questions regarding safety and PK that will be relevant to HIV prevention trials, including MTN-003, the VOICE trial. Data collection that may support the capacity for HIV prevention trials to keep pregnant and lactating women on study product is a high priority for the MTN. The collection of adequate PK and safety data in the Lactation Cohort for MTN-008, if collected in a timely manner, could potentially have significant benefit to improving study power in VOICE and other trials.

Safety

Preliminary data from MTN-002 suggest a favorable safety profile associated with single-dose tenofovir 1% gel prior to scheduled cesarean delivery. Based on these data, as well as the body of evidence reviewed above indicating a similar safety profile associated with oral dosing in the perinatal period, MTN-008 will include repetitive dosing of topical tenofovir in term pregnancy. If repetitive dosing in term gestation meets predefined safety criteria, the protocol will proceed to dosing in near-term gestation.

Pharmacokinetics

Tenofovir was first studied in gravid rhesus monkeys and noted to cross the placenta following subcutaneous administration in a simian model.⁴⁴ Moreover, data from maternal oral dosing in PACTG/IMPACT 394 and HPTN 057 demonstrate that tenofovir does cross the placenta and produces fetal levels that are roughly 60% of maternal levels. Preliminary data from MTN-002 has confirmed that after vaginal tenofovir gel exposure, among women with detectable maternal levels, a proportion of women also had detectable cord blood levels at roughly the same C/M ratio seen

in oral dosing studies. However, it is not clear what impact repetitive dosing in late gestation will have on fetal exposure.

Normal physiologic changes of pregnancy can affect the PK and pharmacodynamics (PD) of drugs. Increased plasma volume, total body water, and body fat may alter the distribution of drugs, while delays in gastric emptying may slow adsorption. Increased renal blood flow and decreased plasma proteins may impact drug excretion. It is conceivable that the absorption and PK of tenofovir 1% gel in the third trimester of pregnancy may differ from non-pregnant women, given some of the normal physiologic changes above, as well as increased blood flow to the pelvic tissues and engorgement of the pelvic vessels. Preliminary data from MTN-002, however, do not support this theory.

Repeat oral dosing in pregnant women has been associated with median maternal serum levels (C_{max}) of 249 ng/mL (range 83-595 ng/mL), and median cord blood levels of 76 ng/mL (range 0-309 ng/mL). In PACTG/IMPAACT 394, the maternal tenofovir concentrations were similar to those seen after chronic oral dosing with 300 mg daily in non-pregnant individuals, despite the use of a single 600 mg dose. The study has proceeded to the use of a maternal single dose of 900 mg at onset of labor or before cesarean delivery, which to date has been associated with comparable safety and pharmacokinetic results. Using data from HPTN 050, we estimate the relative bioavailability into blood to be 2% with vaginal dosing compared to oral tenofovir. If vaginal dosing in pregnant women leads to similar serum levels as in non-pregnant women, and the cord blood/maternal ratio stays the same as is seen after oral dosing, 0.65 (range 0.35-1.0), then we would anticipate cord levels to be less than 10 ng/mL after repetitive vaginal dosing. As documented above, the anticipated level of tenofovir in the fetus/neonate following repeated maternal vaginal dosing is much lower than that seen following repeated maternal oral dosing. Since significant fetal/neonatal adverse effects have not been seen with the higher levels fetal/neonatal following maternal oral dosing, we do not anticipate adverse fetal/neonatal effects in this study. Although there is some potential for individual variability, high levels would not be anticipated in the cord blood or neonatal blood of infants born to women in the Pregnancy Cohort who deliver during their dosing period.

In HPTN 050, 64% of measurable samples fell in the 2- to 6-hour sampling window. Importantly, all participants' peak tenofovir concentrations occurred within this window. Tenofovir was detectable among many participants in HPTN 050, but importantly the magnitude of levels are relatively low (maximal detectable level is < 15 ng/mL). Preliminary data from MTN-002 have shown detectable levels of tenofovir (LLOQ 1 ng/mL) in some women. These levels are relatively consistent with levels observed in non-pregnant women using vaginal gel, suggesting that although tenofovir is absorbed systemically in some women, the absolute levels remain well below the levels seen with oral dosing (roughly ten-fold less) and peak within 8 hours of dosing.

The planned PK analysis will include descriptions of C_{max} and T_{max} . Estimates of vaginal absorption, systemic clearance, volume of distribution, and half-life require accurate assessment of the initial rise in concentration, AUC, and terminal elimination slope. Only the concentration at 1-3 time points near C_{max} in only some women (based on HPTN 050 experience) may be expected to be measurable. Thus, due to this variability in detectable levels among women using topical tenofovir at the 1% concentration, we anticipate that data from this study cannot be used to estimate initial absorption, AUC, or terminal elimination slope.

It is particularly helpful to enroll mother-infant pairs in studies involving planned exposure. This approach allows for the determination of PK in lactating women, the amount of drug excreted into breast milk, and to assess exposure in breast-fed infants in a single study. The FDA also recommends including PD endpoints for the infants in the study.⁴⁵ Obtaining additional data regarding the drug levels detectable in infants will add critical data to the body of knowledge on detectable levels of drug in breast milk.

Overall, evidence indicates that oral tenofovir exposure among pregnant women leads to maternal levels similar to those observed in non-pregnant women. Preliminary studies of perinatal exposure are also consistent with relatively rapid infant clearance of the drug after birth, suggesting that topical tenofovir use in pregnancy may not be associated with clinically significant drug levels in mothers and their infants.

According to FDA Guidance regarding clinical lactation studies, the first step in determining the degree of risk that drug in breast milk poses to a nursing infant, is to ascertain the presence or absence of drug in breast milk.⁴⁵ The FDA notes that presence of drug in breast milk may not necessarily have an adverse impact on the nursing infant. However, it is also important to understand how changes to the mother during the lactation and postpartum period can also impact PK.

Adherence/Behavioral

Adherence data are critical for the interpretation of results from randomized controlled prevention trials where distinguishing poor adherence (a behavioral measure) from method safety or efficacy is essential. Behavioral data may be particularly useful when a safety trial yields no toxicity or minimal AEs result and examining the levels of product use reported by participants in each arm could aid in interpretation of study findings. Similarly, behavioral data would aid in interpreting pharmacokinetic results, when the effects of cumulative dosage of study product is evaluated. While in this study, treatment regimen is short (7 days) and two of the gel dosages will be administered by the clinician (Day 0 and Day 6 Visits), 5 other doses will be taken daily at home. Thus, adherence to daily regimen, missed doses and reasons for non-adherence will be monitored through the Day 1 and Day 3 Phone Calls, and through a behavioral assessment at the Day 6 Visit. In addition to the adherence measures, MTN-008 will evaluate acceptability of product use at the Day 6 Visit, and sexual behavior (Day 0 and Day 6 Visits). The behavioral assessments

fit well within the schedule of study activities and will be minimally burdensome to the participants, as they will be conducted during the two 8-hour clinic visits (collection of blood and breast milk specimens for PK analyses).

Limited studies indicate a wide array of patients' beliefs and perceptions related to drug treatment during pregnancy and lactation.⁴⁶ Acceptability research on the vaginal administration of drugs in pregnancy has primarily focused on misoprostol in medical abortion^{47, 48}, and a limited amount of data largely related to safety and/or efficacy have been published for other drugs.^{49, 50} Acceptability issues, however, are likely to be different for women in HIV prevention trials. Therefore, an important contribution of the adherence/behavioral component of MTN-008 will be to explore willingness to use and acceptability of microbicides in these two populations, and to begin identifying the potential barriers and facilitators to adoption of microbicide use during pregnancy and lactation. Pregnancy may affect both behavior and product acceptability; while term and near-term pregnancy may be a time when sexual intercourse is uncomfortable, frequent sexual intercourse may be used by some couples to induce labor. This activity may increase women's potential exposure to HIV, at a critical time during their pregnancy.

Although the inaccuracy of self-reported adherence data in clinical trials is well documented⁵¹, increasing the privacy of the interview mode has been found to reduce social desirability bias in reporting sensitive behaviors.⁵² We propose here to use a combination of face-to-face interview (when probing responses will be important) and self-administered questionnaire and retrospective logs (when collecting data on socially sensitive behaviors, such as product use and sexual activity).

Vaginal Microenvironment

Pregnancy and the postpartum period are both associated with physiologic changes in the cervicovaginal environment. Kennedy et al⁵³ conducted a prospective, longitudinal descriptive study in pregnant women to identify the prevalence and severity of vaginal and vulvar symptoms during pregnancy as well as three months post-partum. One hundred and three evaluable pregnant women were included in the analysis and were also compared to a historical, non-pregnant cohort. Participants were asked to complete a questionnaire each trimester as well as 3 months post-partum regarding the frequency and severity of vulvar burning, itching, pain, vaginal discharge, and dyspareunia. Participants were asked to grade severity on a scale from 0 – 10, with 0 being none, and 10 being the most severe. With the exception of dyspareunia, study results showed that these symptoms increased in frequency throughout the course of pregnancy and generally decrease postpartum. Dyspareunia, however, was shown to increase in the postpartum period, which is not uncommon following vaginal delivery. The overall reported severity of these symptoms was low (none with median severity > 4).

During pregnancy, vaginal discharge typically increases, due in part to a normal increase in cervical secretions. The postpartum period has been associated with a

hypoestrogenic state of the vaginal epithelium, particularly among lactating women. In some women, these physiologic changes are consistent with clinical criteria for the diagnosis of atrophic vaginitis, a condition associated with a thinning of the epithelial cell layer, decreased vaginal lubrication, dyspareunia, and the detection of fewer lactobacilli on microscopic exam.

A study of vaginal and rectal bacteria was recently conducted in pregnant women.⁵⁴ El Aila and colleagues cultured vaginal specimens from 132 pregnant women at 35 – 37 weeks gestation. The isolates were identified via tRNA intergenic length polymorphism analysis (tDNA-PCR) and were then genotyped via RAPD-analysis. One hundred twenty-one (91.6%) of 132 vaginal samples were positive for lactobacilli, with *L. crispatus* as the most frequently isolated *Lactobacillus* species (40%). This was followed by *L. jensenii* (32%), *L. gasseri* (30%), and *L. iners* (11%).

Potentially, certain changes in the vaginal microenvironment could influence drug absorption, tolerability, and susceptibility to HIV infection, through an alteration in the vagina's natural defenses. Thus, MTN-008 will further examine changes in the vaginal microenvironment as part of the exploratory objectives.

2.11 Justification of Dosing

Choice of the tenofovir 1% gel concentration for MTN-008 is based on both animal and clinical evidence suggesting an appropriate safety profile and potency. Animal and human studies have demonstrated minimal vaginal irritation at this concentration. A rabbit vaginal irritation test identified tenofovir 1% gel as being histopathologically identical to sham or control treatment, while on a qualitative basis 3% gel was more irritating to vaginal epithelia. The tolerability of the 1% gel was confirmed in the HPTN 050 Phase 1 study and the HPTN 059 expanded safety study, as well as the CONRAD tissue PK and male tolerance studies. The 1% gel concentration is currently being evaluated in MTN-001 and MTN-002, and is planned for evaluation in MTN-003, RMP-02/MTN-006, MTN-007, and (via pregnancy registry) in MTN-016.

The second line of evidence is from vaginal transmission inhibition studies performed in non-human primates. Six separate studies provide 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 tenofovir 1% gel as the lowest efficacious concentration tested when given within two hours of infection.

Finally, the amount of tenofovir administered by intravaginal application of 4 grams of a 1% dose (40 mg) is similar to the orally absorbed amount that has been shown to be highly active against HIV and results in a reduction of plasma HIV RNA of 1.5 log₁₀ 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% concentration minimizes the potential risks of vaginal epithelial toxicity while providing the potential benefit of delivering sufficient tenofovir to achieve an initial and possibly durable barrier to infection.

3 OBJECTIVES

3.1 Primary Objectives

1. To assess safety and tolerability of tenofovir 1% gel used daily for 7 days in pregnancy and lactation
2. To assess the pharmacokinetics of tenofovir 1% gel used daily for 7 days in pregnancy and lactation

3.2 Secondary Objectives

1. To assess for the presence of tenofovir in blood among infants of participants in the Pregnancy and Lactation Cohorts
2. To assess the impact of tenofovir gel exposure on the presence of select organisms associated with neonatal sepsis among participants in the Pregnancy Cohort, (e.g., Group B β -hemolytic *Streptococcus*, *E. coli*)
3. To assess the adherence to daily use of tenofovir 1% gel for 7 days and its acceptability among pregnant and lactating women

3.3 Exploratory Objectives

1. To measure vaginal flora characteristics and to descriptively examine changes in these characteristics over the course of daily tenofovir 1% gel use among pregnant and lactating women
2. To assess the effects of tenofovir 1% gel on biomarker expression in vaginal and cervical secretions of pregnant and lactating women

4 STUDY DESIGN

4.1 Identification of Study Design

MTN-008 is an expanded Phase 1, randomized, double-blinded, placebo-controlled safety and pharmacokinetic study of daily use of tenofovir 1% gel in pregnancy and lactation. Accrual into Pregnancy Cohort Group 2 (women between 34 0/7 and 36 6/7 weeks gestation) is contingent upon favorable safety data observed in Pregnancy Cohort Group 1 (women between 37 0/7 and 39 1/7 weeks gestation).

4.2 Summary of Major Endpoints

1. Pregnancy Safety and Tolerability – Maternal Outcomes

- Grade 2 or higher AEs in the following categories

Specific laboratory abnormalities

- ALT
- AST
- Creatinine

Specific genital/pelvic signs/symptoms

- Dyspareunia
- Pain (vulvar, vaginal, and/or pelvic)
- Tenderness (vulvar, vaginal, and/or pelvic)
- Itching (vulvar and/or vaginal)
- Edema (vulvar, vaginal, and/or cervical)
- Erythema (Vulvar, vaginal, and/or cervical)
- Lesions (vulvar, vaginal, and/or cervical)
- Vulvar rash
- Vaginal dryness
- Dysuria
- Vulvovaginitis
- Cervicitis

Specific pregnancy complications

- Postpartum hemorrhage
 - Postpartum endometritis
 - Chorioamnionitis
 - Third trimester bleeding
 - Preterm premature rupture of membranes (prior to onset of labor)
 - Term premature rupture of membranes (prior to onset of labor)
 - Spontaneous preterm delivery
- For adverse events not included above, Grade 3 or higher adverse events judged by the investigator to be related to the study gel or applicator

2. Pregnancy Safety and Tolerability – Infant Outcomes

- Infant in the Pregnancy Cohort diagnosed (and confirmed on review of medical records) with any of the following during the 7 days following delivery
 - Intensive care admission greater than 24 hours. *Note: all intensive care unit admits will be reviewed for toxicities potentially related to study product exposure*
 - Sepsis
3. Lactation Safety and Tolerability-Maternal Outcomes
- Grade 2 or higher AEs in the following categories
 - Specific laboratory abnormalities
 - ALT
 - AST
 - Creatinine
 - Specific genital/pelvic signs/symptoms
 - Dyspareunia
 - Pain (vulvar, vaginal, and/or pelvic)
 - Tenderness (vulvar, vaginal, and/or pelvic)
 - Itching (vulvar and/or vaginal)
 - Edema (vulvar, vaginal, and/or cervical)
 - Erythema (vulvar, vaginal, and/or cervical)
 - Lesions (vulvar, vaginal, and/or cervical)
 - Vulvar rash
 - Vaginal dryness
 - Dysuria
 - Vulvovaginitis
 - Cervicitis
4. Lactation Safety and Tolerability-Infant Outcomes
- Infant in the Lactation Cohort with the following during the period of study participation, confirmed on review of medical records
 - Inpatient admission (confirmed on review of medical records) with diagnosis of AE judged to be related to study product
5. Tenofovir Levels
- Maternal blood of participants in the Pregnancy and Lactation Cohorts
 - Breast milk of participants in the Lactation Cohort

4.3 Description of Study Population

Pregnancy Cohort

- Healthy, 3rd trimester gestation, HIV-uninfected, pregnant women, 18 – 40 years old, without evidence of maternal or fetal complications in the current pregnancy.

- Group 1: approximately 45 participants between 37 0/7 weeks and 39 1/7 weeks gestation (inclusive) on Study Day 0
- Group 2: approximately 45 participants between 34 0/7 and 36 6/7 weeks gestation (inclusive) on Study Day 0
- Infants born to mothers in the Pregnancy Cohort

Lactation Cohort

- Approximately 15 healthy women, 18 – 40 years old, currently exclusively breastfeeding an infant
- Breastfeeding infants of women in the Lactation Cohort (4-26 weeks inclusive)

4.4 Time to Complete Accrual

Accrual is expected to be completed in approximately 14 months.

4.5 Study Groups

Pregnancy Cohort

A total of approximately 90 mother-infant pairs will be enrolled. Additional mother-infant pairs will be enrolled to ensure that a total of 90 evaluable pairs complete the study.

The following will be considered as having incomplete exposure and additional participants will be enrolled:

- Mother-infant pairs in which the mother did not receive at least 4 doses of study gel
- Mother-infant pairs in which the mother did not complete the Day 6 Visit

Lactation Cohort

A total of approximately 15 mother-infant pairs will be enrolled. Additional participants will be enrolled to ensure that a total of 15 evaluable mother-infant pairs complete the study.

The following will be considered as having incomplete exposure and additional participants will be enrolled:

- Mother-infant pairs in which the mother did not receive at least 4 doses of study gel
- Mother-infant pairs in which the mother did not complete the Day 6 Visit

The number of additional participants enrolled due to incomplete follow-up should not exceed 20% of the target sample size (45 in each Pregnancy Cohort and 15 in the Lactation Cohort).

4.6 Sequence of Trial Periods

Acknowledging that it is not always possible to complete study evaluations/visits on the targeted dates/times, evaluations/visits may be completed within a specified window (see MTN-008 SSP Manual) around the target date/time.

Table 7: Sequence of Trial Periods and Target Days for Visits/Calls

	SCR	ENR	Day 1 Phone Call	Day 3 Phone Call	Day 6 Visit	Day 14 Phone Call	Del. Call	Del. Visit	Post-Delivery Assessment
	≤ -Day 28	Day 0	Day 1	Day 3	Day 6	Day 14	May Vary	May Vary	May Vary
Pregnancy Cohort Group 1	X	X	X	X	X	X	X	X	X
Pregnancy Cohort Group 2	X	X	X	X	X	X	X	X	X
Lactation Cohort	X	X	X	X	X	X	N/A	N/A	N/A

Note: Infant participation in the Pregnancy Cohort will begin at birth and continue until the Post-Delivery Assessment.

4.7 Expected Duration of Participation

The expected duration for women enrolled in either of the Pregnancy Cohorts is approximately 3-10 weeks. The expected duration of scheduled participation for an infant who takes part in the Pregnancy Cohort procedures will be from the time of birth until the Post-Delivery Assessment (target 14 days). The expected duration for enrolled mother-infant pairs in the Lactation Cohort is approximately 3 weeks. Mothers participating in the Pregnancy Cohort at participating MTN-016 sites will be encouraged to enroll themselves and their infants into MTN-016 (HIV Prevention Agent Pregnancy Exposure Registry: EMBRACE Study), for follow up during the first year of life.

5 STUDY POPULATION

5.1 Selection of the Study Population

5.1.1 Recruitment

Study site staff will recruit potential participants from clinical care site(s), including prenatal and postnatal care clinics, using IRB-approved materials.

Mothers in both cohorts will consent for participation of both themselves and their infants during the informed consent process. As the informed consent documents will be signed prior to delivery, eligibility criteria will not be assessed for infants in the

Pregnancy Cohort (all infants born to mothers in the Pregnancy Cohort will be considered eligible).

5.1.2 Composition

The racial and ethnic composition of the study population is anticipated to be consistent with the primary patient population at the prenatal and postnatal care sites. Women of other racial and ethnic backgrounds will not be excluded.

5.2 Pregnancy Cohort Inclusion Criteria: Mothers

Women must meet all of the following criteria (by self-report, unless otherwise indicated) to be eligible for inclusion in the study:

1. Age 18 through 40 years (inclusive) at screening, verified per site standard operating procedures (SOP)
2. Willing and able to provide written informed consent to be screened for and take part in the study, including participation of the infant after delivery
3. Willing and able to provide adequate locator information, as defined in site SOP
4. Willing and able to communicate in written and spoken English
5. HIV-uninfected (per HIV Testing Algorithm, Appendix II)
6. Current pregnancy with the following characteristics:
 - a. Viable
 - b. Singleton
7. Gestational age (as determined by criteria in the MTN-008 SSP manual) consistent with the following guidelines:
 - a. For Pregnancy Cohort Group 1, between 37 0/7 and 39 1/7 weeks (inclusive) at the Enrollment Visit (Day 0)
 - b. For Pregnancy Cohort Group 2, between 34 0/7 and 36 6/7 weeks (inclusive) at the Enrollment Visit (Day 0)
8. Pap result consistent with Grade 0 according to the Female Genital Grading Table for Use in Microbicide Studies (Addendum 1 to the DAIDS Table for Grading Adult and Pediatric Adverse Events, Version 1.0, December 2004, Clarification dated August 2009) or satisfactory evaluation of a non-Grade 0 Pap result, per clinical judgment of Site Investigator or Record (IoR)/designee in the 12 calendar months prior to enrollment

9. Willing to abstain from the following during study participation:
 - a. Non-prescribed intravaginal products and practices (including douching and sex toys)
 - b. Other investigational agent or device study

Note: Sexual activity is neither restricted nor required as a condition of study participation

5.3 Pregnancy Cohort Exclusion Criteria: Mothers

Women who meet any of the following criteria (by self-report, unless otherwise indicated) will be excluded from the study:

1. Participant report of any of the following:
 - a. History of adverse reaction to any component of tenofovir 1% gel
 - b. Enrollment in any other investigational drug or device trial within 30 days prior to the Enrollment Visit (Day 0)
 - c. Currently breastfeeding
 - d. Within 48 hours prior to Screening or Enrollment (Day 0), use of vaginal medications (participant may return to complete study procedures after 48 hours have passed since use of vaginal medication)
2. Documented to have any of the following during the current pregnancy:
 - a. Ultrasound evidence of significant fetal congenital anomaly (in the opinion of the IoR or designee)
 - b. Known rupture of the amniotic membranes, as defined in the MTN-008 SSP Manual
 - c. Known placental/fetal abnormalities that could affect placental transfer (e.g., placental abruption, placenta previa, placenta accreta, intrauterine growth restriction, two-vessel cord, etc.)
 - d. Known maternal disease with predictable negative effect on placental function (e.g., hypertension, diabetes mellitus, collagen vascular disease)
3. Has any of the following laboratory abnormalities noted at screening:
 - a. Hemoglobin value of Grade 3 or higher according to DAIDS Toxicity Table
 - b. Serum creatinine greater than 1.0 mg/deciliter (dL)
 - c. AST and/or ALT greater than 1.5 upper limit of normal (ULN)
 - d. Hepatitis B surface antigen (HBsAg) positivity
4. By participant report or review of medical record, in the past 8 weeks prior to enrollment (Day 0), diagnosis of STI, including chlamydia, gonorrhea, and/or trichomoniasis

5. At the time of enrollment (Day 0), symptomatic vaginitis, including BV and vulvovaginal candidiasis (asymptomatic evidence of BV and/or yeast is not exclusionary)
6. Clinically apparent Grade 2 or higher pelvic exam finding (observed by study staff)

Note: Uterine enlargement, increased vaginal discharge, evidence of minor bleeding associated with speculum insertion and/or specimen collection, and other findings consistent with normal pregnancy and judged to be within the range of normal according to the clinical judgment of the IoR/designee are not exclusionary.

7. Use of oral and/or vaginal preparations of antibiotic or antifungal medications at Screening or within 7 days of enrollment (Day 0)
8. At screening or enrollment (Day 0), any social or medical condition that, in the investigator's opinion, would preclude informed consent, make study participation unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the study objectives

Note: Otherwise eligible participants with exclusionary physical exam findings may be enrolled after the findings have improved to a non-exclusionary severity grading or resolved. If improvement to a non-exclusionary grade or resolution is documented within 28 days of providing informed consent for screening, the participant may be enrolled.

5.4 Lactation Cohort Inclusion Criteria: Mothers

Women must meet all of the following criteria (by self-report, unless otherwise indicated) to be eligible for inclusion in the study:

1. Age 18 through 40 years (inclusive) at screening, verified per site SOP
2. Willing and able to provide written informed consent to be screened for and take part in the study
3. Willing and able to provide adequate locator information, as defined in site SOP
4. Willing and able to communicate in spoken and written English
5. HIV-uninfected (per HIV Testing Algorithm in Appendix II)
6. Currently primarily breastfeeding a single healthy infant between the ages of 4 and 26 weeks (inclusive) according to guidelines specified in the MTN-008 SSP Manual

7. Intending to breastfeed during the period of anticipated study participation
8. Using an effective method of contraception at enrollment (Day 0) into Lactation Cohort, and intending to use an effective method for the duration of scheduled study participation; effective methods include hormonal methods, abstinence, male condoms, intrauterine device, and sterilization (of participant or her sexual partner or partners, as applicable and with verification as defined in site SOPs)
9. Pap result consistent with Grade 0 according to the Female Genital Grading Table for Use in Microbicide Studies (Addendum 1 to the DAIDS Table for Grading Adult and Pediatric Adverse Events, Version 1.0, December 2004, Clarification dated August 2009) or satisfactory evaluation of non-Grade 0 Pap result per clinical judgment of Site IoR/designee) in the 12 calendar months prior to enrollment (Day 0)
10. Willing to abstain from the following:
 - a. Non-prescribed intravaginal products and practices (including douching and sex toys) during study participation
 - b. Other drug or device study during study participation

5.5 Lactation Cohort Exclusion Criteria: Mothers

Women who meet any of the following criteria (by self-report, unless otherwise indicated) will be excluded from the study:

1. Participation in Pregnancy Cohort
2. Infant excluded from participation in MTN-008 Lactation Cohort
3. Participant report of any of the following:
 - a. History of adverse reaction to any component of tenofovir 1% gel
 - b. Participation in investigational drug or device trial within 30 days prior to the Enrollment Visit (Day 0)
 - c. Within 48 hours prior to Screening or Enrollment (Day 0), use of vaginal medication(s) (participant may return to complete study procedures after 48 hours have passed since use of vaginal medication)
 - d. Within 7 days prior to Screening or Enrollment (Day 0), more than two infant feedings in a single day with nutrition other than own breast milk (e.g., formula, solids)
4. At the time of enrollment (Day 0), participant report or clinical evidence according to the judgment of the IoR/designee of any of the following conditions:

- a. Insufficient milk supply
 - b. Mastitis
5. As determined by the IoR/designee, any significant uncontrolled active or chronic cardiovascular, renal, liver, hematologic, neurologic, gastrointestinal, psychiatric, endocrine, respiratory, immunologic disorder or infectious disease
 6. Has any of the following laboratory results:
 - a. Positive urine pregnancy test
 - b. Serum creatinine at screening greater than 1.0 mg/dL
 - c. AST and/or ALT at screening greater than 1.5 ULN
 - d. HBsAg positivity at screening
 7. By participant report or review of medical record, in the 8 weeks prior to Day 0, STI, including chlamydia, gonorrhea, and/or trichomoniasis
 8. At the time of enrollment (Day 0), symptomatic vaginitis, including BV and vulvovaginal candidiasis (asymptomatic evidence of BV and/or yeast is not exclusionary)
 9. On pelvic exam, any of the following findings:
 - a. Incomplete postpartum involution of the uterus
 - b. Clinically apparent Grade 2 or higher pelvic exam finding (observed by study staff)

Note: Cervical bleeding associated with speculum insertion and/or specimen collection judged to be within the range of normal according to the clinical judgment of the IoR/designee is considered expected non-menstrual bleeding and is not exclusionary.

10. Use of oral and/or vaginal preparations of antibiotic or antifungal medications at Screening or within 7 days of enrollment (Day 0)
11. At screening or enrollment (Day 0), any social or medical condition that, in the investigator's opinion, would preclude informed consent, make study participation unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the study objectives

Note: Otherwise eligible participants with exclusionary physical or pelvic exam findings may be enrolled after findings have improved to a non-exclusionary severity grading or resolved. If improvement to a non-exclusionary grade or resolution is documented within 28 days of providing informed consent for screening, the participant may be enrolled.

5.6 Lactation Cohort Inclusion Criteria: Infants

Infants must meet the following criteria to be eligible for inclusion in this study:

1. Mother consents for participation of both self and infant in Lactation Cohort
2. In general good health, as determined by clinical judgment of IoR/designee
3. Between the ages of 4 and 26 weeks (inclusive)

5.7 Lactation Cohort Exclusion Criterion: Infants

Infants who meet the following criterion will be excluded from the study:

1. At screening or enrollment (Day 0), any social or medical condition that, in the investigator's opinion, would make study participation unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the study objectives

6 STUDY PRODUCT

6.1 Regimen

Participants will participate in either the Pregnancy Cohort or Lactation Cohort. Participants in the Pregnancy Cohort will be randomized to receive tenofovir 1% gel or placebo gel in a 2:1 ratio. Participants in the Lactation Cohort will receive tenofovir 1 % gel.

6.1.1 Pregnancy Cohort

There will be approximately 90 evaluable participants in the Pregnancy Cohort. Each participant in the Pregnancy Cohort will be randomized to one of two blinded study regimens. Participants will be randomized to receive either tenofovir 1% gel or placebo gel. The Pregnancy Cohort will be further divided into Group 1 or Group 2 based on gestation. Participants will first be enrolled in Group 1 and will be women between 37 0/7 and 39 1/7 weeks gestation (inclusive). Once enrollment is complete, accrual into Group 2 will begin and will be contingent upon reassuring safety data obtained from Group 1. Study participants in Group 2 will be women between 34 0/7 and 36 6/7 weeks gestation (inclusive).

Each participant will receive one applicatorful of tenofovir 1% gel or placebo gel vaginally for 7 consecutive days. The first dose (Day 0) and the seventh and final dose (Day 6) will be administered by the IoR/authorized clinician at the study site.

The second through sixth doses of study product will be administered by the study participant at home.

Table 8: Study Regimen-Pregnancy Cohort

Arm	Description	N	Dose, Route, and Frequency
1	tenofovir 1% gel	Group 1: 30 Group 2: 30	Entire contents of an applicator will be inserted vaginally, for 7 consecutive days
2	placebo gel	Group 1: 15 Group 2: 15	Entire contents of an applicator will be inserted vaginally, for 7 consecutive days

6.1.2 Lactation Cohort

The Lactation Cohort will include approximately 15 evaluable participants currently breastfeeding an infant 4 – 26 weeks old (inclusive). Each mother will receive one applicatorful of tenofovir 1% gel vaginally for 7 consecutive days. The first dose (Day 0) and the seventh and final dose (Day 6) will be administered by the IoR/authorized clinician at the study site. The second through sixth doses of study product will be administered by the study participant (mother) at home.

Table 9: Study Regimen-Lactation Cohort

Arm	Description	N	Dose, Route, and Frequency
1	tenofovir 1% gel	15	Entire contents of an applicator will be inserted vaginally, for 7 consecutive days

6.2 Administration

Study staff will instruct participants in the proper method of administering and storing study product. Participants will be instructed to insert their gel at approximately the same time every day. If a daily dose is missed, the participant will be instructed to administer the missed dose as soon as possible, unless the next dose is due within 6 hours. If the next dose is due within 6 hours, the missed dose will be skipped and the next dose will be administered as originally scheduled.

6.3 Study Product Formulation

6.3.1 Tenofovir 1% Gel

Tenofovir 1% gel is a gel formulation of tenofovir (PMPA, 9-[(R)-2-(phosphonomethoxy)propyl]adenine monohydrate), formulated in purified water with edetate disodium, citric acid, glycerin, methylparaben, propylparaben, HEC, and pH adjusted to 4-5. Tenofovir 1% gel is a transparent, viscous gel that will be supplied in pre-filled, single-use applicators. Each pre-filled applicator will contain a dose of approximately 4 mL of tenofovir gel, equal to 4.4 g of gel. Tenofovir 1% gel should be stored at 25°C (77°F). Excursions are permitted between 15°C and 30°C (59°F and 86°F).

6.3.2 Placebo Gel

The placebo gel, sometimes called the “Universal Placebo Gel” contains hydroxyethylcellulose as the gel thickener, purified water, sodium chloride, sorbic acid and sodium hydroxide.²⁵ The gel is isotonic and formulated at a pH of 4.4 to avoid disrupting the normal vaginal pH and has minimal buffering capacity to avoid the inactivation of sexually transmitted pathogens. Hydroxyethylcellulose, the gelling agent, is used to approximate the viscosity of other microbicide gel candidates. Each pre-filled applicator will contain approximately 4 mL of Placebo gel for delivery.

Placebo gel should be stored at 25°C (77°F). Excursions are permitted between 15°C and 30°C (59°F and 86°F).

6.4 Study Product Supply and Accountability

All study products will be available through the DAIDS Clinical Research Products Management Center (CRPMC). The Clinical Research Site (CRS) Pharmacist of Record (PoR) can obtain the study products for this protocol by following the instructions in the *Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks*. All study products must be stored in the pharmacy.

6.4.1 Study Product Supply

Tenofovir 1% vaginal gel and placebo gel will be supplied by CONRAD (Arlington, VA, USA).

6.4.2 Study Product Accountability

The CRS PoR is required to maintain complete records of all study products received from the NIAID CRPMC and subsequently dispensed. All unused study products must be returned to the NIAID CRPMC after the study is completed or terminated unless otherwise instructed by the DAIDS Protocol Pharmacist. The procedures to be followed are provided in the manual, *Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks*.

6.5 Study Product Dispensing

Study products will be dispensed only for enrolled participants, upon receipt of a written prescription signed by an authorized prescriber. Seven pre-filled applicators of tenofovir 1% gel or placebo gel (for Pregnancy Cohort) will be dispensed on Day 0. This will provide participants one “extra” applicator should one of the pre-filled applicators not be usable. An additional pre-filled applicator will be dispensed on Day 6 (for administration at the site by the IoR/authorized clinician).

6.6 Retrieval of Unused Study Products

It is anticipated that one unused applicator will be returned to the study site on Day 6, unless a replacement applicator is needed by the participant during Days 1 through 5. Study participants will be instructed to return all unused applicators to the site on Day 6. Unused applicators will be counted and documented, then sent to the pharmacy and will be placed in quarantine until returned to the CRPMC.

6.7 Concomitant Medications

With the exception of those prohibited by exclusion criteria, concomitant medications will be permitted. These include both prescription and non-prescription medications. All concomitant medications reported for mothers and infants throughout the course of the study will be recorded on case report forms designated for that purpose, including any medications administered in the hospital setting.

6.8 Prohibited Medications and Practices

To protect the integrity of the lower genital tract and reduce the possibility of AEs due to agents other than the study product, vaginal douching and use of sex toys are not permitted. In the event that prohibited practices are reported following administration of study gel, the protocol-specified visit schedule will continue for safety assessment through study exit.

6.9 Recommended Medications and Procedures

Study sites will be provided a single brand of latex condoms to be distributed to study participants for use during study participation. These condoms will contain an aqueous lubricant and will not be impregnated or coated with spermicide. Instructions and counseling on the use of these products will be provided as needed. In the event that a participant needs additional male condoms between visits, she may request these from clinic staff at any time.

Study sites will offer participants a single brand of panty liners (unscented and breathable) to study participants for use during study participation. Panty liners may be requested from clinic staff at any time including in between visits.

7 STUDY PROCEDURES

An overview of the study visit and evaluations schedule is presented in Appendix I. Presented in this section is additional information on visit-specific study procedures. Detailed instructions to guide procedures are provided in the MTN-008 SSP Manual available at www.mtnstopshiv.org. Unless otherwise specified, the laboratory procedures listed in this section are performed at the local study site laboratories.

The following visits will be scheduled for all adult study participants in both cohorts:

- Screening Visit
- Enrollment Visit (Day 0)
- Day 1 Phone Call
- Day 3 Phone Call
- Day 6 Visit (including gel administration and PK measures)
- Day 14 Phone Call

The Pregnancy Cohort will also have the following visits:

- Delivery Call
- Delivery Visit
- Post-Delivery Assessment (may be by phone or in-person visit, approximately 14 days [\pm 7 days] following delivery)

Mothers in the Pregnancy Cohort will be instructed to call the study site when they anticipate that delivery may be imminent. Infant participation in the Pregnancy Cohort begins at birth and continues until the Post-Delivery Assessment. Infant participation in the Lactation Cohort begins at the Enrollment (Day 0) Visit and continues through the Lactation Cohort Final Phone Call. A participant who is not administered any study gel will not complete subsequent scheduled laboratory procedures or assessments of adherence/behavior.

At any time, with the permission of the participant, a physician investigator or designee will be available to answer questions of the primary obstetrician or other clinical staff regarding the requirements of study participation. Appropriate written materials for clinical staff members describing screening procedures will be placed in the mother's clinical care chart after written informed consent has been obtained from the participant.

MTN-008 visits may be combined with MTN-016 visits, where applicable.

7.1 Pre-Screening

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

7.2 Screening Visit: Pregnancy Cohort and Lactation Cohort

A Screening Visit may take place up to 4 weeks prior to the Enrollment Visit (Day 0) in the Pregnancy Cohort. Multiple visits may be conducted to complete all required screening procedures, if necessary. Written informed consent for screening will be obtained before any screening procedures are initiated. For participants who do not meet the eligibility criteria, screening will be discontinued once ineligibility is determined.

Table 10: Screening Visit: Pregnancy and Lactation Cohorts

Screening Visit: Pregnancy and Lactation Cohorts		
Component	Procedure/Analysis (Mothers)	Lactation Cohort: Procedures/Analysis (Infants)
Administrative, Behavioral, and Regulatory	<ul style="list-style-type: none"> • Informed consent for screening • Demographic information • Locator information • Eligibility assessment • Obtain signed records release* • Assign participant identification number (PTID) • HIV pre- and post-test counseling • Reimbursement • Schedule next visit* • Provision of condoms and pantyliners* 	<ul style="list-style-type: none"> • Assign participant identification number (PTID) • Eligibility assessment
Clinical	<ul style="list-style-type: none"> • Medical eligibility information (including exclusionary medical conditions and medications) • Medical history • Concomitant medications • Review medical records • Blood collection • Urine collection (Lactation Cohort) • Targeted physical exam • Pelvic exam • Vaginal swabs • Cervical swabs • Treatment for reproductive tract infection (RTI)/urinary tract infection (UTI) or mastitis* • Provision of contraception and contraception education* • Disclosure of available test results 	<ul style="list-style-type: none"> • Medical eligibility determination • Medical history
Laboratory	<ul style="list-style-type: none"> • Cervical Nucleic Acid Amplification Test (NAAT) for chlamydia and gonorrhea (GC/CT) • Urine HCG (Lactation Cohort) • CBC • HIV serology • Serum creatinine • Hemoglobin • AST • ALT • Syphilis serology if not documented within past year • HBsAg test if not documented within past year • Trichomonas test • Pap smear* • Wet prep and vaginal pH* • Vaginal and cervical biomarkers (Pregnancy Cohort) • Quantitative vaginal culture (Pregnancy Cohort) • Gram stain (Pregnancy Cohort) • Herpes culture* 	

* if indicated

7.3 Enrollment Visit: Pregnancy and Lactation Cohorts

Table 11: Enrollment Visit: Pregnancy and Lactation Cohorts

Enrollment Visit (Day 0)			
Component	Pregnancy Cohort: Procedure/Analysis (Mothers)	Lactation Cohort: Procedure/Analysis (Mothers)	Lactation Cohort: Procedure/Analysis (Infants)
Administrative, Behavioral, and Regulatory	<ul style="list-style-type: none"> • Informed consent for enrollment • Locator information • Eligibility confirmation • Randomization • Baseline acceptability questionnaire • Coital log • Adherence counseling • Reimbursement • Schedule follow-up visits/calls • Disclosure of available test results* • Provision of condoms and panty liners* 	<ul style="list-style-type: none"> • Informed consent for enrollment • Locator information • Eligibility confirmation • Baseline acceptability questionnaire • Coital log • Adherence counseling • Participant education on collection of 2 breast milk samples at home (target 4 hours post dosing on two different days when study product was inserted) • Supplies for breast milk collection • Reimbursement • Schedule follow-up visits/calls • Disclosure of available test results* • Provision of condoms and panty liners* 	<ul style="list-style-type: none"> • Eligibility confirmation
Clinical	<ul style="list-style-type: none"> • Review medical records • Medical history • Concomitant medications • Targeted physical exam • Pelvic exam • Vaginal swabs • Cervical swabs • Insert saline lock** • Blood collection at PK time points (pre-gel, 1, 2, 4, 6, and 8 hours) • Supply 7 pre-filled study product applicators • Single dose of study gel to be administered by IoR/designee at clinic • Collect AEs 	<ul style="list-style-type: none"> • Review medical records • Medical history • Concomitant medications • Targeted physical exam • Pelvic exam • Vaginal swabs • Cervical swabs • Urine collection • Insert saline lock** • Blood collection at PK time points (target pre-gel, 1, 2, 4, 6, and 8 hours) • Breast milk collection at PK time points (target 2,4,6 hours) • Supply 7 pre-filled study product applicators • Single dose of study gel to be administered by IoR/designee at clinic • Collect AEs 	<ul style="list-style-type: none"> • Medical history • Concomitant medications • Collect AEs (self-reported, medical records, direct evaluation) • Blood collection via heelstick (target 6 hours <u>following maternal dosing</u>)

Laboratory	<ul style="list-style-type: none"> • CBC with differential (pre-gel) • Maternal blood tenofovir level (pre-gel, 1, 2, 4, 6, and 8 hours) • Flow cytometry (pre-gel)*** • PBMCs*** • Vaginal pH • Vaginal and cervical biomarkers • Quantitative vaginal culture • Gram stain • Wet prep* • Trichomonas* • Herpes culture* 	<ul style="list-style-type: none"> • Urine HCG • CBC with differential (pre-gel) • Maternal blood tenofovir levels (pre-gel, 1, 2, 4, 6, and 8 hours) • Flow cytometry (pre-gel)*** • PBMCs*** • Breast milk tenofovir level(target pre-gel, 2,4,6 hours) • Vaginal pH • Vaginal and cervical biomarkers • Quantitative vaginal culture • Gram stain • Wet prep* • Trichomonas test* • Herpes culture* 	<ul style="list-style-type: none"> • Blood tenofovir levels
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* if indicated ** if applicable *** if site capacity allows

7.4 Day 1 and Day 3 Phone Calls: Pregnancy and Lactation Cohorts

Table 12: Day 1 and Day 3 Phone Calls: Pregnancy and Lactation Cohorts

Day 1 and Day 3 Phone Calls: Pregnancy and Lactation Cohorts	
Component	Procedure/Analysis
Administrative, Behavioral, and Regulatory	<ul style="list-style-type: none"> • Update locator information • Schedule study visit* • Brief adherence assessment Adherence counseling
Clinical	<ul style="list-style-type: none"> • Collect AEs for mothers and infants (if born)

* if indicated

7.5 Day 6 Visit: Pregnancy and Lactation Cohorts

Table 13: Day 6 Visit: Pregnancy and Lactation Cohorts

Day 6 Visit: Pregnancy and Lactation Cohorts			
Component	Pregnancy Cohort Procedure/Analysis (Mothers)	Lactation Cohort Procedure/Analysis (Mothers)	Lactation Cohort: Procedure/Analysis (Infants)
Administrative, Behavioral, and Regulatory	<ul style="list-style-type: none"> • Update locator information • Follow-up acceptability and adherence questionnaire • Coital and product use log • HIV pre-and post-test counseling* • Reimbursement • Schedule Day 14 Phone Call • Provision of condoms and panty liners* 	<ul style="list-style-type: none"> • Update locator information • Follow-up acceptability and adherence questionnaire • Coital and product use log • HIV pre-and post-test counseling* • Reimbursement • Schedule Day 14 Phone Call • Provision of condoms and panty liners* 	<ul style="list-style-type: none"> • Disclosure of available test results
Clinical and Study Product	<ul style="list-style-type: none"> • Medical history • Concomitant medications • Targeted physical exam • Pelvic exam • Vaginal and cervical swabs • Urine collection* • Single dose of study gel to be administered by IoR/designee at clinic • Insert saline lock** • Blood collection at PK time points (target pre-gel and 1, 2, 4, 6, and 8 hours) • Collect AEs • Collect unused study gel • Disclosure of results* 	<ul style="list-style-type: none"> • Medical history • Concomitant medications • Targeted physical exam • Pelvic exam • Vaginal and cervical swabs • Urine collection* • Single dose of study gel administered by IoR/designee at clinic • Insert saline lock** • Blood collection at PK time points (target pre-gel and 1, 2, 4, 6, and 8 hours) • Breast milk collection (target pre-dose and 2,4, 6 hours) • Collect AEs • Collect unused study gel • Disclosure of results* 	<ul style="list-style-type: none"> • Medical history • Concomitant medications • Collect AEs • Blood collection via heelstick (target 6 hours <u>following maternal dosing</u>)
Laboratory	<ul style="list-style-type: none"> • Cervical NAAT for GC/CT* • CBC with differential (pre-gel) • Serum creatinine • AST and ALT • HIV serology* • HBsAg* • Maternal blood tenofovir level (target pre-gel, 1, 2, 4, 6, and 8 hours) • Flow cytometry (pre-gel)*** • PBMCs*** • Vaginal pH • Vaginal/cervical biomarkers • Quantitative vaginal culture • Vaginal Gram stain • Trichomonas test* • Wet prep* • Herpes culture* 	<ul style="list-style-type: none"> • Cervical NAAT for GC/CT* • CBC with differential (pre-gel) • Serum creatinine • AST and ALT • HIV serology* • HBsAg* • Maternal blood tenofovir level (target pre-gel, 1, 2, 4, 6, and 8 hours) • Breast milk tenofovir levels (target pre-dose and 2,4, 6 hours) • Flow cytometry (pre-gel)*** • PBMCs*** • Vaginal pH • Vaginal/cervical biomarkers • Quantitative vaginal culture • Vaginal Gram stain • Trichomonas test* • Wet prep* • Herpes culture* 	<ul style="list-style-type: none"> • Blood tenofovir levels

*if indicated ** if applicable *** if site capacity allows

7.6 Day 14 Phone Call: Pregnancy and Lactation Cohorts

Table 14: Day 14 Phone Call: Pregnancy and Lactation Cohorts

Day 14 Phone Call: Pregnancy and Lactation Cohorts	
Component	Procedure/Analysis
Administrative, Behavioral, and Regulatory	<ul style="list-style-type: none"> • Update locator information • Schedule study visit if indicated
Clinical	<ul style="list-style-type: none"> • Record AEs for mothers and infants (if born)

7.7 Delivery Phone Call (Pregnancy Cohort only)

Participants will be instructed to call the study site when they think they may be in labor, or if they are admitted with anticipation of delivery (e.g., for labor evaluation or induction of labor).

Table 15: Delivery Phone Call

Delivery Phone Calls: Pregnancy Cohort	
Component	Procedure/Analysis
Administrative, Behavioral, and Regulatory	<ul style="list-style-type: none"> • Confirm destination institution

7.8 Delivery Visit (Pregnancy Cohort only)

Table 16: Delivery Visit: Pregnancy Cohort

Delivery Visit: Pregnancy Cohort	
Component	Procedure/Analysis
Administrative, Behavioral, and Regulatory	<ul style="list-style-type: none"> • Update locator information (Mothers) • Reimbursement (Mothers) • Assign PTID for infant
Clinical	<ul style="list-style-type: none"> • Cord blood collection • Blood collection (Mothers, single time point when cord blood is taken) • Collect AEs for mothers and infants • Targeted physical exam (Mothers)*
Laboratory	<ul style="list-style-type: none"> • Cord blood tenofovir level • CBC*** • Maternal blood tenofovir level • Flow cytometry *** • PBMCs***

*As needed, e.g., to evaluate a reported AE *** if site capacity allows

7.9 Post-Delivery Assessment (Pregnancy Cohort only)

The Post-Delivery Visit will occur approximately 2 weeks (+/- 7 days) following delivery.

Table 17: Post-Delivery Assessment: Pregnancy Cohort

Post-Delivery Assessment: Pregnancy Cohort	
Component	Procedure/Analysis
Administrative, Behavioral, and Regulatory	<ul style="list-style-type: none"> Update locator information Reimbursement
Clinical	<ul style="list-style-type: none"> Collect AEs for mothers and infants

7.10 Interim Visit

An interim visit may occur at any time during study participation, and may be triggered by participant report of an AE, need for participant education, or other purpose at the discretion of the IoR/designee.

Table 18: Interim Visits

Interim Visit			
Component	Pregnancy Cohort: Procedure/Analysis (Mothers)	Lactation Cohort: Procedure/Analysis (Mothers)	Pregnancy and Lactation Cohort: Infants
Administrative, Behavioral, and Regulatory	<ul style="list-style-type: none"> Update locator information Schedule next visit/call* Provision of condoms and panty liners* 	<ul style="list-style-type: none"> Update locator information Schedule next visit/call* Provision of condoms and panty liners* 	<ul style="list-style-type: none"> Schedule next visit/call*
Clinical	<ul style="list-style-type: none"> Update concomitant medications Update AEs Perform targeted physical exam* Perform pelvic exam* Vaginal swabs* Cervical swabs* Urine collection* Blood collection* Chart review* Treatment for RTI/UTI* Disclosure of available results* 	<ul style="list-style-type: none"> Update concomitant medications Update AEs Perform targeted physical exam* Perform pelvic exam* Vaginal swabs* Cervical swabs* Urine collection* Blood collection* Treatment for RTI/UTI or mastitis* Disclosure of available results* 	<ul style="list-style-type: none"> Update AEs Blood collection via heelstick* (Lactation Cohort only)
Laboratory	<ul style="list-style-type: none"> Cervical NAAT for GC/CT* Serum creatinine* AST and ALT* HIV serology* HBsAg* Vaginal pH* Trichomonas test* Wet prep* Herpes culture* 	<ul style="list-style-type: none"> Cervical NAAT for GC/CT* Serum creatinine* AST and ALT* HIV serology* HBsAg* Vaginal pH* Trichomonas test* Wet prep* Herpes culture* 	

*if indicated

7.11 HIV Test Results

Participants will receive their HIV test results in person in the context of a post-test counseling session with a trained study staff member. As rapid testing will be used, results are expected to be available during the study visit.

In the unlikely event that an indeterminate or positive HIV test result occurs, participants will be informed of their results in person by a physician investigator able to participate in a thorough discussion of follow-up testing and treatment options, including prevention of mother-to-child transmission (PMTCT), as appropriate, and recommendation against breastfeeding. Confirmation of HIV infection will occur according to guidelines presented in Appendix II. If applicable, study staff members will make every effort to refer the participant to appropriate clinical and social support resources for HIV-infected pregnant women.

7.12 Pharmacokinetic Measures

For the purposes of scheduling subsequent evaluation and follow-up, the date of first gel administration will be considered Day 0, which is also the Enrollment Visit. The time of gel administration will be considered Time 0. Timed pharmacokinetic measures are timed by hours passed since gel administration. The study regimen and PK schedule is outlined in the table below.

Table 19: Overview of Study Regimen and Pharmacokinetic (PK) Procedures

	ENR Visit/Day 0	Day 6 Visit	Delivery Visit (Pregnancy Cohort)
Tenofovir 1% gel: vaginal dosing	First dose at clinic Participants will also receive enough study product for 5 self-administered doses for consecutive daily dosing at home	Final dose at clinic	
PK (mothers in Pregnancy and Lactation Cohorts)	Blood (target pre-gel, 1, 2, 4, 6, and 8 hours post-dose)	Blood (target pre-gel, 1, 2, 4, 6, and 8 hours post-dose)	Blood (single time point when cord blood is taken)
PK (infants in Pregnancy Cohort)			Cord blood Infant blood (if cord blood cannot be collected)
PK measurements (mothers in Lactation Cohort)	Breast milk (target 2,4, and 6 hours post-dose)	Breast milk (target pre-gel, 2,4, and 6 hours post-dose)	
PK measurements (infants in Lactation Cohort)	Infant blood (target 6 hours <u>following maternal dosing</u>)	Infant blood(target 6 hours <u>following maternal dosing</u>)	

Flow cytometry will be obtained pre-dose at the Enrollment Visit (Day 0), at the Day 6 Visit, and may also be obtained at the Delivery Visit for participants in the Pregnancy Cohort if site capacity allows. This will be required as a surrogate for cell activation and proliferation markers, to serve as covariates in the intracellular model building.

If not already in place, a saline lock (or similar device) may be inserted to facilitate the collection of multiple blood specimens. This device may be replaced as needed. Participants may also opt to have separate venipunctures for the PK blood draws.

As in MTN-002, MTN-008 will use a more sensitive tenofovir assay than the one used for HPTN 050. This assay was approved on 7 August 2009 by Clinical Pharmacology Quality Assurance (CPQA), which operates under contract with DAIDS. The validated liquid chromatography mass spectrometry (LCMS) method planned for MTN-008 has a LLOQ of 5 ng/mL, but uses only 50µL of fluid. Previous methods have had a LLOQ of 3 ng/mL but used 250µL. Since assay sensitivity is largely determined by mass in the sample, not concentration, this likely represents a 3-fold improvement in sensitivity if a similar volume to previous tenofovir gel studies is used. To improve even further, 1 mL serum for the blood tenofovir assay may be used to further increase the sensitivity, possibly below 1 ng/mL. This may increase the number of samples quantifiable in the blood assuming similar PK as in HPTN 050 and HPTN 059.

7.13 Clinical Evaluations and Procedures

Clinical evaluation of participants will include a targeted physical exam as described in Appendix III.

7.14 Laboratory Evaluations

Participants who temporarily hold or permanently discontinue use of study product will not routinely be withdrawn from the study. Rather, every effort will be made to complete all protocol-specified visits. In the event that a participant does not receive study gel, the IoR/designee will consult PSRT for further guidance.

7.14.1 Local Laboratory

The local laboratory, site investigator, or designee will run the following maternal laboratory evaluations, as indicated per protocol:

- Urine pregnancy test
- Serum creatinine
- AST
- ALT
- HIV test
- Confirmatory testing for HIV

- CBC with differential
- HBsAg
- Rapid plasma reagin
- Cervical NAAT for chlamydia and gonorrhea
- Confirmatory testing for syphilis
- Vaginal pH
- Wet prep
- Herpes culture
- Trichomonas test
- Flow cytometry

7.14.2 Network Laboratory

The Network Laboratory (NL) will run the following maternal laboratory evaluations:

- Quantitative vaginal cultures
- Gram stains
- Vaginal and cervical biomarkers (may be shipped to laboratory affiliated with NL)
- Pharmacokinetic analyses (at NL Pharmacology Core)

The following specimens will be collected for pharmacokinetic analyses of tenofovir according to guidelines outlined in the MTN-008 SSP Manual.

- Maternal blood (Pregnancy and Lactation Cohorts)
- Umbilical cord blood (Pregnancy Cohort)
- Infant blood (Lactation Cohort)
- Expressed breast milk (Lactation Cohort)

Pharmacokinetic samples may be batch shipped from the NL to the NL Pharmacology Core for assay.

7.15 Specimen Collection and Processing

The study site will adhere to the standards of good clinical laboratory practice, the HPTN-MTN Network Laboratory Manual (www.mtnstopshiv.org), DAIDS Laboratory Requirements(<http://www3.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/Laboratories.htm>), MTN-008 Study Specific Procedures Manual (www.mtnstopshiv.org), and site SOPs for proper collection, processing, labeling, transport, and storage of specimens at the local laboratory. Specimen collection, testing, and storage at the site laboratories will be documented when applicable using the Laboratory Data Management System (LDMS). In cases where laboratory results are not available due to administrative or laboratory error, sites are permitted to re-draw specimens.

7.16 Specimen Handling

Specimens will be handled in accordance with current DAIDS policies.

7.17 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. All dangerous goods materials, including diagnostic specimens and infectious substances, must be transported according to instructions detailed in the International Air Transport Association (IATA) Dangerous Goods Regulations. Biohazard waste will be contained according to institutional, transportation/carrier, and all other applicable regulations.

7.18 Behavioral Assessments

Using questionnaires, the following behaviors will be assessed:

- Study product adherence
- Sexual activity, including frequency of vaginal sex and condom use
- Intravaginal practices

At the Enrollment Visit (Day 0) and the Day 6 Visit, participants will provide data about their sexual behavior (recorded in a 7-day retrospective Coital Log) and their acceptability and use of the study gel. A Baseline Behavioral and Acceptability Questionnaire will be administered in the clinic after initial insertion of gel has occurred at the Enrollment Visit (Day 0). Participants will be asked questions about sexual behavior and intravaginal practice history, and about their attitudes towards the physical properties of the gel, their attitudes and perceptions about using gel during pregnancy and lactation, and other acceptability measures of the gel related to the sexual partner(s), sexual pleasure attributes, etc. During Day 1 and Day 3 Phone Calls, staff will ask participants about their gel use. At the Day 6 Visit, participants will report their use of gel and coital frequency using a 7-day retrospective Coital and Product Use Log. After clinical procedures and gel insertion are completed, they will be asked to complete the Follow-up Acceptability and Adherence questionnaire which will include acceptability questions at the Enrollment Visit (Day 0), as well as additional questions about their experiences using the gel, including reasons for non-use, if applicable, willingness to use in the future and beliefs around use of vaginal products during pregnancy and lactation, and any perceived effect on fetus/infant.

8 ASSESSMENT OF SAFETY

8.1 Safety Monitoring

The study site investigators are responsible for continuous close safety monitoring of all study participants, and for alerting the Protocol Team if unexpected concerns arise. A sub-group of the Protocol Team, including the Protocol Chair or designee, DAIDS and NICHD Medical Officers, Statistical Data Management Center (SDMC) Clinical Affairs Safety Associate, and Protocol Statistician, will serve as the Protocol Safety Review Team (PSRT). The PSRT will be co-chaired by the protocol safety physicians in the MTN CORE. Close cooperation among the PSRT and other study team members will be necessary to monitor participant safety and respond to occurrences of toxicity in a timely manner. The MTN SDMC prepares routine AE and clinical data reports for review by the PSRT, which meets via conference call approximately every month or as needed throughout the period of study implementation to review safety data, discuss product use management and address any potential safety concerns.

8.2 Clinical Data and Safety Review

A multi-tiered safety review process will be followed for the duration of this study. 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, the PSRT and study sponsors. Additional reviews may be conducted at each of these levels as dictated by the occurrence of certain events.

MTN SDMC Clinical Affairs staff will review incoming safety data on an ongoing basis. Events identified as questionable, inconsistent, or unexplained will be queried for verification. AE reports submitted in an expedited manner to the DAIDS Safety Office will be forwarded to the NICHD and DAIDS Medical Officers and SDMC Clinical Affairs staff for review.

The PSRT will meet approximately every month via conference call to review clinical data reports generated by the MTN 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, and may include posting on ATLAS. 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 MTN, representing 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.

In the unlikely event that the protocol team has serious safety concerns that lead to a decision to permanently stop accrual into the study, the protocol team will request a review of the data by the Study Monitoring Committee (SMC) before deciding that the study be stopped. Members of the SMC will be independent investigators with no financial interest in the outcomes of this study. If at any time a decision is made to discontinue enrollment, DAIDS will notify the FDA and the CRS Principal Investigator (PI) will notify the responsible IRB expeditiously.

8.3 Adverse Events Definitions and Reporting Requirements

8.3.1 Adverse Events

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

Study participants will be provided instructions for contacting the study site to report any untoward medical occurrences they may experience, except for possible life-threatening events, for which they will be instructed to seek immediate emergency care. Where feasible and medically appropriate, participants will be encouraged to seek evaluation where a study clinician is based, and to request that the clinician be contacted upon their arrival. With appropriate permission of the participant, whenever possible, records from all non-study medical providers related to untoward medical occurrences will be obtained and required data elements will be recorded on study CRFs. All participants reporting (or, in the case of infants, observed to have) an untoward medical occurrence will be followed clinically until the occurrence resolves (returns to baseline) or stabilizes.

Study site staff will document in source documents and the appropriate AE Log CRF all AEs reported by or observed in enrolled study participants regardless of severity and presumed relationship to study product. AE severity will be graded per the DAIDS Table for Grading Adult and Pediatric Adverse Events, Version 1.0, December 2004 (Clarification dated August 2009) and the Female Genital Grading Table for Use in Microbicide Studies (Addendum 1 to the DAIDS Table for Grading Adult and Pediatric Adverse Events, Version 1.0, December 2004, Clarification dated August 2009), except that asymptomatic BV will not be a reportable AE. AEs not included in the Female Genital Grading Table will be graded by the DAIDS AE Grading Table Version 1.0, December 2004 (Clarification dated August 2009). In cases where a genital AE is covered in both tables, the Female Genital Grading Table for Use in Microbicide Studies will be the grading scale utilized.

For hypertensive disorders of pregnancy, the following grading scale will be employed, using diagnostic criteria consistent with the ACOG Practice Bulletin on Diagnosis and Management of Preeclampsia and Eclampsia (Number 33, January 2002):

Table 20: Grading Scale for Hypertensive Disorders of Pregnancy

Parameter	Grade 1	Grade 2	Grade 3	Grade 4
Hypertensive disorder of Pregnancy	Pregnancy-induced hypertension	Mild preeclampsia	Severe preeclampsia	HELLP syndrome, eclampsia, or life-threatening sequelae of preeclampsia (e.g., pulmonary edema)

8.3.2 Serious Adverse Events

SAEs will be defined per US Code of Federal Regulations (CFR) 312.32, as AEs occurring at any dose that:

- Results in death
 - Is life-threatening
 - Results in persistent or significant disability/incapacity
 - Is a congenital anomaly/birth defect
 - Requires inpatient hospitalization or prolongation of existing hospitalization
- Note:* Per International Conference on Harmonisation (ICH) SAE definition, hospitalization itself is not an AE, but is an outcome of the event. Thus, hospitalization in the absence of an AE is not regarded as an AE, and is not subject to expedited reporting. The following examples are not considered to be AEs:
- Protocol-specified admission (e.g., for procedure required by study protocol)
 - Admission for treatment of target disease of the study, or for pre-existing condition (unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator)
 - Diagnostic admission (e.g., for a work-up of an existing condition such as persistent pretreatment lab abnormality)
 - Administrative admission (e.g., for annual physical)
 - Social admission (e.g., placement for lack of place to sleep)
 - Elective admission (e.g., for elective surgery)

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

8.3.3 Adverse Event Relationship to Study Product

Relatedness is an assessment made by a study clinician of whether or not the event is related to the study agent. Per the Manual for Expedited Reporting of Adverse Events to DAIDS (Version 2.0, January 2010), the relationship categories that will be used for this study are:

- *Related:* There is a reasonable possibility that the AE may be related to the study agent(s)
- *Not Related:* There is not a reasonable possibility that the AE is related to the study agent(s)

8.4 Expedited Adverse Event Reporting Requirements

For this study, the requirements and definitions for reporting AEs to the DAIDS Regulatory Compliance Center (RCC) Safety Office are defined and are available in the Manual for Expedited Reporting of Adverse Events to DAIDS, Version 2.0 (January 2010) on the RCC website: <http://rcc.tech-res.com/safetyandpharmacovigilance/>.

The AEs that must be reported in an expedited fashion to the DAIDS RCC Safety Office include all SAEs as defined by ICH of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines regardless of relationship to the study agent(s), and outlined in Section 8.3.2.

The timeframe for expedited reporting of individual AEs begins when the CRS recognizes that an event fulfills the protocol-defined criteria for expedited reporting to DAIDS. Clinical research sites must submit AEs to the DAIDS RCC Safety Office immediately and no later than 3 reporting days after the site becomes aware of an event requiring expedited reporting.

For all SAEs submitted to RCC, sites must file an update to RCC with the final or stable outcome unless the initial SAE submitted had a final or stable outcome noted already.

Sites using the internet-based DAIDS Adverse Event Reporting System (DAERS) for submission of AEs to DAIDS will follow the DAERS processes as outlined in the DAERS training information. For questions about DAERS, please contact DAIDS-ES at DAIDS-ESSupport@niaid.nih.gov or from within the DAERS application itself.

If the site cannot use DAERS to report an AE on an expedited basis, the AE must be documented on the EAE Form available on the RCC website: <http://rcc.tech-res.com>, and submitted as specified by DAIDS. For questions about AE reporting, please continue to contact the RCC Safety Office at RCCSafetyOffice@tech-res.com.

The study products that must be considered when AE relationships are assessed are:

For mothers

- tenofovir 1% gel
- HEC placebo gel (Pregnancy Cohort)
- study gel applicator

For infants (for maternal exposure to study gel)

- tenofovir 1% gel
- HEC placebo gel (Pregnancy Cohort)

Grading Severity of Events and Reporting Period

The grading of severity of events and the reporting period will be the same as for all AEs, as described in Section 8.3.1. After the end of the Protocol-defined AE Reporting Period, the study site 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.

The AE reporting period for this study comprises the entire study duration for each individual participant (from study enrollment until study completion or discontinuation of the participant from study participation for any reason). The NIAID/DAIDS will report all unexpected SAEs associated with the study products observed in this clinical trial to the FDA.

8.5 Pregnancy Outcomes

Pregnancy outcomes will be collected for all study participants. After the participant's final study contact, any pregnancy outcomes that meet criteria for SAE reporting as described above (e.g., congenital anomalies) occurring among participants will continue to be expeditiously reported.

8.6 Regulatory Requirements

Information on all reported AEs will be included in reports to the FDA and other applicable government and regulatory authorities. The site IoR/designee will submit AE information in accordance with local regulatory agencies' or other local authorities' requirements. The site IoR/designee also will submit AE information and any other relevant safety information to the IRB in accordance with IRB requirements.

9 CLINICAL MANAGEMENT

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

9.1 Toxicity Management

Based on results from previous clinical trials, significant toxicity in study participants is not expected in MTN-008. Based on preliminary results from MTN-002 and previous clinical trials, minimal systemic absorption is expected from this vaginal gel product.

Lactic acidosis and severe hepatomegaly with steatosis, including fatal cases have been reported with the use of orally administered nucleoside analogs alone or in combination with other antiretrovirals. A majority of these cases have been in women. Obesity and prolonged nucleoside exposure may be risk factors. Particular caution is advised when administering nucleotide analogs to patients with known risk factors for liver disease; however, cases have been reported in patients with no known risk factors.

9.2 Dose Modification Instructions

No dose modifications will be undertaken in this study.

9.3 Criteria for Withholding Study Product

Participants may decline administration of study product. The IoR/designee may withhold study product from participants to protect their safety.

The withholding of study product will occur only under certain criteria. At least one of the following criteria must be met to withhold study product.

- Request by participant to not receive study product
- Decision by the investigator to protect the participant's safety
- Participant is unable or unwilling to comply with study procedures

9.4 Criteria for Early Termination of Study Participation

A participant may voluntarily withdraw from the study for any reason at any time. The IoR/designee may, with the approval of the PSRT, withdraw a participant to protect their safety.

Early (premature) termination of a study participant will occur only under certain criteria. At least one of the following criteria must be met to implement early termination from the study:

- Request by participant to withdraw
- Request by the IoR/designee, to protect the participant's safety

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

A participant will be temporarily held or permanently discontinued from study product for the following reasons:

- Participant is unable or unwilling to comply with required study procedures, or otherwise might be put at undue risk to their safety and well-being by continuing product use, according to the judgment of the IoR/designee. The IoR/designee must consult the PSRT on all temporary product holds instituted for this reason for further guidance on resuming product use, continuing the temporary hold, or progressing to permanent discontinuation. If product use is temporarily held/permanently discontinued for this reason, but the underlying reason for the temporary hold later resolves, the IoR/designee should consult the PSRT to resume product use at that time.

9.6 Temporary Product Hold/Permanent Discontinuation in Response to Observed Adverse Events

Grade 1

In general, a participant mother who develops a Grade 1 AE regardless of relatedness to study product may continue product use.

Grade 2

A participant mother who develops a Grade 2 AE judged by the IoR/designee to be not related to study product may continue product use.

For Grade 2 AEs judged to be related to study product, a participant mother may continue product use at the discretion of the clinical judgment of the IoR/designee, while a response to PSRT consultation is pending. The PSRT will be consulted in such cases for recommendations regarding product use management.

Grade 3

Participant mothers who develop a Grade 3 AE that is judged by the IoR/designee to be not related to study product may continue product use.

For Grade 3 AEs judged to be related to study product, the IoR/designee will:

- Temporarily hold the study product.

- Consult the PSRT regarding resumption of study product or permanent discontinuation.

Grade 4

A participant mother who develops a Grade 4 AE must have the study product held. The IoR/designee must consult the PSRT and continue the temporary product hold minimally until a recommendation is obtained from the PSRT. In general, product use will not be resumed with the exception of cases for which the IoR/designee can identify a definitive cause for the AE other than study product. If, in consultation with the PSRT, product use is resumed and the same Grade 4 AE recurs at Grade 4 level at any time, study product must then be permanently discontinued. Product hold for mothers in the Lactation Cohort and consultation with the PSRT may also be triggered by infant AEs, according to the clinical judgment of the IoR/designee.

9.7 Management of Specific Toxicities

Specific temporary product hold requirements are specified here in the context of clinical management of toxicities.

9.7.1 Nausea, Vomiting, and/or Diarrhea

The IoR/designee may treat a participant with Grade 1 or 2 nausea, vomiting, and/or diarrhea symptomatically (e.g., diet changes, antiemetics, and/or supportive fluids).

9.7.2 AST and/or ALT Elevations

Careful assessments should be done to rule out preeclampsia, alcohol, non-study medication-related drug toxicity, herbal agents, or viral hepatitis as the cause of elevation in AST and/or ALT of any grade. The IoR/designee must carefully assess the participant for any symptoms or signs of hepatotoxicity, including fatigue, malaise, anorexia and nausea, jaundice, acholic stools, right upper quadrant pain or hepatomegaly. If the AST and/or ALT elevation is considered most likely to be due to concomitant illness or medication, standard management, including discontinuation of the likely causative agent (if clinically indicated), should be undertaken.

9.8 Allergic Reaction and/or Suspected Local Toxicity

If deemed clinically appropriate by the IoR/designee, product exposure may be minimized to the extent possible via cervicovaginal lavage.

9.9 Suspected Complication of Pregnancy or Lactation

Participants judged by the IoR/designee to have a suspected complication of pregnancy or lactation (e.g., preterm contractions, preeclampsia, mastitis, etc.) will be referred to appropriate clinical management in a timely fashion, regardless of

relatedness to study participation. Treatment for mastitis or clinical evaluation for suspected rupture of membranes may be undertaken at the study site, at the discretion of the IoR/designee. Every effort will be made to facilitate the participant's access to appropriate clinical management.

10 STATISTICAL CONSIDERATIONS

10.1 Overview and Summary of Design

This is an expanded Phase 1, randomized, double-blinded, placebo-controlled, safety and pharmacokinetic study of tenofovir 1% gel used daily for 7 days in HIV-uninfected pregnant and lactating women. Two groups are considered within the Pregnancy Cohort: the first group contains 45 pregnant women during 37 0/7 and 39 1/7 weeks gestation (inclusive) on Day 0; the second group contains 45 pregnant women during 34 0/7 and \leq 36 6/7 weeks gestation (inclusive) on Day 0. In both groups, 45 pregnant women will be randomized to the active gel arm and the placebo gel arm with ratio 2:1. Opening accrual into the second group within the Pregnancy Cohort will be contingent upon reassuring safety data obtained from the first group.

Fifteen lactating women with infants 4 – 26 weeks old will be recruited into the Lactation Cohort. Accrual into the Lactation Cohort may occur simultaneously with accrual into the Pregnancy Cohort. For both Pregnancy and Lactation Cohorts, the first dose will be administered at the study site on Day 0. The second through sixth doses of study product will be administered at home. The seventh and final dose of study product will be administered at the study site at the Day 6 Visit.

10.2 Study Endpoints

Consistent with the primary study objectives, the following endpoints will be assessed:

1. Pregnancy Safety and Tolerability – Maternal Outcomes

- Grade 2 or higher AEs in the following categories

Specific laboratory abnormalities

- ALT
- AST
- Creatinine

Specific genital/pelvic signs/symptoms

- Dyspareunia
- Pain (vulvar, vaginal, and/or pelvic)
- Tenderness (vulvar, vaginal, and/or pelvic)
- Itching (vulvar and/or vaginal)
- Edema (vulvar, vaginal, and/or cervical)

- Erythema (Vulvar, vaginal, and/or cervical)
- Lesions (vulvar, vaginal, and/or cervical)
- Vulvar rash
- Vaginal dryness
- Dysuria
- Vulvovaginitis
- Cervicitis

Specific Pregnancy Complications

- Postpartum hemorrhage
- Postpartum endometritis
- Chorioamnionitis
- Third trimester bleeding
- Preterm premature rupture of membranes (prior to onset of labor)
- Term premature rupture of membranes (prior to onset of labor)
- Spontaneous preterm delivery
- For AEs not included above, Grade 3 or higher AEs judged by the investigator to be related to the study gel or applicator

2. Pregnancy Safety and Tolerability – Infant Outcomes

- Infant in the Pregnancy Cohort diagnosed (and confirmed on review of medical records) with any of the following during the 7 days following delivery
 - Intensive care admission greater than 24 hours. *Note: all intensive care unit admits will be reviewed for toxicities potentially related to study product exposure*
 - Sepsis

3. Lactation Safety and Tolerability – Maternal Outcomes

- Grade 2 or higher AEs in the following categories

Specific laboratory abnormalities

- ALT
- AST
- Creatinine

Specific genital/pelvic signs/symptoms

- Dyspareunia
- Pain (vulvar, vaginal, and/or pelvic)
- Tenderness (vulvar, vaginal, and/or pelvic)
- Itching (vulvar and/or vaginal)
- Edema (vulvar, vaginal, and/or cervical)
- Erythema (vulvar, vaginal, and/or cervical)
- Lesions (vulvar, vaginal, and/or cervical)
- Vulvar rash
- Vaginal dryness
- Dysuria
- Vulvovaginitis

- Cervicitis
4. Lactation Safety and Tolerability – Infant Outcomes
 - Infant in the Lactation Cohort with the following during the period of study participation, confirmed on review of medical records
 - Inpatient admission (confirmed on review of medical records) with diagnosis of AE judged to be related to study product
 5. Tenofovir Levels
 - Maternal blood of participants in the Pregnancy and Lactation Cohorts
 - Breast milk of participants in the Lactation Cohort

Consistent with the secondary study objectives, the following endpoints will be assessed:

- The presence of tenofovir in the cord blood and/or venous blood of infants in the Pregnancy Cohort
- The presence of tenofovir in the blood of infants in the Lactation Cohort
- The presence of selected organisms in the vagina at Day 0 and Day 6 (for both Pregnancy Cohort and Lactation Cohort)
- Self-reported gel use during home administration (including last time used), frequency of sexual activity (including vaginal sex and condom use), intravaginal practices (ever, during study period), unused applicator counts; acceptability of gel use.

Consistent with the exploratory study objectives, the following endpoints will be assessed:

- Vaginal flora characteristics in Gram stain and quantitative culture
- Biomarker expression in vaginal and cervical secretions

10.3 Primary Study Hypotheses

We hypothesize that:

- Repeated exposure via daily use of tenofovir 1% vaginal gel by pregnant and lactating women for 7 days will be well-tolerated and not associated with significant toxicity
- Blood tenofovir concentrations will be detectable in approximately 80% of women in the Pregnancy Cohort who receive study gel
- Among women in the Pregnancy Cohort, only women who deliver during their study product dosing period will have detectable levels of tenofovir in cord blood and/or neonatal blood
- In the Lactation Cohort, tenofovir will only be detectable at low levels in breast milk, and when detectable, only in a minority of participants

- Blood tenofovir levels among women in the Lactation Cohort will be similar to those in the Pregnancy Cohort
- Tenofovir will not be detectable in the blood of nursing infants in the Lactation Cohort

Note: There are no hypotheses associated with the adherence/behavioral secondary objective as this is a purely descriptive objective.

10.4 Sample Size and Power Calculations

For the proposed study sample size, the statistical properties of this study in assessing the safety and PK of study products are summarized below. An event is defined as a subject either having at least one safety endpoint or having a detectable level of tenofovir. With approximately 15 participants in the Lactation Cohort or the placebo control arm in the Pregnancy Cohort, and approximately 30 in the active gel arm in the Pregnancy Cohort (Group 1 and Group 2), the probabilities of observing various numbers of events are listed in the following table, assuming various true event rates. For instance, if the true rate is 5%, the probability of observing that endpoint in at least one participant out of 15 participants is 0.537; the probability of observing at least one participant out of 30 participants is 0.785. A higher true event rate will result in a larger probability to observe at least one event. So if the true rate is 10%, the probability of observing that endpoint in at least one participant out of 15 participants is 0.794; the probability of observing at least one participant out of 30 participants is 0.958. Note that the focus in the Lactation Cohort is on the PK of tenofovir gel, therefore a smaller sample size (15 participants) is designated.

Table 21: The Probability of Observing a Number of Participants of Having an Event Given the True Event Rate and the Sample Size in the Group

True rate	n=15/group			n=30/group		
	0 event	1 or more events	2 or more events	0 event	1 or more events	2 or more events
1%	0.860	0.140	0.010	0.740	0.260	0.036
5%	0.463	0.537	0.171	0.215	0.785	0.446
10%	0.206	0.794	0.451	0.042	0.958	0.816
15%	0.087	0.913	0.681	0.008	0.992	0.952
20%	0.035	0.965	0.833	0.001	0.999	0.989
25%	0.013	0.986	0.920	0.000	1.000	0.998

When evaluating the safety and tolerability in the pregnancy cohorts, we will use the data from the randomized control arm as the primary baseline data for comparison. However due to a smaller sample size (15) in the control arm, relatively rare AE (rates <10%), such as some pregnancy-related complications, may not be observed at all in the controls. In this case we will use rates from historical controls from local databases. Table 21 shows the rates of pregnancy related endpoints from local databases in Pittsburgh and Birmingham. For the smaller Lactation Cohort, the primary and secondary endpoints of laboratory and genital AEs will be compared to historical control data from HPTN 059. These rates are for descriptive purposes, serving as approximate baseline estimates for informal comparison.

Table 22: Rates of pregnancy-related endpoints

Pregnancy-related Endpoint	Rate (Pittsburgh)	Rate (Birmingham)****
Postpartum hemorrhage	10.2%***	2.49%
Term premature rupture of membranes (prior to onset of labor)	10%**	16.23%
Spontaneous preterm delivery	3.3%*	7.8%
Chorioamnionitis	3.4%*	5.11%
Preterm premature rupture of membranes (prior to onset of labor)	3.0%*	7.31%
Third trimester bleeding	1.5%***	Data not available
Postpartum endometritis	Vaginal delivery: 1%*** Caesarean delivery: 15%**	Vaginal delivery: 0.61% Caesarean delivery: 0.76%
Neonatal sepsis	0.1%*	0.2%

* MOMI Database 2006-2008

** Gabbe SG, Niebyl JR, Simpson JL. *Obstetrics: Normal and Problem Pregnancies*. 5th ed: Churchill Livingstone 2007

*** MOMI data corroborated US rates reported in Gabbe et al.

**** OBARS data 2007-2009

This is a Phase 1 study and therefore not powered to detect small increases in rates of AE endpoints as compared to the control arm or baseline historical control. Rather we are only able to detect significantly large deviations from the control rates. The table below shows the minimal detectable increased rate with 80% power for the target sample size (30 treatment/15 control) in the Pregnancy Cohort groups and a range of baseline rates. For relatively rare events with a baseline rate of 5%, our targeted sample size provides 80% power to detect a statistically significant increase for a minimal 21% observed event rate. For a higher baseline event rate such as 10% and 15%, our targeted sample size provides 80% power or detect a 2-3-fold increase compared to the baseline 10% rate.

Table 23: The Minimal Event Rate that is Detected to be Significantly Higher Than the Control Arm Given Power of 0.8 and One-Sided p-value of 0.5 for a Sample Size of 30 Treatment/15 Control

	Control p=5%	Control p=10%	Control p=15%
N1=30/N0=15	0.21	0.27	0.32

Additional participants may enroll in the study, at the discretion of the protocol team, to replace currently enrolled participants who are non-adherent to the study product and/or the study visit schedule. Thus, in the event that additional participants are recruited for this purpose, the total sample size at the end of the study may slightly exceed 30 participants in each pregnancy group or 15 in the Lactation Cohort.

10.5 Participant Accrual, Randomization, Follow-up and Retention

The Pregnancy Cohort and Lactation Cohort will be recruited simultaneously. The study site is expected to enroll approximately 4 participants per month. Therefore accrual is anticipated to take approximately 6 months for Pregnancy Cohort 1, 2 months for the Lactation Cohort, and an additional 6 months for Pregnancy Cohort 2. The target for retention will be 95% of enrolled participants over the study period.

Women in the Pregnancy Cohort will be randomized to the active gel and the placebo gel by the ratio of 2:1. The SDMC will provide each study site with a series of numbered, sealed envelopes containing the randomization assignment for each participant. The envelopes will be assigned sequentially by site staff. Each participant will be assigned a product code number. Using a blinded list of product codes and assigned products, the pharmacist at each site will supply the study product. Multiple codes will be utilized to protect the randomization assignments in this study.

10.6 Data and Safety Monitoring and Analysis

10.6.1 Study Monitoring Committee (SMC)

In addition to the safety monitoring done by the PSRT, the MTN SMC will conduct interim reviews of study progress, including rates of participant accrual, retention, completion of primary and main secondary endpoint assessments, and study or lab issues. These reviews will take place approximately every 120 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.

It is not expected that this investigation will predispose pregnant women to higher rates of untoward pregnancy outcomes, given the favorable safety profile demonstrated among non-pregnant women, and among pregnant women to date in MTN-002. However, an interim review of the safety data by the SMC is planned after Group 1 of the Pregnancy Cohort completes scheduled participation, and prior to beginning accrual in to Group 2.

The SMC will review safety data collected in Pregnancy Cohort Group 1 to look for a significantly higher rate (e.g., approximately three times higher for events with a baseline rate of 5%) of pregnancy complications compared to controls. If this were noted at interim review, serious consideration would be given as to the prudence of enrolling and exposing women at preterm gestational ages to this product.

Outcomes among women in Pregnancy Cohort Group 1 to be reviewed by the SMC include rates of the following diagnoses:

- **Preeclampsia**
- **Chorioamnionitis**
- **Endometritis**
- **Placental abruption**
- **Intrauterine fetal demise**
- **Postpartum hemorrhage**
- **Premature rupture of membranes**
- **Neonatal sepsis**

Although the predominant consideration would be pregnancy outcomes, if there are some repetitive significant high grade genital or lab toxicities that are related to the product, they would become part of the consideration.

10.6.2 Data Analysis

Descriptive statistics will be used to summarize the characteristics of study endpoints. For categorical variables, the numbers and the proportions will be tabulated with 95% confidence interval; for continuous variables, the mean, median, standard deviation, and quartiles will be reported.

All AE data will be used to assess safety. In particular, since the first dose is administered at the study site, the number and the percentages of participants experiencing at least one AE, and the number and percentage experiencing each specific AE will be tabulated for all women who completed the first dose of study product and for all women who complete the study. To account for potential discontinuation of gel use, the AE rates will also be computed based on person-day use of gel, so that all AE data will be used. The number and percentage of participants experiencing each type of AE (including AEs leading to study discontinuation) will be tabulated by severity and relationship to treatment for each cohort. AEs that lead to study product discontinuation will be listed in a separate data listing. Changes from baseline to follow-up within each cohort will be assessed

by a McNemar's test for dichotomous variables, or by a Wilcoxon signed rank test for continuous variables. The event rates will also be compared across treatment arms by Chi-square test.

Blood PK of tenofovir will be evaluated after vaginal administration. The concentration of tenofovir will be plotted against time since gel administration. Pharmacokinetic parameter estimates will include peak concentration (C_{max}) and time to peak concentration (T_{max}) for tenofovir in the blood. Descriptive statistics will be used to summarize these PK parameters in the cohort. The ratio of concentrations of tenofovir in maternal blood relative to temporally matched cord blood, and ratio of maternal blood plasma relative to intracellular peripheral blood mononuclear cell (PBMC) tenofovir and tenofovir diphosphate levels will be calculated and summarized using descriptive statistics. If a substantial number of women are below the limit of detection of the assay, a proportion of participants with detectable levels will be computed along with 95% confidence interval.

To assess the effect of tenofovir gel on the presence of certain organisms, the proportion of women who have these organisms present in their vagina will be computed for Day 0 and Day 6. The difference between Day 0 and Day 6 will be evaluated by a paired two-sample test. To assess the effect of tenofovir gel on vaginal flora, clinically significant changes in vaginal flora will be evaluated by the Nugent score with shift tables from baseline (Enrollment) to follow-up visits. The Nugent score is graded 0 to 10 as follows:

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

Any shift from normal at baseline to intermediate or BV at a follow-up visit, or intermediate at baseline to BV at a follow-up visit, will be considered a clinically meaningful change in vaginal flora. Summary statistics will be also computed for quantitative measures of vaginal flora (defined by ≥ 1 log change in dominant members of the microflora, including *Lactobacillus* (H_2O_2 positive and negative strains), anaerobic gram negative rods, *Gardnerella vaginalis*, *Escherichia coli*, *Staphylococcus aureus*, *Candida* species, Group B *Streptococcus*, and *Enterococcus* species).

Adherence, Acceptability, and Sexual Behavior

An important secondary analysis will focus on adherence to study treatment. Self-reported adherence to product use will be measured at 3 time points through clinician interview (Day 1 and Day 3 Phone Calls; Day 6 Visit), and through self-completed retrospective product log (Day 6 Visit). Study staff will also collect data on returned unused applicators. This data structure will permit the estimation of adherence rates and a comparison of differences in adherence by modality (self-report, returned applicators, PK levels). Furthermore, we will assess self-reported

sexual behavior as the count and proportion of vaginal sexual acts unprotected by condom.

11 DATA HANDLING AND RECORDKEEPING

11.1 Data Management Responsibilities

Study case report forms will be developed by the SDMC. Quality control reports and queries routinely will be generated and distributed by the SDMC to the study site for verification and resolution.

11.2 Source Documents and Access to Source Data/Documents

All study sites will maintain source data/documents in accordance with [current](#) DAIDS policies. Each IoR/designee will maintain, and store securely, complete, accurate and current study records throughout the study. In accordance with US regulations, the IoR/designee will maintain all study documentation for at least two years following the date of marketing approval for the study product for the indication in which it was studied. If no marketing application is filed, or if the application is not approved, the records will be retained for two years after the investigation is discontinued and the FDA is notified.

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

11.3 Quality Control and Quality Assurance

All study sites will conduct quality control and quality assurance procedures in accordance with current DAIDS policies.

11.4 Study Coordination

DAIDS holds the IND application for this study (#55,690). Assignment of all sponsor responsibilities for this study will be specified in a Clinical Trial Agreement (CTA) executed by DAIDS and CONRAD. Training and written instructions outlining management and reporting, study gel dispensing, product accountability, and other study operations will be provided by MTN CORE, Family Health International (FHI), the Statistical Center for HIV/AIDS Research & Prevention (SCHARP), and the MTN Network Laboratory (NL).

12 CLINICAL SITE MONITORING

Study monitoring will be carried out in accordance with [current](#) DAIDS policies. Study monitors will visit the site to do the following:

- Review informed consent forms, procedures, and documentation
- Assess compliance with the study protocol, Good Clinical Practices (GCP) guidelines, and applicable regulatory requirements (US and non-US), including CFR Title 45 Part 46 and Title 21 Parts 50, 56, and 312.
- Perform source document verification to ensure the accuracy and completeness of study data
- Verify proper collection and storage of biological specimens
- Verify proper storage, dispensing, and accountability of investigational study products

The IoR/designee will allow study monitors to inspect study facilities and documentation (e.g., informed consent forms, clinic and laboratory records, other source documents, CRFs), as well as observe the performance of study procedures. The IoR/designee also will allow inspection of all study-related documentation by authorized representatives of the MTN CORE, SDMC, NL, NIAID, and US regulatory authorities. A site visit log will be maintained at the study site to document all visits.

13 HUMAN SUBJECTS PROTECTIONS

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

13.1 Institutional Review Boards

The participating institution is responsible for assuring that this protocol and the associated informed consent documents and study-related documents are reviewed and approved by the IRB prior to implementation of the protocol. Any amendments to the protocol and/or informed consent documents must be approved by the IRB and DAIDS prior to implementation.

13.2 Protocol Registration

Each study site will complete protocol registration with the DAIDS RCC Protocol Registration Office. Protocol registration material can be sent electronically to epr@tech-res.com. The following site can be accessed for information regarding

protocol registration: <http://rcc.tech-res.com/protocolregistration/>. For questions regarding protocol registration, please call (301) 897-1707 or email Protocol@tech-res.com. For additional information, refer to the protocol registration documents located at <http://rcc.tech-res.com/forms.htm>. Protocol registration must occur as a condition for site-specific study activation; no participants may be screened or enrolled in this study prior to obtaining protocol registration approval and completing all other study activation requirements. MTN CORE FHI staff, will notify the study site when all activation requirements have been met by issuing a site-specific study activation notice. Study implementation may not be initiated until the activation notice is issued. The study will be conducted in full compliance with the protocol. The protocol will not be amended without prior written approval by the Protocol Chairs and DAIDS Medical Officer. All protocol amendments must be submitted to and approved by the relevant IRB and the RCC prior to implementing the amendment.

13.3 Risk Benefit Statement

13.3.1 Risks

Waiting for test results may lead to anxiety in participants. Disclosure of STI status may cause sadness or depression in participants. Disclosure of HIV-positive status has been associated with depression, suicidal ideation, and denial, as well as social isolation.

The risks associated with tenofovir disoproxil fumarate include upset stomach, vomiting, gas, loose or watery stools, weakness, dizziness, depression, headache, abdominal pain, worsening or new kidney damage or failure, inflammation or swelling and possible damage to the pancreas and liver, shortness of breath, rash, allergic reaction including fever, rash, upset stomach, vomiting, loose or watery stools, abdominal pain, achiness, shortness of breath, general feeling of illness or a potentially serious swelling of the face, lips, and/or tongue, muscle pain and muscle weakness, and bone pain and bone changes such as thinning and softening which may increase the risk of breakage.

The risks associated with tenofovir gel are believed to be less than those identified for systemic use. Side effects which have been associated with tenofovir gel include dryness, itching, burning, or pain in the genital area. Administration of tenofovir gel intravaginally at 0.3% and 1% concentrations in the HPTN 050 Phase 1 study resulted in minimal local irritation and little or no systemic adverse effects. Although 92% of participants reported at least one AE, 87% of those reported AEs were mild, and 77% 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. In the HPTN 050 Phase 1 study of tenofovir gel, PK analysis in a subset of participants demonstrated that there is no clinically significant systemic toxicity. Fourteen of 25 women with PK results had low, but detectable, tenofovir levels.

The most common AEs in patients receiving TDF with other antiretroviral (ARV) therapy in clinical trials were mild to moderate gastrointestinal events, such as nausea, diarrhea, vomiting, and flatulence. Less than 1% of patients discontinued participation in the clinical studies because of gastrointestinal AEs. Laboratory abnormalities observed in studies occurred with similar frequency in the TDF and placebo-treated groups.

In rare cases, hypophosphatemia, proteinuria, glycosuria, and reduced creatinine clearance have been seen, and several cases of renal tubular injury have been reported. In a previous study, discontinuation for renal toxicity was equally infrequent in the TDF and d4T treated patients; all patients had normal baseline renal function. In a retrospective review, the rate of TDF discontinuation due to increased creatinine was evaluated in a review of a clinical database which included drug treatment, demographic, and laboratory data of 563 HIV-1-infected subjects who had been treated with TDF. Of these subjects, 11 (2%) had discontinued TDF due to elevated creatinine after a median of four months (range 2-9); of the nine for whom renal biopsy was available, all showed evidence of acute tubular injury. Lactic acidosis and severe hepatomegaly with steatosis, including fatal cases have been reported with the use of nucleoside analogues alone or in combination, including TDF, and other ARVs.

Given that Phase 1 data demonstrates measurable plasma concentrations of tenofovir in some participants, participants with hepatitis B infection might be at risk for development of tenofovir-resistant hepatitis B. However, participants with known hepatitis B infection will not be eligible for enrollment.

Maternal blood tenofovir levels are expected to be inconsistent and low-level, if detected. Available data suggest no risk to the fetus after exposure to higher maternal systemic levels associated with oral dosing. In the unlikely event that appreciable neonatal levels occur, potential side effects may include diarrhea, nausea, vomiting, and flatulence.

The effects of maternal tenofovir gel exposure on a nursing infant are unknown. However, the low levels observed in breast milk following maternal exposure to oral TDF would suggest a low risk of clinically significant exposure to the nursing infant following maternal exposure to topical tenofovir.

Tenofovir gel is not expected to pose a significant risk to male sexual partners. No evidence of significant safety concerns for sexual partners has been identified, based on a study of male tolerance³⁸, as well as multiple studies to date in which vaginal sexual intercourse was permitted by the protocol. Potential risk to female sexual partners has not been assessed in studies to date.

13.3.2 Benefits

Participation in this study likely will have no direct benefit to participants, yet the participant may appreciate the opportunity to contribute to the body of knowledge in the field of microbicide research.

13.4 Informed Consent Process

Written informed consent will be obtained from all potential study participants prior to the initiation of any study-related procedures. In obtaining and documenting informed consent, the investigators and their designees will comply with applicable local and domestic regulatory requirements and will adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki. Study staff must document the informed consent process in accordance with all DAIDS requirements. Participants are provided with copies of the informed consent forms if they are willing to receive them. Each study site is responsible for developing study informed consent forms for local use, based on the templates in the Appendices that describe the purpose of screening and of the study, the procedures to be followed, and the risks and benefits of participation, in accordance with all applicable regulations.

Prior to the beginning of the trial, site investigators will have the IRB's written approval/favorable opinion of the protocol, informed consent forms, and any other study-related information to be provided to participants.

The informed consent process will give individuals all of the relevant information they need to decide whether to participate, or to continue participation, in this study. Study staff will also use IRB approved methods/materials to fully inform potential participants of what they can expect by participating in the study during the Screening and Enrollment Visits. Potential research participants will be permitted to ask questions and to exchange information freely with the study investigators. Listed study investigators or their designees will obtain informed consent from potential study participants. The investigators will keep research participants fully informed of any new information that could affect their willingness to continue study participation. Additionally, the father's permission for infant participation in the study may be sought per determination of the local IRB.

The informed consent process covers all elements of informed consent required by research regulations. In addition, the process specifically addresses the following topics of import to this study:

- The importance of adherence to the study visit and procedures schedule.
- The potential risks of study participation (and what do if such risks are experienced).
- The potential social harms associated with study participation (and what do if such harms are experienced).
- The distinction between research and clinical care.
- The right to withdraw from the study at any time.

13.5 Participant Confidentiality

All study procedures will be conducted in private, and every effort will be made to protect participant privacy and confidentiality to the extent possible. The study site will establish a standard operating procedure for confidentiality protection that reflects the local study implementation plan. In addition to local considerations, the protections described below will be implemented at the site.

All study-related information will be stored securely at the study site. All participant information will be stored in locked areas with access limited to study staff. All local databases will be secured with password-protected access systems. Participants' study information will not be released without their written permission, except as necessary for monitoring (see Section 12).

The MTN has obtained a Certificate of Confidentiality from the US Department of Health and Human Services that is applicable for this study. This Certificate protects study staff from being compelled to disclose study-related information by any US Federal, State or local civil, criminal, administrative, legislative or other proceedings. It thus serves to protect the identity and privacy of study participants.

Participants' study information will not be released without their written permission, except as necessary for review, monitoring, and/or auditing by the following:

- Representatives of the US Federal Government, including the FDA, the US Office for Human Research Protections (OHRP), NIH, and/or contractors of the NIH
- Representatives of CONRAD
- Representatives of the MTN CORE, SDMC, and/or NL
- Site IRB

13.6 Special Populations

13.6.1 Pregnant Women

During the informed consent process, women will be informed that the data on oral TDF exposure in pregnancy thus far, do not suggest any deleterious effects. At the time of this writing, oral TDF is classified by the FDA as a pregnancy category B drug. Animal studies have failed to demonstrate a risk to the fetus. No significant AEs in the women or infants have been attributed to tenofovir. Preliminary data from PACTG 394, which investigated fourteen mother-infant pairs using a single oral dose of TDF 600 mg showed no significant AEs in the women or infants.

Additional safety data regarding the use of tenofovir in pregnancy are available from the APR. Through January 31, 2009, a total of 1063 cases of tenofovir exposure during pregnancy have been reported. Of these 1063 exposures 678 occurred

during the first trimester and 385 occurred later in pregnancy. There has been no increase in birth defects or unusual pattern of defects detected, first trimester: 16 of 678 (2.4%); later in pregnancy: 6 of 385 (1.6%).²⁷ These rates fall below the generally accepted background rate of 3% for major malformations, and thus add to the growing body of evidence supporting the safety of tenofovir exposure during pregnancy.

13.6.2 Children

The NIH has mandated that children be included in research trials when appropriate. This protocol will include infants less than approximately 6 months old and may include participants aged 18 to 21 years old.

13.7 Compensation

Pending IRB approval, participants will be compensated for time and effort.

13.8 Communicable Disease Reporting

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

13.9 Access to HIV-Related Care

13.9.1 HIV Counseling and Testing

HIV pre-test and post-test counseling will be provided to all study participants who consent to undergo HIV screening. Participants must receive their HIV test results to take part in this study. Participants who have positive or indeterminate results will have standard post-test counseling as well as limited follow-up confirmatory testing provided by the study; the participant's primary obstetrician will also be notified with the participant's permission. Referral for additional counseling related to testing or diagnosis will occur if needed or requested by the participant.

13.9.2 Care for Participants Identified as HIV-Infected

Study staff will provide participants with their HIV test results in the context of post-test counseling. In compliance with local regulations and in accordance with site SOPs, study staff will refer participants found to be HIV-infected to available sources of medical and psychological care, social support, and local research studies for HIV-infected women.

13.10 Study Discontinuation

This study may be discontinued at any time by NIAID, the MTN, CONRAD, the FDA, the OHRP, other government or regulatory authorities, or the site IRB.

14 PUBLICATION POLICY

DAIDS and MTN policies and a CTA between CONRAD and NIAID will govern publication of the results of this study. Any presentation, abstract, or manuscript will be submitted by the Investigator to the MTN Manuscript Review Committee, DAIDS, NICHD and CONRAD for review prior to submission.

15 APPENDICES

APPENDIX I: SCHEDULE OF STUDY VISITS AND EVALUATIONS

PREGNANCY									
	SCR Visit	ENR Visit (Day 0)	Day 1 and Day 3 Calls	Day 6 Visit	Day 14 Call	Del. Call	Del.	Post-Del.	Interim
ADMINISTRATIVE, BEHAVIORAL, AND REGULATORY									
Informed Consent	X	X							
Demographic Info.	X								
Locator Information	X	X	X	X	X		X	X	X
Eligibility Assessment/Confirmation	X	X							
Randomization		X							
Record Release for Mother	▲								
Assign PTID	X								
Schedule Next Visit/Phone Call	▲	X	▲	X	▲				▲
Participant to Call Clinic						◇			
Acceptability Assessment		X		X					
Adherence Assessment			X	X					
Adherence Counseling		X	X						
Coital Log		X		X					
Product Use Log				X					
Reimbursement	X	X		X			X	X	
HIV Pre/Post-Test Counseling	X			▲					
Provision of Condoms	▲	▲		▲					▲
Provision of Panty Liners		▲							
CLINICAL									
Medical Eligibility Assessment	X								
Disclosure of Available Test Results	X	▲		▲					▲
Review Medical Records	X	X							
Targeted Physical Exam	X	X		X					▲
Pelvic Exam	X	X		X					▲
Vaginal and Cervical Swabs	X	X		X					▲
Urine Collection				▲					▲
Blood Collection	X	X		X			X		▲
Treatment for RTI/UTI	▲								▲
Contraception/Education	▲								
Medical History	X	X		X					
Chart Review		▲							▲
Concomitant Medications	X	X		X					X
Insert/Replace Saline Lock or Similar		●		●					
Observed Dose of Study Gel		X		X					
Provision of Study Gel		X							
Collect Unused Study Gel				X					
Collect/Record AEs		X	X	X	X		X	X	X
LABORATORY									
Cervical NAAT for GC/CT	X			▲					▲
HIV Serology	X			▲					▲
Syphilis Serology	■								
HBsAg test	■			▲					▲
Serum Creatinine	X			X					▲
CBC with Differential	X*	X		X			**		
Hemoglobin	X								
Flow Cytometry and PBMCs		**		**			**		
AST/ALT	X			X					▲
Tenofovir Levels		X		X			X		
Trichomonas Test	X	▲		▲					▲
Vaginal pH	▲	X		X					▲
Gram Stain	X	X		X					
Vaginal and Cervical Biomarkers	X	X		X					
Quantitative Vaginal Culture	X	X		X					
Wet Prep	▲	▲		▲					▲
Herpes Culture	▲	▲		▲					▲
Pap Smear	▲								

▲ if indicated, ● if applicable ■ if not documented within past year, X* CBC only; **if site capacity allows, ◇ if able to do so, participants to call clinic when they anticipate delivery or labor evaluation

LACTATION COHORT						
	SCR Visit	ENR Visit Day 0	Day 1 and Day 3 Calls	Day 6 Visit	Day 14 Call	Interim
ADMINISTRATIVE, BEHAVIORAL, AND REGULATORY						
Informed Consent	X	X				
Demographic Information	X					
Locator Information	X	X	X	X	X	X
Eligibility Assessment/Confirmation	X	X				
Record Release for Mother and Infant	▲					
Assign PTID	X					
Schedule Next Visit/ Phone Calls	▲	X	▲	X	▲	▲
Acceptability Assessment		X		X		
Product Use Assessment			X	X		
Adherence Counseling		X	X			
Coital Log		X		X		
Product Use Log				X		
Reimbursement	X	X		X		
HIV Pre/Post-Test Counseling	X			▲		
Education on Collection of Milk		X				
Supplies for Milk Collection		X				
Provision of Condoms	▲	▲		▲		▲
Provision of Panty Liners		▲		▲		▲
CLINICAL						
Medical Eligibility Assessment	X					
Disclosure of Available Test Results	X	▲		▲		▲
Review Medical Records	X	X				
Targeted Physical Exam	X	X		X		▲
Pelvic Exam	X	X		X		▲
Vaginal and Cervical Swabs	X	X		X		▲
Urine Collection	X	X		▲		▲
Blood Collection	X	X		X		▲
Treatment for RTI/UTI	▲					▲
Contraception/Education	▲					
Medical History		X		X		
Concomitant Medications		X		X		X
Insert/Replace Saline Lock or Similar		●		●		
Observed Dose of Study Gel		X		X		
Provision of Study Gel		X				
Collected Unused Study Gel				X		
Collect/Record AEs		X	X	X	X	X
Breast Milk Collection		X		X		
LABORATORY						
Cervical NAAT for GC/CT	X			▲		▲
HIV Serology	X			▲		▲
Syphilis Serology	■					
AST/ALT	X			X		▲
Serum Creatinine	X			X		▲
HBsAg test	■			▲		▲
Hemoglobin	X					
CBC with Differential	X*	X		X		
Flow Cytometry (pre-gel) and PBMCs		**		**		
Blood Tenofovir Levels		X		X		
Urine HCG	X	X				
Trichomonas Test	X	▲		▲		▲
Wet Prep	▲	▲		▲		▲
Vaginal pH	▲	X		X		▲
Gram Stain		X		X		
Quantitative Vaginal Culture		X		X		
Vaginal and Cervical Biomarkers		X		X		
Herpes Culture	▲	▲		▲		▲
Pap Smear	▲					
Breast Milk Tenofovir Levels		X		X		

▲ if indicated, ● if applicable ■ if not documented within past year, X* CBC only; **if site capacity allows

Pregnancy Cohort: Infants

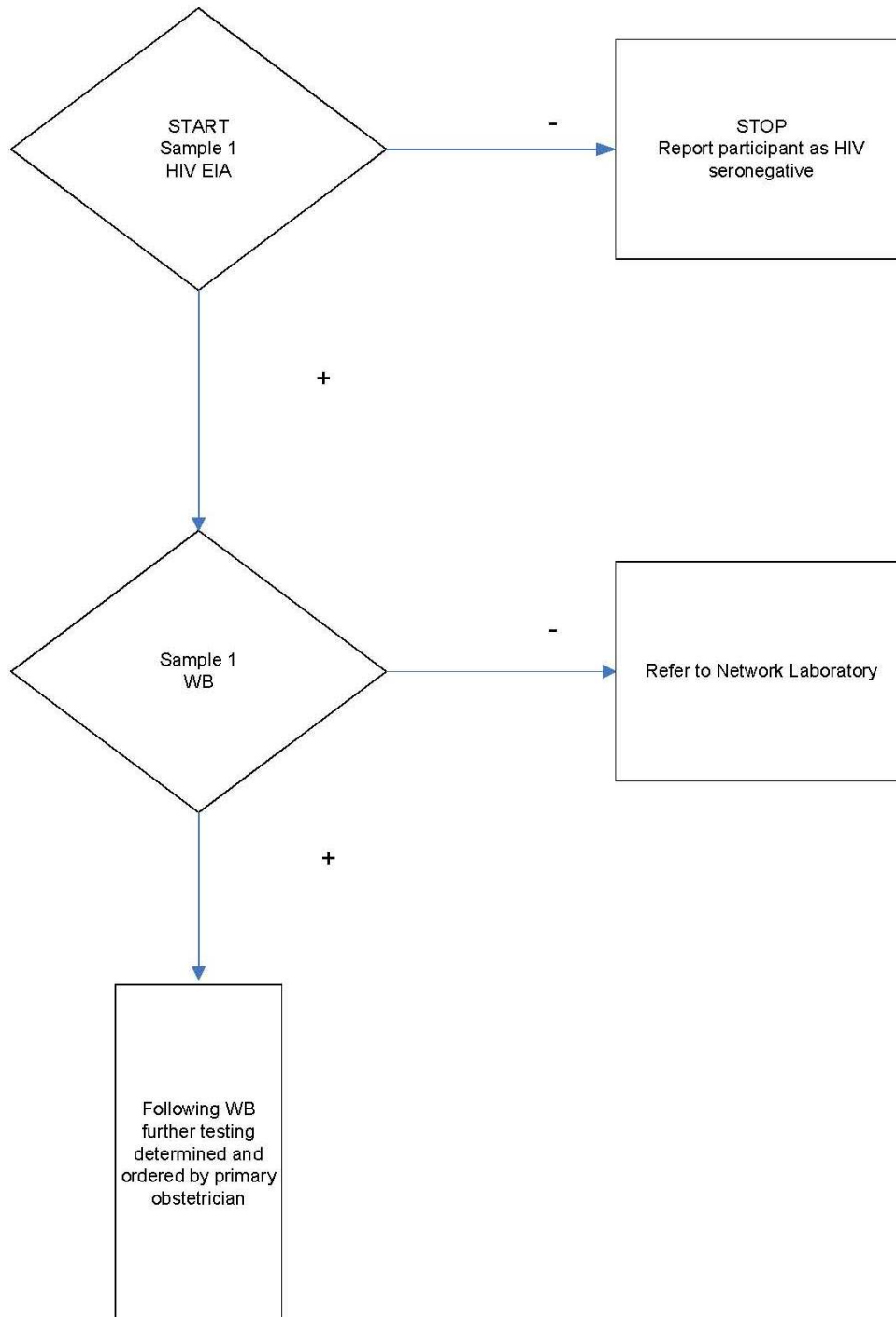
	Delivery Visit	Post-Delivery	Interim
Administrative, Behavioral, Regulatory			
Assign PTID	X		
Schedule Next Visit			▲
Clinical			
Cord Blood Collection	X		
Infant Blood Collection	▲		
Collect/Record AEs	X	X	X
Laboratory			
Tenofovir Level	X		

Lactation Cohort: Infants

	SCR Visit	ENR Visit Day 0	Day 1 and Day 3 Calls	Day 6 Visit	Day 14 Call	Interim
Administrative, Behavioral, and Regulatory						
Assign PTID	X					
Eligibility Assessment/Confirmation	X	X				
Schedule Next Visit						▲
Clinical						
Medical History	X	X	X	X		
Concomitant Medications	X	X	X	X		
Collect Blood		X		X		▲
Collect/Record AEs		X	X	X	X	X
Disclosure of Available Results			X			
Laboratory						
Tenofovir Level		X		X		▲

▲ If indicated

APPENDIX II: ALGORITHM FOR HIV ANTIBODY TESTING



APPENDIX III: COMPONENTS OF EXAMINATIONS

Targeted Physical Exam (Mothers)

- Vital signs (may be transcribed from chart if taken in past hour)
 - Temperature
 - Pulse
 - Blood pressure
- General appearance
- Abdomen
- Breasts (for Lactation Cohort)
- Other components as indicated by participant symptoms

Pelvic Exam

- Vulva
- Perianal area
- Speculum exam
 - Vagina (including vaginal discharge)
 - Cervix (including cervical discharge)
- Bimanual exam
 - Cervix
 - Uterus
 - Adnexae (for Lactation Cohort)

**APPENDIX IV: SAMPLE INFORMED CONSENT FORM (SCREENING –
PREGNANCY COHORT)**

**SAMPLE INFORMED CONSENT FORM
DIVISION OF AIDS, NIAID, NIH**

MTN-008

**Expanded Safety Investigation of Tenofovir 1% Gel in Pregnancy and
Lactation**

March 29, 2010

PRINCIPAL INVESTIGATOR: [insert name]

PHONE: [insert number]

**Short Title for the Study: Tenofovir Gel in Pregnancy and Lactation -
[Pregnancy Cohort]**

INTRODUCTION

You are being asked to screen for the research study named above. This study is for healthy women who are pregnant or breastfeeding and their babies. Before you decide whether to be screened for this study, we would like to explain the screening procedures, review their risks and benefits, discuss what is expected of you, and help you understand what you can expect from the study site. The United States National Institutes of Health is funding this study.

YOUR PARTICIPATION IS VOLUNTARY

This form gives information about the test(s) that will be done during this study. Once you understand and agree to take part in screening tests, you will be asked to sign your name on this form. You will be offered a copy of this form to keep.

Before you learn about the study, it is important to know the following:

- It is your choice whether or not you join this study
- You may decide not to join this study, or you may choose to leave this study at any time, without losing the benefits of your regular medical care
- If you decide not to join this study, you can still choose to join another study later, if you qualify, and if one is available

PURPOSE OF THE SCREENING TESTS AND THE STUDY

The main purpose of this study is to find out if using vaginally applied tenofovir gel is safe for you and your baby during the end of pregnancy and during breastfeeding. This study will also check to see how much tenofovir passes from gel to the blood and breast milk. Tenofovir gel is an “experimental” gel that is being studied for prevention of HIV infection (in other studies). Tenofovir gel is not approved for use in the general community. Tenofovir gel is made from the same active drug as tenofovir tablets. Tenofovir tablets are generally safe when used as treatment for

HIV. Tenofovir tablets have been tested in pregnant women with HIV infection, and so far have been shown to be generally safe for pregnant women and their babies. HIV-infected pregnant women who took tenofovir tablets prior to delivery generally have been shown to have low levels of tenofovir in breast milk.

Tenofovir gel has been tested in one other study in pregnant women so far. In that study, called MTN-002, pregnant women receive a single dose of tenofovir gel before their cesarean delivery. No significant health problems have been determined to be caused by tenofovir gel for pregnant women or their babies.

The side effects from tenofovir gel will be compared to the side effects of HEC gel, which is a placebo gel. The placebo gel does not have any medicine in it. The placebo gel will only be used in the pregnancy portion of the study.

In this consent form, you are being asked for your consent for you to be screened for the *pregnancy* portion of the study.

STUDY GROUPS

There are two study groups. If you decide to take part in the study, you will be placed in one of the two groups: tenofovir gel or placebo gel. There will be approximately 30 participants assigned to receive placebo gel and approximately 60 participants assigned to receive tenofovir gel. Your group will be chosen “by lot” [or other equivalent term, for example, like flipping a coin or throwing dice] to be in one of the two groups. You cannot choose your group, and the study staff cannot choose your group for you. Once you are in a group, you cannot change to another group. Study procedures will be the same for all pregnant participants. The study staff and study doctor will not know what group you are in. Both groups are very important to the results of this study.

PROCEDURES

If you agree to have the screening tests, you will have at least one visit to complete the screening process. Screening tests must be done within 4 weeks of the Enrollment Visit. Screening procedures may begin after you read, discuss, understand and sign your name on this form. Study staff will help you understand the form and answer your questions before you sign or mark this form. The procedures done at this visit will take about *[study site to insert estimated time]*. During screening procedures, you will:

- Answer questions about where you live and questions about you and your health. Study staff will also review your medical records
- Give blood *[site to insert amount]* to check the health of your blood, liver and kidneys
- Have a test for HIV infection – HIV is the virus that causes AIDS *[site to insert language regarding local testing guidelines if applicable]*
- Have a physical exam, pelvic exam, and have fluid collected from your vagina and cervix with swabs to check for

- different kinds of normal and abnormal bacteria
- signs of inflammation and changes in the contents of the vaginal fluid
- Have tests for genital infections, including infections passed through sex
- Receive available test results and referrals for other medical care if you need it
- Receive condoms, if you need them

[Site to insert following section if required by Site IRB]

What happens in the HIV test?

If you agree to participate and you qualify for the study, your blood sample will be tested for the antibody to HIV. An antibody is a substance that blood cells make to fight infection. Exposure (contact) to the HIV virus produces antibodies. A positive HIV test means that your blood sample tested positive for antibodies to HIV and that repeat testing will be performed to confirm (prove) this finding. If your sample is positive for antibodies to HIV, it means that you have been exposed to HIV and that you are a carrier of HIV. It also means that you can pass the virus to others by intimate sexual contact, by sharing needles, through donating blood and organs, and your baby would also be at risk of HIV infection. A negative HIV test means that at this time, no antibody to HIV was found in your blood sample based on the result of the initial screening test, repeat screening tests, or a confirmatory test.

There can be individuals who have HIV test results that are called “false positive,” (for some reason the test shows that HIV antibodies are present in the blood when they are not). There can also be false negative results which can have two possible meanings; 1) the person has been infected with HIV, but that person’s body has not yet made antibodies to the virus, or 2) HIV antibody is present in the person’s blood, but for some reason the test failed to detect it.

The HIV test used for this study is a rapid test and the results will be available in about an hour from the time the sample is taken to the lab. We will give you your results today. If you test positive for HIV antibody, you will be asked to give an additional 2 tablespoons of blood for a repeat HIV antibody test. You will also be counseled as to the risks for transmitting HIV to others, risks for developing AIDS, the available treatments for HIV infection and for reducing the chances that your baby will become infected with the virus. You will return to the clinic to receive results from the repeat test, but will no longer be tested in this office for HIV antibody. You will not be able to participate in this study. This study does not provide treatment for HIV, but study staff will refer you to available sources of medical care, counseling, and other services you may need if your test is positive. Study staff also will be available to talk with other doctors that you see for your medical care and share your test results (with your permission).

We will also ask for your written permission to get copies of your and your baby’s medical records. Study staff will also place a copy of this informed consent document and other information about the study in your regular medical records so

that your doctor or health care provider knows that you are participating in this research study.

The results of the tests that may affect your medical care will be available within *[study site to insert estimated time]*, and will be shared with you, in person, by study staff.

RISKS AND/OR DISCOMFORTS

When your blood is drawn, you may feel discomfort, pain, or dizziness, or have a bruise, swelling, clot, or possible infection where the needle goes in. When you have a pelvic exam, you may feel discomfort in your genital area and/or have a small amount of genital spotting which should stop shortly after the exam. You may become worried or nervous while waiting for your STI and HIV test results. If you have HIV, knowing your HIV status could make you depressed, worried or nervous. You will talk with a trained staff member who will help you deal with any feelings or questions you have.

We will make every effort to protect your privacy and confidentiality while you are in the study. Your visit(s) here will take place in private. However, discrimination may occur from others who learn you are participating in this study. If you have any problems like this, study staff will talk with you to try to help resolve them.

BENEFITS

You may get no direct benefit from being screened for this study. However, you will have a physical exam, pelvic exam, and tests to check on the health of your blood, liver and kidneys. If these exams or tests show that you might have health problems, you will be referred for medical care and other available services. You will get counseling and testing for HIV and other infections that may be passed through sex. If you are infected with HIV, you will be referred for medical care, counseling, and other available services. If you have other infections passed through sex [site to specify infections], you will be offered treatment for them, if needed. You or others may benefit in the future from information learned in this study. You may also get personal satisfaction from taking part in HIV research.

NEW INFORMATION

You will be told about new information from this or other studies that may affect your health, welfare or willingness to be in this study. You will also be told when the results of the study may be available, and how to learn about them.

WHY YOU MAY BE WITHDRAWN FROM SCREENING TESTS WITHOUT CONSENT

You may be withdrawn from the screening tests without your consent if:

- You are found not to be eligible for this study
- This study is stopped or canceled
- The study staff feel that having screening tests would be harmful to you
- You are not willing to find out your HIV test results
- You are not able to attend clinic visits or complete the screening tests

COSTS TO YOU

[Site to complete according to site capacity] There is no cost to you for screening tests. Treatments available to you from the study site for infections passed through sex will be given to you free of charge or you will be referred for available treatment.

REIMBURSEMENT

[Site to insert information about local reimbursement:] You will receive [insert amount] for your time and effort in this study. You will also receive compensation for activities affected by your participation, including [site to complete].

CONFIDENTIALITY

Efforts will be made to keep personal information confidential. However, absolute confidentiality cannot be guaranteed. Personal information may be disclosed if required by law. Any publication of this study will not use your name or identify you personally.

Your records will not be given to anyone without your permission except as needed for review by any or all of the following:

- Representatives of the US Federal Government, including the US Food and Drug Administration (FDA), the US Office for Human Research Protections (OHRP), NIH, and/or contractors of the NIH
- The organization that supplies the study gel
- Site IRB
- Study staff

If during the course of the study, we find out that you have *[insert applicable reportable diseases]*, we must report it to *[insert the name(s) of the local health authorities]*.

In addition to the efforts made by the study staff to keep your personal information confidential, a Certificate of Confidentiality has been obtained from the US Federal Government for this study. This Certificate protects study staff from being forced to tell people who are not connected with this study, such as the court system, about your participation or information you give for study purposes. However, if the study staff learns of possible child abuse and/or neglect or a risk of harm to you or others, they will be required to tell the proper authorities. This Certificate does not prevent you from releasing information about yourself and your participation in the study.

RESEARCH-RELATED INJURY

[Site to specify institutional policy:] If you are injured as a result of being in this study, *[institution]* will give you immediate necessary treatment for injuries. You *[will/will not]* have to pay for this treatment. You will be told where you can get additional treatment for injuries. The US NIH does not have a program to pay money or give other forms of compensation for such injuries. You do not give up legal rights by signing this form.

PROBLEMS OR QUESTIONS

If you ever have any questions about this study, or if you have a research-related injury, you should contact *[insert name of the investigator or other study staff]* at *[insert telephone number and/or physical address]*. If you have questions about your rights as a research participant, you should contact *[insert name or title of person on the IRB or other organization appropriate for the site]* at *[insert telephone number and/or physical address of above]* *[Site to insert required HIPAA language if required by site IRB]*.

SIGNATURES

[Insert signature blocks as required by the local IRB:] If you have read this consent form, or had it read and explained to you, and you understand the information, and you voluntarily agree to participate in the screening procedures, please sign your name below.

_____	_____	_____
Participant Name (print)	Participant Signature/Mark	Date

_____	_____	_____
Study Staff Conducting Consent Discussion (print)	Study Staff Signature	Date

_____	_____	_____
Witness Name (print)	Witness Signature	Date

**APPENDIX V: SAMPLE INFORMED CONSENT FORM (SCREENING –
LACTATION COHORT)**

**SAMPLE INFORMED CONSENT FORM
DIVISION OF AIDS, NIAID, NIH**

MTN-008

**Expanded Safety Investigation of Tenofovir 1% Gel in Pregnancy and
Lactation**

March 29, 2010

PRINCIPAL INVESTIGATOR: [insert name]

PHONE: [insert number]

**Short Title for the Study: Tenofovir Gel in Pregnancy and Lactation - [Lactation
Cohort]**

INTRODUCTION

You are being asked to screen for the research study named above. This study is for healthy women who are pregnant or breastfeeding and their babies. Before you decide whether to be screened for this study, we would like to explain the screening procedures, review their risks and benefits, discuss what is expected of you, and help you understand what you can expect from the study site. The United States National Institutes of Health is funding this study.

YOUR PARTICIPATION IS VOLUNTARY

This form gives information about the test(s) that will be done during this study. Once you understand and agree to take part in the screening procedures, you will be asked to sign your name on this form. You will be offered a copy of this form to keep.

Before you learn about the study, it is important to know the following:

- It is your choice whether or not you join this study
- You may decide not to join this study, or you may choose to leave this study at any time, without losing the benefits of your regular medical care
- If you decide not to join this study, you can still choose to join another study later, if you qualify, and if one is available

PURPOSE OF THE SCREENING TESTS AND THE STUDY

The main purpose of this study is to find out if using vaginally applied tenofovir gel is safe for you and your baby during the end of pregnancy and during breastfeeding. This study will also check to see how much tenofovir passes from gel to the blood and breast milk. Tenofovir gel is an “experimental” gel that is being studied for prevention of HIV infection (in other studies). Tenofovir gel is not approved for use in the general community.

Tenofovir gel is made from the same active drug as tenofovir tablets. Tenofovir tablets are generally safe when used as treatment for HIV. Tenofovir tablets have been tested in pregnant women with HIV infection, and so far have been shown to be generally safe for pregnant women and their babies. HIV-infected pregnant women who took tenofovir tablets prior to delivery generally have been shown to have low levels of tenofovir in breast milk.

Tenofovir gel has been tested in one other study in pregnant women so far. In that study, called MTN-002, pregnant women receive a single dose of tenofovir gel before their cesarean delivery. No significant health problems have been determined to be caused by tenofovir gel for pregnant women or their babies.

The side effects from tenofovir gel will be compared to the side effects of HEC gel, which is a placebo gel. The placebo gel does not have any medicine in it. The placebo gel will only be used in the pregnancy portion of the study.

In this consent form, you are being asked for your consent to be screened for the *breastfeeding* portion of the study.

PROCEDURES

If you agree to have the screening tests, you will have at least one visit to complete the screening process. All screening tests must be done within 4 weeks of the Enrollment Visit.

Screening procedures may begin after you read, discuss, understand and sign your name on this form. Study staff will help you understand the form and answer your questions before you sign or mark this form. The procedures done at this visit will take about [*study site to insert estimated time*]. During screening procedures, you will:

- Answer questions about where you live and about you and your health. Study staff will also review your medical records
- Give urine for a pregnancy test
- Give blood [*site to insert amount*] to check the health of your blood, liver and kidneys
- Have a test for HIV infection – HIV is the virus that causes AIDS [*site to insert language regarding local testing guidelines if applicable*]
- Have a physical exam, pelvic exam and have fluid collected from your vagina and cervix with swabs
- Have tests for genital infections, including infections passed through sex
- Receive available test results and referrals for other medical care if you need it
- Receive condoms, if you need them

[Site to insert following section if required by Site IRB]

What happens in the HIV test?

If you agree to participate and you qualify for the study, your blood sample will be tested for the antibody to HIV. An antibody is a substance that blood cells make to fight infection. Exposure (contact) to the HIV virus produces antibodies. A positive HIV test means that your blood sample tested positive for antibodies to HIV and that repeat testing will be performed to confirm (prove) this finding. If your sample is positive for antibodies to HIV, it means that you have been exposed to HIV and that you are a carrier of HIV. It also means that you can pass the virus to others by intimate sexual contact, by sharing needles, through donating blood and organs, and your baby would also be at risk of HIV infection. A negative HIV test means that at this time, no antibody to HIV was found in your blood sample based on the result of the initial screening test, repeat screening tests, or a confirmatory test.

There can be individuals who have HIV test results that are called “false positive,” (for some reason the test shows that HIV antibodies are present in the blood when they are not). There can also be false negative results which can have two possible meanings; 1) the person has been infected with HIV, but that person’s body has not yet made antibodies to the virus, or 2) HIV antibody is present in the person’s blood, but for some reason the test failed to detect it.

The HIV test used for this study is a rapid test and the results will be available in about an hour from the time the sample is taken to the lab. We will give you your results today. If you test positive for HIV antibody, you will be asked to give an additional 2 tablespoons of blood for a repeat HIV antibody test. You will also be counseled as to the risks for transmitting HIV to others, risks for developing AIDS, the available treatments for HIV infection and for reducing the chances that your baby will become infected with the virus. You will return to the clinic to receive results from the repeat test, but will no longer be tested in this office for HIV antibody. You will not be able to participate in this study. This study does not provide treatment for HIV, but study staff will refer you to available sources of medical care, counseling, and other services you may need if your test is positive. Study staff also will be available to talk with other doctors that you see for your medical care and share your test results (with your permission).

We will also ask for your written permission to get copies of your medical records. Study staff will also place a copy of this informed consent document and other information about the study in your regular medical records so that your doctor or health care provider knows that you are participating in this research study.

The results of the tests that may affect your medical care will be available within [study site to insert estimated time], and will be shared with you, in person, by study staff.

RISKS AND/OR DISCOMFORTS

When your blood is drawn, you may feel discomfort, pain, or dizziness, or have a bruise, swelling, clot, or possible infection where the needle goes in. When you have a pelvic exam, you may feel discomfort in your genital area and/or have a small amount of genital spotting which should stop shortly after the exam.

You may become worried or nervous while waiting for your STI and HIV test results. If you have HIV, knowing your HIV status could make you depressed, worried or nervous. You will talk with a trained staff member who will help you deal with any feelings or questions you have.

We will make every effort to protect your privacy and confidentiality while you are in the study. Your visit(s) here will take place in private. However, discrimination may occur from others who learn you are participating in this study. If you have any problems like this, study staff will talk with you to try to help resolve them.

BENEFITS

You may get no direct benefit from being screened for this study. However, you will have a physical exam, pelvic exam, and tests to check on the health of your blood, liver and kidneys. If these exams or tests show that you might have health problems, you will be referred for medical care and other available services. You will get counseling and testing for HIV and other infections that may be passed through sex. If you are infected with HIV, you will be referred for medical care, counseling, and other available services. If you have other infections [site to specify infections] passed through sex, you will be offered treatment for them, if needed. You or others may benefit in the future from information learned in this study. You may also get personal satisfaction from taking part in HIV research.

NEW INFORMATION

You will be told about new information from this or other studies that may affect your health, welfare or willingness to be in this study. You will also be told when the results of the study may be available, and how to learn about them.

WHY YOU MAY BE WITHDRAWN FROM SCREENING TESTS WITHOUT CONSENT

You may be withdrawn from the screening tests without your consent if:

- You are found not to be eligible for this study
- This study is stopped or canceled
- The study staff feel that having the screening tests would be harmful to you
- You are not willing to find out your HIV test results
- You are not able to attend clinic visits or complete the screening tests

COSTS TO YOU

[Site to complete according to site capacity] There is no cost to you for screening tests. Treatments available to you from the study site for infections passed through sex will be given to you free of charge or you will be referred for available treatment.

REIMBURSEMENT

[*Site to insert information about local reimbursement:*] You will receive [insert amount] for your time and effort in this study. You will also receive compensation for activities affected by your participation, including [*site to complete*].

CONFIDENTIALITY

Efforts will be made to keep personal information confidential. However, absolute confidentiality cannot be guaranteed. Personal information may be disclosed if required by law. Any publication of this study will not use your name or identify you personally.

Your records will not be given to anyone without your permission except as needed for review by any or all of the following:

- Representatives of the US Federal Government, including the US Food and Drug Administration (FDA), the US Office for Human Research Protections (OHRP), NIH, and/or contractors of the NIH
- The organization that supplies the study gel
- Site IRB
- Study staff

If during the course of the study, we find out that you have [*insert applicable reportable diseases*], we must report it to [*insert the name(s) of the local health authorities*].

In addition to the efforts made by the study staff to keep your personal information confidential, a Certificate of Confidentiality has been obtained from the US Federal Government for this study. This Certificate protects study staff from being forced to tell people who are not connected with this study, such as the court system, about your participation or information you give for study purposes. However, if the study staff learns of possible child abuse and/or neglect or a risk of harm to you or others, they will be required to tell the proper authorities. This Certificate does not prevent you from releasing information about yourself and your participation in the study.

RESEARCH-RELATED INJURY

[*Site to specify institutional policy:*] If you are injured as a result of being in this study, [*institution*] will give you immediate necessary treatment for injuries. You [*will/will not*] have to pay for this treatment. You will be told where you can get additional treatment for injuries. The US NIH does not have a program to pay money or give other forms of compensation for such injuries. You do not give up legal rights by signing this form.

PROBLEMS OR QUESTIONS

If you ever have any questions about this study, or if you have a research-related injury, you should contact [*insert name of the investigator or other study staff*] at [*insert telephone number and/or physical address*]. If you have questions about your

rights as a research participant, you should contact *[insert name or title of person on the IRB or other organization appropriate for the site]* at *[insert telephone number and/or physical address of above]* *[Site to insert required HIPAA language if required by site IRB]*.

SIGNATURES

[Insert signature blocks as required by the local IRB:] If you have read this consent form, or had it read and explained to you, and you understand the information, and you voluntarily agree to participate in the screening procedures, please sign your name below.

_____	_____	_____
Participant Name (print)	Participant Signature/Mark	Date

_____	_____	_____
Study Staff Conducting Consent Discussion (print)	Study Staff Signature	Date

Witness Name
(print)

Witness Signature

Date

**APPENDIX VI: SAMPLE INFORMED CONSENT DOCUMENT FOR PREGNANCY
COHORT – MOTHERS AND INFANTS (ENROLLMENT)**

**SAMPLE INFORMED CONSENT FORM
DIVISION OF AIDS, NIAID, NIH**

MTN-008

**Expanded Safety Investigation of Tenofovir 1% Gel in Pregnancy and
Lactation**

March 29, 2010

PRINCIPAL INVESTIGATOR: [insert name]

PHONE: [insert number]

Short Title for the Study: Tenofovir Gel in Pregnancy and Lactation

INTRODUCTION

You are being asked to volunteer for the research study named above. This study is for healthy women who are pregnant or breastfeeding and their babies (after they are born). Before you decide whether to be in this study, we would like to explain its purpose, review its risks and benefits, discuss what is expected of you and your baby, and help you understand what you can expect from the study site. The United States National Institutes of Health is funding this study.

YOUR PARTICIPATION IS VOLUNTARY

This form gives information about the test(s) that will be done during this study. Once you understand and agree to take part in the study, you will be asked to sign your name on this form. You will be offered a copy of this form to keep.

Before you learn about the study, it is important to know the following:

- It is your choice whether or not you join this study
- You may decide not to join this study, or you may choose to leave this study at any time, without losing the benefits of your regular medical care
- If you decide not to join this study, you can still choose to join another study later, if you qualify, and if one is available

PURPOSE OF THE STUDY

The main purpose of this study is to find out if using vaginally applied tenofovir gel is safe for you and your baby during the end of pregnancy and during breastfeeding. This study will also check to see how much tenofovir passes from the gel to blood and breast milk. Tenofovir gel is an “experimental” gel being studied for prevention of HIV infection (in other studies). Tenofovir gel is not approved for use in the general community.

Tenofovir gel is made from the same active drug as tenofovir tablets. Tenofovir tablets are generally safe when used as treatment for HIV. Tenofovir tablets have been tested in pregnant women with HIV infection, and so far have been shown to be generally safe for pregnant women and their babies. HIV-infected pregnant women who take tenofovir tablets before delivery generally have low levels of tenofovir in breast milk.

Tenofovir gel has been tested in one other study in pregnant women so far. In that study called MTN-002, pregnant women received a single dose of tenofovir gel before their cesarean delivery. No significant health problems have been thought to be caused by tenofovir gel for pregnant women or their babies.

The side effects from tenofovir gel will be compared to the side effects of HEC placebo gel, which is a placebo gel. The placebo gel does not have any medicine in it. The placebo gel will only be used in the pregnancy portion of this study.

In this consent form, you are being asked for consent for you and your baby to be in the **PREGNANCY** portion of the study

Approximately 90 mother-infant pairs are planned to be enrolled in the pregnancy portion of the study. It may take up to about 14 months to enroll all participants. You will be in this study for about 3-10 weeks.

STUDY GROUPS

There are two study groups in the pregnancy portion of this study.

1. Group 1: Women who are between 37 and 39 weeks pregnant (about 45 women)
2. Group 2: Women who are between 34 and 37 weeks pregnant (about 45 women)

The study will enroll women in Group 1 first. After Group 1 has finished scheduled study tests, a committee of experts will review the safety information from that part of the study. If the study gel appears safe and well-tolerated among women and babies in Group 1, the study will enroll Group 2. If the expert committee has concerns about the safety data, the study will pause for more review of the data. The study doctors do not expect significant safety concerns in either group, but it is possible that the study would not enroll into Group 2 if there were safety concerns seen in Group 1. Women in both groups will use one full applicator of tenofovir gel vaginally once a day for 7 days and will have the same schedule of study visits and procedures.

You are being asked to be in Group *[study site to insert 1 or 2, as appropriate]*.

STUDY PROCEDURES

If you decide to be in this study, your first visit will occur after you read, discuss, understand, and sign your name on this form. Study staff will help you understand the form and answer your questions. Study staff may also review your medical records at this visit. Study staff will place a copy of this consent document and other information about the study in your regular medical records so that your doctor or health care provider knows that you are participating in this research study.

This study has the following visits and phone calls.

- Enrollment Visit (Day 0) (probably around 9 hours)
- Phone Calls (probably less than 15 minutes) on Day 1, Day 3, and Day 14
- Day 6 Visit (probably around 9 hours)
- Delivery Phone Call
- Delivery Visit (probably around 1 hour)
- Post-Delivery Assessment (probably around 30 minutes, but may be in person or a phone call. This visit will take place approximately 2 weeks after delivery)

During most visits and calls, you will:

- Tell us new information about where you live and how to keep in contact with you
- Tell us if you had any health problems since your last visit
- Tell us about any medications, herbal treatments or supplements you are taking
- Tell us about your gel use
- Be asked to come to the clinic for a visit if the study staff thinks you need to

During most scheduled visits on Day 0 and Day 6, you may also:

- Have a physical exam, a pelvic exam and have fluid collected from your vagina and cervix with swabs to test for
 - infections, if study staff think you may have an infection
 - different kinds of normal and abnormal bacteria
 - signs of inflammation and changes in the contents of the vaginal fluid
- Receive a vaginally applied dose of study gel
- Give blood [site to insert amount] to check the health of your blood, liver and kidneys
- [Site to insert if applicable] Before you give blood for tests of tenofovir in your blood, the study staff may put a special kind of device under your skin, in your arm vein called a lock that will let them draw blood without putting a needle into your skin every time. This lock will be removed after draws are completed
- Give blood [site to insert amount] approximately 6 times per visit to test for tenofovir in your blood
- Receive available test results and referrals for other medical care if you need it

- Talk with study staff about how to get, store, use and return study gel
- Have an interview (answer questions) about your sexual activity, your gel use, and what you think about using the gel. You may be asked to answer some of these questions using a computer or by filling out a form
- Receive condoms, if you need them
- Receive panty liners from the study staff, if you use panty liners and if you need them.

During the Delivery Visit, you will have some of the same procedures listed above, except you will not get study gel, and will not have samples of vaginal or cervical fluid taken. Study staff will visit you and your baby in the hospital (unless you have already been discharged from the hospital) within the first few days after delivery. If you decide to take part in this study, the first procedures for your baby will occur at this visit. Please call the study site at [SITES TO INSERT NUMBER] if you think you may be in labor, or if you are going to the hospital to be checked or have your labor induced.

During the Delivery Visit, study staff will:

- Take a sample of umbilical cord blood [site to insert amount] to test for tenofovir. The sample of cord blood will be taken after delivery directly from the from the placenta (not the baby) once the cord has been cut
- Take a sample of blood [site to insert amount] to test for tenofovir in your blood
- Check on your baby's health

A few days after you deliver your baby, study staff will contact you to check on you and your baby. This may be done in person or by phone.

AT ANY TIME IN THE STUDY

If you have health problems, please tell the study staff, and you may:

- Be checked by a study doctor, have a physical exam and/or pelvic exam
- Give blood, urine and/or vaginal fluid to check for health problems or infections
- Receive treatment or learn where you can get treatment for these problems

If you think you may be going into labor, or if you are being checked for health problems at a hospital or clinic, please let us know as soon as possible.

RISKS AND/OR DISCOMFORTS

When your blood is drawn, you may feel discomfort, pain, or dizziness, or have a bruise, swelling, clot, or possible infection where the needle goes in. When you have pelvic exams, you may feel discomfort in your genital area and/or have a small amount of genital spotting which should stop shortly after the exam.

It is possible you could have side effects from the gel. Some, but not all, women who used tenofovir gel in other studies have had:

- Dryness, itching, burning feeling, or pain in the genital area
- Vaginal yeast infection
- Discharge from the vagina

You could have these effects or other effects that we do not yet know about. Some side effects have been associated with the use of tenofovir tablets, including upset stomach, vomiting, gas, loose or watery stools, weakness, dizziness, depression, headache, abdominal pain, worsening or new kidney damage or failure, inflammation or swelling and possible damage to the pancreas and liver, shortness of breath, rash, allergic reaction (may include fever, rash, upset stomach, vomiting, loose or watery stools, abdominal pain, achiness, shortness of breath, feeling of illness or a potentially serious swelling of face, lips, and/or tongue), bone pain and bone thinning/softening which may increase risk of breakage, and muscle pain and muscle weakness.

A small amount of tenofovir may pass from the vaginal gel into your blood or breast milk. Even though this may be possible, there is only a small chance that any tenofovir may pass from the gel into the breast milk. If this happens, we do not expect it to cause bad effects, but bad effects may be possible. If tenofovir passed into your breast milk, it is not expected that the amount would be high enough to affect the baby. The effects of exposure on a nursing baby are unknown. If your breast milk did absorb some tenofovir, possible side effects in the baby could include increased liver function tests, diarrhea, nausea, vomiting, and flatulence (gas), but we would expect those side effects to be brief and resolve quickly if they occurred. You should let your baby's pediatrician and the study staff know if you believe your baby is experiencing any problems.

There may also be risks related to your pregnancy or fetus due to study product or procedures that we do not yet know about.

Your male sexual partners will be protected from potential risks associated with exposure to tenofovir gel through consistent use of approved male condoms during penile-vaginal sex and avoidance of oral-vaginal sex. Potential risks to female sexual partners have not been studied and are not known.

We will make every effort to protect your privacy and confidentiality while you are in the study. Your visit(s) here will take place in private. However, discrimination may occur from others who learn you are participating in this study. If you have any problems like this, study staff will talk with you to try to help resolve them.

BENEFITS

You and your baby may get no direct benefit from being in this study. You or others may benefit in the future from information learned in this study. You may also get some personal satisfaction from taking part in HIV research.

NEW INFORMATION

You will be told about new information from this or other studies that may affect your health, welfare or willingness to be in this study. You will also be told when the results of the study may be available, and how to learn about them.

WHY YOU MAY HAVE TO STOP TAKING THE STUDY DRUG EARLY

You may have to stop taking the study drug early for the following reasons:

- This study is stopped or canceled
- The study staff feels that the study drug would be harmful to you
- You are unable to follow the rules of the study

Even if you have to stop taking the study drug early, study staff may ask you to come in for some of the same study visits and procedures if you agree.

WHY YOU MAY BE WITHDRAWN FROM THE STUDY WITHOUT YOUR CONSENT

You may be removed from this study without your consent for the following reasons:

- This study is stopped or canceled
- The study staff feels that the study would be harmful to you
- Other administrative reasons

Even if you have to stop taking the study drug early, study staff may ask you to come in for some of the same study visits and procedures if you agree.

ALTERNATIVES TO PARTICIPATION

There may be other studies going on here or in the community that you may be eligible for or other ways you could obtain the same test results available through this study. If you wish, we will tell you about other studies and testing locations that we know about.

COSTS TO YOU

There is no cost to you for being in this study. Costs related to prenatal care, labor, and delivery will not be provided through this study.

REIMBURSEMENT

[Site to insert information about local reimbursement:] You will receive *[insert amount]* for your time and effort in this study. You will also receive compensation for activities affected by your participation *[site to complete]*.

CONFIDENTIALITY

Efforts will be made to keep personal information confidential. However, absolute confidentiality cannot be guaranteed. Personal information may be disclosed if required by law. Any publication of this study will not use your name or identify you personally.

Your (and your baby's) records will not be given to anyone without your permission except as needed for review by any or all of the following:

- Representatives of the US Federal Government, including the US Food and Drug Administration (FDA), the US Office for Human Research Protections (OHRP), NIH, and/or contractors of the NIH
- The organization that supplies tenofovir gel
- Site IRB
- Study staff

If during the course of the study, we find out that you have *[insert applicable reportable diseases]*, we must report it to *[insert the name(s) of the local health authorities]*.

In addition to the efforts made by the study staff to keep your personal information confidential, a Certificate of Confidentiality has been obtained from the US Federal Government for this study. This Certificate protects study staff from being forced to tell people who are not connected with this study, such as the court system, about your participation or information you give for study purposes. However, if the study staff learns of possible child abuse and/or neglect or a risk of harm to you or others, they will be required to tell the proper authorities. Having this Certificate does not prevent you from releasing information about yourself and your participation in the study.

RESEARCH-RELATED INJURY

[Site to specify institutional policy:] If you are (or your baby is) injured as a result of being in this study, the *[institution]* will give immediate necessary treatment for injuries. You *[will/will not]* have to pay for treatment. You will be told where you can get additional treatment for injuries. The US NIH does not have a program to provide money or other compensation for such injuries. You do not give up any legal rights by signing this consent form.

PROBLEMS OR QUESTIONS

If you ever have any questions about this study, or if you or your baby has a research-related injury, you should contact *[insert name of the investigator or other study staff]* at *[insert telephone number and/or physical address]*. If you have questions about your rights as a research participant, you should contact *[insert name or title of person on the IRB or other organization appropriate for the site]* at *[insert telephone number and/or physical address of above]* *[Site to insert required HIPAA language if required by site IRB]*.

SIGNATURES

[Insert signature blocks as required by the local IRB:] If you have read this consent form, or had it read and explained to you, and you understand the information, and you voluntarily agree to have the study, please sign your name below.

_____ Participant Name (print)	_____ Participant Signature/Mark	_____ Date
_____ Study Staff Conducting Consent Discussion (print)	_____ Study Staff Signature	_____ Date
_____ Witness Name (print)	_____ Witness Signature	_____ Date

**APPENDIX VII: CONSENT DOCUMENT FOR LACTATION COHORT
(ENROLLMENT)**

**SAMPLE INFORMED CONSENT FORM
DIVISION OF AIDS, NIAID, NIH**

MTN-008

**Expanded Safety Investigation of Tenofovir 1% Gel in Pregnancy and
Lactation**

March 29, 2010

PRINCIPAL INVESTIGATOR: [insert name]

PHONE: [insert number]

Short Title for the Study: Tenofovir Gel in Pregnancy and Lactation: Lactation Cohort

INTRODUCTION

You are being asked to volunteer for the research study named above. This study is for women who are pregnant, breastfeeding and their babies. Before you decide whether to be in this study, we would like to explain its purpose, review its risks and benefits, discuss what is expected of you and help you understand what you can expect from the study site. This part of the study will include mother-infant pairs. This means that both you and your baby will be asked to join this study together. The United States National Institutes of Health is funding this study.

YOUR PARTICIPATION IS VOLUNTARY

This form gives information about the test(s) that will be done during this study. Once you understand and agree to take part in the study, you will be asked to sign your name on this form. You will be offered a copy of this form to keep. You will be asked to sign a separate consent form giving permission for your baby to be in this study.

Before you learn about the study, it is important to know the following:

- It is your choice whether or not you join this study
- You may decide not to join this study, or you may choose to leave this study at any time, without losing the benefits of your regular medical care
- If you decide not to join this study, you can still choose to join another study later, if you qualify, and if one is available

PURPOSE OF THE STUDY

The main purpose of this study is to find out if using vaginally applied tenofovir gel is safe for you and your baby during the end of pregnancy and during breastfeeding. This study will also check to see how much tenofovir passes from the gel to the blood and breast milk. Tenofovir gel is an “experimental” gel that is being studied for prevention of HIV infection (in other studies). Tenofovir gel is not approved for use in the general community. Tenofovir gel is made from the same active drug as

tenofovir tablets. Tenofovir tablets are generally safe when used as treatment for HIV. Tenofovir tablets have been tested in pregnant women with HIV infection, and so far have been shown to be generally safe for pregnant women and their babies. HIV-infected pregnant women who took tenofovir tablets prior to delivery generally have been shown to have low levels of tenofovir in breast milk.

Tenofovir gel has been tested in one other study in pregnant women so far. In that study, called MTN-002, pregnant women receive a single dose of tenofovir gel before their cesarean delivery. No significant health problems have been thought to be caused by tenofovir gel for pregnant women or their babies.

The side effects from tenofovir gel will be compared to the side effects of HEC placebo gel. The placebo gel does not have any medicine in it. The placebo gel will only be used in the pregnancy portion of the study.

In this consent form, you are being asked for your consent to be in the **BREASTFEEDING** portion of the study.

Approximately 15 mother-infant pairs are planned to be enrolled in the breastfeeding portion of the study. It may take up to about 14 months to enroll all participants. Mother-infant pairs will be in the study for about 3 weeks.

Women in the breastfeeding portion of the study will use one full applicator of tenofovir gel vaginally once a day for 7 days.

STUDY PROCEDURES

This part of the study is for women who are primarily breastfeeding their babies, and who intend to continue primarily breastfeeding while they participate in the study. If you decide to be in this study, your first visit will occur today after you read, discuss, understand, and sign your name on this form. Study staff will also review your medical records at this visit. Study staff will place a copy of this informed consent document and other information about the study in your regular medical records so that your doctor or health care provider knows that you are participating in this research study. Study staff will help you understand the form and answer your questions. This study has the following visits and phone calls.

- Enrollment Visit (Day 0) (probably around 9 hours)
- Phone Calls (probably less than 15 minutes) on Day 1, Day 3, and Day 14
- Day 6 Visit (probably around 9 hours)

During most visits and calls, you will:

- Tell us new information about where you live and how to keep in contact with you
- Tell us if you had any health problems since your last visit

- Tell us about any medications, herbal treatments or supplements you are taking
- Tell us about your gel use

During most scheduled visits on Day 0 and Day 6, you will also bring your baby with you as your baby will be examined at these visits, and you may also:

- Have a physical exam
- Have a pelvic exam and have fluid collected from your vagina with swabs to test for
 - infections, if study staff think you may have an infection
 - different kinds of normal and abnormal bacteria
 - signs of inflammation and changes in the contents of the vaginal fluid
- Receive a vaginally applied dose of study gel
- Give urine for a pregnancy test (Day 0 only)
- Give blood [site to insert amount] to check the health of your blood, liver and kidneys
- [Site to insert if applicable] Before you give blood for tests of tenofovir in your blood, the study staff may put a special kind of device under your skin, in your arm vein called a lock that will let them draw blood without putting a needle into your skin every time. This lock will be removed after blood draws are completed
- Give blood [site to insert amount] approximately 6 times per visit for tests of tenofovir in your blood
- Provide breast milk [site to insert amount] approximately 4 times to check the amount of tenofovir in your breast milk
- Receive available test results and referrals for other medical care if you need it
- Talk with study staff about how to get, store, use and return study gel
- Have an interview (answer questions) about your sexual activity, your gel use, and what you think about using the gel. You may be asked to answer some of these questions using a computer or by filling out a form

Between your scheduled visits, you will collect samples of breast milk at home. At today's visit, the study staff will talk to you about how much and when to collect, store, and return the breast milk. If milk that you express for the study ends up being needed by your baby, you should give that milk to your baby, provided it has been stored properly for that purpose. If you have any questions regarding the proper storage or handling of breast milk, you should contact the study site or your pediatrician's office.

AT ANY TIME IN THE STUDY

If you have health problems, please tell the study staff, and you may:

- Be checked by a study doctor
- Have a physical exam and/or pelvic exam
- Give blood, urine and/or vaginal fluid to check for health problems or infections

- Receive treatment or learn where you can get treatment for these problems

RISKS AND/OR DISCOMFORTS

Whenever blood is drawn, you may:

- Feel discomfort, pain, or dizziness
- Have a bruise, swelling, clot, or possible infection where the needle goes in for the blood draw

When you have pelvic exams, you may:

- Feel discomfort in your genital area and inside your vagina
- Have a small amount of genital spotting which should stop shortly after the exam

It is possible you could have side effects from the gel. Some, but not all, women who used tenofovir gel in other studies have had:

- Dryness, itching, burning feeling, or pain in the genital area
- Vaginal yeast infection
- Discharge from the vagina

You could have these effects or others that we do not yet know about. Some side effects have been associated with the use of tenofovir tablets, including upset stomach, vomiting, gas, loose or watery stools, weakness, dizziness, depression, headache, abdominal pain, worsening or new kidney damage or failure, inflammation or swelling and possible damage to the pancreas and liver, shortness of breath, rash, allergic reaction (may include fever, rash, upset stomach, vomiting, loose or watery stools, abdominal pain, achiness, shortness of breath, feeling of illness or a potentially serious swelling of face, lips, and/or tongue), bone pain and bone thinning/softening which may increase risk of breakage, and muscle pain and muscle weakness.

A small amount of tenofovir may pass from the vaginal gel into your blood or breast milk. We do not know if tenofovir passes into breast milk after women receive the vaginal form of tenofovir. Even though this may be possible, there is only a small chance that any tenofovir may pass from the gel into the breast milk. If tenofovir passed into your breast milk, it is not expected that the amount would be high enough to affect the baby. The effects of exposure on a nursing baby are unknown. If your breast milk did absorb some tenofovir, possible side effects in the baby could include increased liver function tests, diarrhea, nausea, vomiting, and flatulence (gas), but we would expect those side effects to be brief and resolve quickly if they occurred. You should let your baby's pediatrician and the study staff know if you believe your baby is experiencing any problems.

Your male sexual partners will be protected from potential risks associated with exposure to tenofovir gel through consistent use of approved male condoms during penile-vaginal sex and avoidance of oral-vaginal sex. Potential risks to female sexual partners have not been studied and are not known.

We will make every effort to protect your privacy and confidentiality while you are in the study. Your visit(s) here will take place in private. However, discrimination may occur from others who learn you are participating in this study. If you have any problems like this, study staff will talk with you to try to help resolve them.

BENEFITS

You may get no direct benefit from being in this study. You or others may benefit in the future from information learned in this study. You may also get some personal satisfaction from taking part in HIV research.

NEW INFORMATION

You will be told about new information from this or other studies that may affect your health, welfare or willingness to be in this study. You will also be told when the results of the study may be available, and how to learn about them.

WHY YOU MAY HAVE TO STOP TAKING THE STUDY DRUG EARLY

You may have to stop taking the study drug early for the following reasons:

- This study is stopped or canceled
- The study staff feels that the study drug would be harmful to you
- You are unable to follow the rules of the study

Even if you have to stop taking the study drug early, study staff may ask you to come in for some of the same study visits and procedures if you agree.

WHY YOU MAY BE WITHDRAWN FROM THE STUDY WITHOUT YOUR CONSENT

You may be removed from this study without your consent for the following reasons:

- This study is stopped or canceled
- The study staff feels that the study would be harmful to you
- Other administrative reasons

Even if you have to stop taking the study drug early, study staff may ask you to come in for some of the same study visits and procedures if you agree.

ALTERNATIVES TO PARTICIPATION

There may be other studies going on here or in the community that you may be eligible for or other ways you could obtain the same test results available through this study. If you wish, we will tell you about other studies and testing locations that we know about.

COSTS TO YOU

There is no cost to you for being in this study.

REIMBURSEMENT

[Site to insert information about local reimbursement:] You will receive *[insert amount]* for your time and effort in this study. You will also receive compensation for activities affected by your participation *[site to complete]*.

CONFIDENTIALITY

Efforts will be made to keep personal information confidential. However, absolute confidentiality cannot be guaranteed. Personal information may be disclosed if required by law. Any publication of this study will not use your name or identify you personally.

Your (and your baby's) records will not be given to anyone without your permission except as needed for review by any or all of the following:

- Representatives of the US Federal Government, including the US Food and Drug Administration (FDA), the US Office for Human Research Protections (OHRP), NIH and/or contractors of the NIH
- The organization that supplies tenofovir gel
- Site IRB
- Study staff

If during the course of the study, we find out that you have *[insert applicable reportable diseases]*, we must report it to *[insert the name(s) of the local health authorities]*.

In addition to the efforts made by the study staff to keep your personal information confidential, a Certificate of Confidentiality has been obtained from the US Federal Government for this study. This Certificate protects study staff from being forced to tell people who are not connected with this study, such as the court system, about your participation or information you give for study purposes. However, if the study staff learns of possible child abuse and/or neglect or a risk of harm to you or others, they will be required to tell the proper authorities. Having this Certificate does not prevent you from releasing information about yourself and your participation in the study.

RESEARCH-RELATED INJURY

[Site to specify institutional policy:] If you are injured as a result of being in this study, the *[institution]* will give you immediate necessary treatment for injuries. You *[will/will not]* have to pay for this treatment. You will be told where you can get additional treatment for injuries. The US NIH does not have a program to pay money or give other forms of compensation for such injuries. You do not give up any legal rights by signing this consent form.

PROBLEMS OR QUESTIONS

If you ever have any questions about this study, or if you have a research-related injury, you should contact *[insert name of the investigator or other study staff]* at *[insert telephone number and/or physical address]*. If you have questions about your

rights as a research participant, you should contact *[insert name or title of person on the IRB or other organization appropriate for the site]* at *[insert telephone number and/or physical address of above]* *[Site to insert required HIPAA language if required by site IRB]*.

SIGNATURES

[Insert signature blocks as required by the local IRB:] If you have read this consent form, or had it read and explained to you, and you understand the information, and you voluntarily agree to be in the study, please sign your name below.

Participant Name
(print)

Participant Signature

Date

Study Staff Conducting Consent
Discussion
(print)

Study Staff Signature

Date

Witness Name
(print)

Witness Signature

Date

**APPENDIX VIII: SAMPLE INFORMED CONSENT DOCUMENT FOR INFANTS IN
THE LACTATION COHORT (SCREENING AND ENROLLMENT)**

**SAMPLE INFORMED CONSENT FORM
DIVISION OF AIDS, NIAID, NIH**

MTN-008

**Expanded Safety Investigation of Tenofovir 1% Gel in Pregnancy and
Lactation**

March 29, 2010

PRINCIPAL INVESTIGATOR: [insert name]

PHONE: [insert number]

Short Title for the Study: Tenofovir Gel in Pregnancy and Lactation

INTRODUCTION

This study is for women who are pregnant or breastfeeding, and their babies. Even though you may be pregnant or may have delivered more than one baby, we will be using the term “baby” throughout this consent. Before you decide whether to allow your baby to be in this study, we would like to explain its purpose, review its risks and benefits, discuss what is expected of you and your baby, and help you understand what you can expect from the study site. You are being asked to allow your baby to be in this part of the study so that we can look at how HIV prevention studies might affect the breastfeeding period, and the amount of study product, if any, is passed from the mother to the baby. HIV is the virus that causes AIDS. This study is being paid for by the United States National Institutes of Health.

YOUR BABY’S PARTICIPATION IS VOLUNTARY

This form gives information about the test(s) that will be done during this study. You are free to ask questions at any time. Once you understand the study, and if you agree to let your baby take part, you will be asked to sign your name on this form. You will be offered a copy of this form to keep.

Before you learn about the study, it is important to know the following:

- It is your choice whether or not you let your baby join this study
- You may decide not to let your baby join this study, or you may choose to take your baby out of this study at any time, without losing the benefits of your baby’s regular medical care
- If you decide not to join this study, you can still choose to join another study later, if you qualify, and if one is available

PURPOSE OF STUDY

The main purpose of this study is to find out if using tenofovir gel is safe during the end of pregnancy and during breastfeeding. This study will also check to see how

much tenofovir passes from the gel to the blood and breast milk. Tenofovir gel is an “experimental” gel that is being studied for prevention of HIV infection (in other studies). Tenofovir gel is not approved for use in the general community.

Tenofovir gel is made from the same active drug as tenofovir tablets. Tenofovir tablets are used to treat people who have HIV infection and are generally safe when used as treatment for HIV. Tenofovir tablets have been tested in pregnant women with HIV infection, and so far have been shown to be generally safe for pregnant women and their babies. HIV-infected pregnant women who took tenofovir tablets prior to delivery generally have been shown to have low levels of tenofovir in breast milk.

Tenofovir gel has been tested in one other study in pregnant women so far. In that study, called MTN-002, pregnant women received a single dose of tenofovir gel before cesarean delivery. No significant health problems have been thought to be caused by tenofovir gel for pregnant women or their babies.

The side effects from tenofovir gel will be compared to the side effects of HEC placebo gel. The placebo gel does not have any medicine in it. The placebo gel will only be used in pregnancy portion of this study.

WHAT DO I HAVE TO DO IF MY BABY IS IN THIS STUDY?

This study is planned to continue for approximately 2 years, but your baby will only stay in the study for approximately 3 weeks. If you decide to let your baby take part in this study, you will be asked to bring your baby back for visits for as long as the baby is in the study. At study visits you will answer questions about your baby’s health. The visits your baby will have in this study are described in detail below.

STUDY PROCEDURES

If you decide to allow your baby to take part in this study, the first visit will occur after you read, discuss, understand, and sign your name on this form. Study staff will help you understand the form and answer your questions.

This study has the following visits and phone calls:

- Screening Visit
- Enrollment Visit (Day 0)
- Phone Calls-Day 1, Day 3, and Day 14
- Day 6 Visit

During most visits and calls, you will:

- Tell us if your baby has had any health problems since your last visit
- Tell us about any medications, herbal treatments or supplements your baby is taking

During the Screening and Enrollment (Day 0) Visits:

- Study staff will also make sure your baby can be in the study

During the Enrollment (Day 0) and Day 6 Visits, study staff will:

- Ask you questions about your baby's health and any medicines your baby might be taking
- Take a small sample of blood from your baby's heel to check for tenofovir

During the Day 6 Visit study staff will:

- Give you any test results for your baby

AT ANY TIME IN THE STUDY

If you think your baby has health problems, please tell the study staff, and your baby may:

- Be checked by a study doctor and have a physical exam
- Receive treatment or learn where your baby can get treatment for any problems

RISKS AND/OR DISCOMFORTS

Whenever blood is drawn, your baby may:

- Feel discomfort, pain, or dizziness
- Have a bruise, swelling, clot, or possible infection where the needle goes in for the blood draw

Your baby could have these effects or other effects that we do not yet know about. A small amount of tenofovir may pass from vaginal gel into your breast milk. Even though this may be possible, there is only a small chance that any tenofovir may pass from the gel into the breast milk. If tenofovir passed into your breast milk, it is not expected that the amount would be high enough to affect the baby. The effects of exposure on a nursing baby are unknown. If your breast milk did absorb some tenofovir, possible side effects in the baby could include increased liver function tests, diarrhea, nausea, vomiting, and flatulence (gas), but we would expect those side effects to be brief and resolve quickly if they occurred. You should let your baby's pediatrician and the study staff know if you believe your baby is experiencing any problems.

We will make every effort to protect your privacy and confidentiality while you are in the study. Your visit(s) here will take place in private. However, discrimination may occur from others who learn you are participating in this study. If you have any problems like this, study staff will talk with you to try to help resolve them.

BENEFITS

You and your baby may get no direct benefit from being in this study. You or others may benefit in the future from information learned in this study. You may also get some personal satisfaction from taking part in HIV research.

NEW INFORMATION

You will be told about new information from this or other studies that may affect your baby's health, welfare or willingness to let your baby take part in this study. You will also be told when the results of the study may be available, and how to learn about them.

WHY YOUR BABY MAY BE WITHDRAWN FROM THE STUDY WITHOUT YOUR CONSENT

Your baby may be removed from this study without your consent for the following reasons:

- This study is stopped or canceled
- The study staff feels that the study would be harmful to you and your baby
- Other administrative reasons

ALTERNATIVES TO PARTICIPATION

There may be other studies going on here or in the community that your baby may be eligible for or other ways you could obtain the same test results available through this study. If you wish, we will tell you about other studies and testing locations that we know about.

COSTS TO YOU

There is no cost to you for study visits or exams of your baby. This study will not provide or pay for others to provide routine infant care.

REIMBURSEMENT

[Site to insert information about local reimbursement:] You will receive *[insert amount]* for your time and effort in this study. You will also receive compensation for activities affected by your baby's participation in this study *[site to complete]*.

CONFIDENTIALITY

Efforts will be made to keep personal information confidential. However, absolute confidentiality cannot be guaranteed. Personal information may be disclosed if required by law. Any publication of this study will not use your baby's name or identify your baby personally.

Your baby's records will not be given to anyone without your permission except as needed for review by any or all of the following:

- Representatives of the US Federal Government, including the US Food and Drug Administration (FDA), the US Office for Human Research Protections (OHRP), NIH, and/or contractors of the NIH
- The organization that supplies tenofovir gel
- Site IRB
- Study staff

If during the course of the study, we find out that your baby has *[insert applicable reportable diseases]*, we must report it to *[insert the name(s) of the local health authorities]*.

In addition to the efforts made by the study staff to keep your and your baby's personal information confidential, a Certificate of Confidentiality has been obtained from the US Federal Government for this study. This Certificate protects study staff from being forced to tell people who are not connected with this study, such as the court system, about your participation or information you give for study purposes. However, if the study staff learns of possible child abuse and/or neglect or a risk of harm to you or others, they will be required to tell the proper authorities. Having this Certificate does not prevent you from releasing information about yourself or your study participation.

RESEARCH-RELATED INJURY

[Site to specify institutional policy:] If your baby is injured as a result of being in this study, the *[institution]* will give your baby immediate necessary treatment for injuries. You *[will/will not]* have to pay for this treatment. You will be told where you can take your baby for additional treatment for injuries. The US NIH does not have a program to pay money or give other forms of compensation for such injuries. You do not give up any legal rights by signing this consent form.

PROBLEMS OR QUESTIONS

If you ever have any questions about this study, or if your baby has a research-related injury, you should contact *[insert name of the investigator or other study staff]* at *[insert telephone number and/or physical address]*. If you have questions about your or your baby's rights as a research participant, you should contact *[insert name or title of person on the IRB or other organization appropriate for the site]* at *[insert telephone number and/or physical address of above]* *[Site to insert required HIPAA language if required by site IRB]*.

SIGNATURES

[Insert signature blocks as required by the local IRB:] If you have read this consent form, or had it read and explained to you, and you understand the information, and you voluntarily agree to let your baby be in the study, please sign your name below.

_____ Participant Name (print)	_____ Participant Signature	_____ Date
_____ Father's Name (optional) (print)	_____ Father's Signature/Mark (optional)	_____ Date
_____ Study Staff Conducting Consent Discussion (print)	_____ Study Staff Signature	_____ Date
_____ Witness Name (print)	_____ Witness Signature	_____ Date

APPENDIX IX: SAMPLE INFORMED CONSENT (STORAGE AND FUTURE TESTING OF SPECIMENS)

**SAMPLE INFORMED CONSENT FORM
DIVISION OF AIDS, NIAID, NIH**

MTN-008

Expanded Safety Investigation of Tenofovir 1% Vaginal Gel in Pregnancy and Lactation

March 29, 2010

PRINCIPAL INVESTIGATOR: [insert name]

PHONE: [insert number]

Short Title for the Study: Tenofovir Gel in Pregnancy and Lactation

STORAGE AND FUTURE TESTING OF LEFTOVER SPECIMENS

The researchers would like to keep any leftover blood, vaginal fluid, and/or breast milk to use for research in the future. If you agree to this, no additional samples will be taken from you or your baby. Only leftover samples from the specimens already taken for this study will be kept and used for future research. This consent form gives you information about the collection, storage and use of your samples. If you agree to the Storage and Future Testing of Specimens you will not be compensated for donated leftover samples.

If you choose not to have leftover samples stored for future testing, you and your baby will still be able to participate in this study. Any leftover samples will be destroyed after all research related tests have been performed.

HOW WILL YOU USE MY LEFTOVER SAMPLES?

The specific research to be done on your or your baby's samples is not known at this time. Research studies wishing to use your samples must gain approval by the United States National Institutes of Health (NIH) and a special committee at the researcher's institution (an Institutional Review Board). The role of this committee is to protect you and other research volunteers from harm.

HOW LONG WILL YOU KEEP MY LEFTOVER SAMPLES AND HOW WILL THEY BE STORED?

There is no time limit on how long samples will be stored. Samples will be stored safely and securely in a storage facility at this site. [Site should modify the previous sentence to identify where long-term samples are being stored.] Only approved researchers and staff who work at the facility will have access to your samples. The people who work at the facility will not have any information that identifies you. The approved researchers may be given information about you such as your age and sex, but they will not be given your name or any other information that identifies you.

Your samples may be shipped to approved researchers who work outside of your country.

DOES STORAGE OF MY LEFTOVER SAMPLES BENEFIT ME?

There are no direct benefits to you or your baby. The benefit of doing research on stored samples includes helping researchers learn more about HIV infection and its prevention.

WHAT ARE THE RISKS RELATED TO STORAGE OF LEFTOVER SAMPLES?

There are few risks related to storing your samples. When tests are done on the stored samples there is a small but possible risk to your privacy. It is possible but unlikely that if others find out information about you that is learned from tests, it could cause you problems with your family, getting a job or insurance.

WHAT ABOUT CONFIDENTIALITY?

To keep your information private, your samples will be labeled with a code that can only be traced back to your research clinic. Your personal information (name, address, phone number) will be protected by the research clinic. When researchers are given your stored samples to study they will not be given your personal information, they will only be given the code. The research staff will do everything they can to protect your privacy. Every effort will be made to keep your personal information confidential, but absolute confidentiality cannot be guaranteed.

Your records will not be given to anyone without your permission except as needed for review by any or all of the following:

- Representatives of the US Federal Government, including the US Food and Drug Administration (FDA), the US Office for Human Research Protections (OHRP), NIH and/or contractors of the NIH
- The organization that supplies tenofovir gel
- Site IRB
- Study staff

WILL THE RESULTS OF FUTURE TESTS BE SHARED?

The results of future tests will not be included in your health records nor will the results of these tests be shared with you or your doctors. Also, any publication of the research will not use your name or identify you personally.

WHAT ARE MY RIGHTS REGARDING STORAGE OF LEFTOVER SAMPLES?

Allowing your samples to be stored is completely voluntary. You may decide not to have any samples stored other than what is needed to complete this study and still be in this research study or any future study. If you decide that your samples can be stored for future research, you may change your mind at any time. If you change your mind about storage of your samples you must contact the study staff by telling them or writing a letter to let them know that you do not want your samples used for future research. Your samples will not be used and will be destroyed as per laboratory guidelines.

PROBLEMS OR QUESTIONS

If you ever have any questions about this study or specimen storage, you should contact *[insert name of the investigator or other study staff]* at *[insert telephone number and/or physical address]*. If you have questions about your rights as a research participant, you should contact *[insert name or title of person on the IRB or other organization appropriate for the site]* at *[insert telephone number and/or physical address of above]* *[Site to insert required HIPAA language if required by site IRB]*.

SIGNATURES

[Insert signature blocks as required by the local IRB:] If you have read this consent form, or had it read and explained to you, and you understand the information, and voluntarily agree to the long-term storage of your samples, please sign your name below.

_____ Participant Name (print)	_____ Participant Signature	_____ Date
_____ Study Staff Conducting Consent Discussion (print)	_____ Study Staff Signature	_____ Date
_____ Witness Name (print)	_____ Witness Signature	_____ Date

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