MTN-002
Phase I Study of the Maternal Single-Dose Pharmacokinetics and Placental Transfer of Tenofovir 1% Vaginal Gel among Healthy Term Gravidas

A Study of the Microbicide Trials Network

Sponsored by:
Division of AIDS, US National Institute of Allergy and Infectious Diseases
US National Institute of Child Health and Human Development
US National Institutes of Health

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CONRAD

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LIST OF ABBREVIATIONS AND ACRONYMS

AE	adverse event
AIDS	acquired immunodeficiency syndrome
ALT	alanine transaminase
APR	Antiretroviral Pregnancy Registry
ARV	antiretroviral
AST	aspartate aminotransferase
AUC	area under the curve
BUN	blood urea nitrogen
CDC	Centers for Disease Control
CFR	code of federal regulations
cGMP	current Good Manufacturing Practices
CI	confidence interval
CONRAD	Contraceptive Research and Development Organization
CRF	case report form
C_{max}	maximum concentration
CRS	Clinical Research Site
C/S	cesarean section
CT	Chlamydia trachomatis
CTA	clinical trial agreement
DAIDS	Division of AIDS
DNA	deoxyribonucleic acid
EAE	expedited adverse event
EC_{50}	50% effective concentration
FDA	(United States) Food and Drug Administration
GC	gonococcus
GCP	Good Clinical Practices
GU	genitourinary
HBsAg	Hepatitis B surface antigen
HIV	human immunodeficiency virus
HPTN	HIV Prevention Trials Network
IATA	International Air Transport Association
IND	investigational new drug
IoR	Investigator of Record
IRB	Institutional Review Board
LCMS	liquid chromatography mass spectrometry
LDMS	Laboratory Data Management System
LLOQ	lower limit of quantitation
MTN	Microbicide Trials Network
NIAID	National Institute of Allergy and Infectious Disease
NICHD	National Institute of Child Health and Development
NIH	(United States) National Institutes of Health
OHRP	Office of Human Research Protection
PACTG	Pediatric AIDS Clinical Trials Group
PAB	(DAIDS) Pharmaceutical Affairs Branch
PI	principal investigator
PK	pharmacokinetic
PPD	Pharmaceutical Product Development, Inc
PMTCT	prevention of mother-to-child transmission
PSRT	Protocol Safety Review Team
RCC	Regulatory Compliance Center

RNA	ribonucleic acid
RPR	Rapid Plasma Reagin
SAE	serious adverse event
SCHARP	Statistical Center for HIV/AIDS Research & Prevention
SDA	strand displacement assay
SDMC	Statistical Data Management Center
SIV	simian immunodeficiency virus
SMC	Study Monitoring Committee
SOP	standard operating procedure(s)
ss	steady state
STI	sexually transmitted infection
TDF	tenofovir disoproxil fumarate
UNAIDS	Joint United Nations Program on AIDS
UPMC	University of Pittsburgh Medical Center

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INVESTIGATOR SIGNATURE FORM

Final Version 1.0
29 August 2007
A Study of the Microbicide Trials Network (MTN)

Sponsored by:
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US National Institute of Child Health and Human Development
US National Institutes of Health

Co-Sponsored 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 and/or tablets for the indication in which it was/they were 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-002

Phase I Study of the Maternal Single-dose Pharmacokinetics and Placental Transfer of Tenofovir 1% Vaginal Gel among Healthy Term Gravidas

PROTOCOL SUMMARY

Short Title: Maternal Pharmacokinetics and Placental Transfer of Tenofovir 1% Vaginal Gel

Clinical Phase: I

IND Sponsor: Division of AIDS (DAIDS)

Protocol Chair: Richard Beigi, MD, MSc

Sample Size: 16

Study Population: Healthy, HIV-uninfected, term gravidas planning elective cesarean section (C/S) between ≥37 and <42 completed gestational weeks and lacking evidence of placental disease

Participating Site: Magee-Womens Hospital of the University of Pittsburgh Medical Center (UPMC)

Study Design: Phase I, single-site, open label, pharmacokinetic (PK) and placental transfer evaluation

Study Duration: Approximately three to six weeks per participant
Approximately 18 total months for planned accrual and study duration

Study Product Regimen:

N	Regimen	Follow-Up		
16	Tenofovir 1% vaginal gel, 4 grams per vagina once	Pharmacokinetic measures (1-12 hours)	24 Hour Evaluation	Two Week Phone Call

Primary Objective:

1. Assess term pregnancy maternal single-dose pharmacokinetics of tenofovir 1% vaginal gel

Secondary Objectives:

1. Characterize the systemic safety profile of single-dose tenofovir 1% vaginal gel in term gravidas

2. Compare 3rd trimester absorption of tenofovir 1% vaginal gel to absorption in non-pregnant recent historic controls

3. Assess amniotic fluid, cord blood, endometrial tissue and placental tissue levels following single-dose tenofovir 1% vaginal gel

1 KEY ROLES

1.1 Protocol Identification

Protocol Title:	Phase I Study of the Maternal Single-dose Pharmacokinetics and Placental Transfer of Tenofovir 1% Vaginal Gel among Healthy Term Gravidas
Protocol Number:	MTN-002
Date:	29 August 2007

1.2 Sponsor and Monitor Identification

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Sponsor:	NICHD Pediatric Adolescent & Maternal AIDS Branch National Institute of Child Health & Human Development National Institutes of Health, DHHS 6100 Executive Blvd., Rm. 4B11 Bethesda, MD 20892-7510
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2 INTRODUCTION

2.1 Oral Pre-Exposure Prophylaxis and Microbicides in HIV/AIDS Prevention

According to UNAIDS, an estimated 38.6 million [33.4 million–46.0 million] people worldwide were living with HIV in 2005. An estimated 4.1 million [3.4 million–6.2 million] became newly infected with HIV and an estimated 2.8 million [2.4 million–3.3 million] lost their lives to AIDS in 2005.[1] Given these statistics, it is clear that available prevention options today have been insufficient to stem the tide of the AIDS epidemic, particularly for women, who continue to comprise a growing proportion of new HIV infections around the world. There is an urgent need for prevention methods that women can initiate and control themselves. Topical microbicides represent one such method, and a growing body of data suggests that a safe and effective topical microbicide will be a real option for women in the future. Many candidate microbicides are currently in various stages of preclinical and clinical investigation; tenofovir 1% vaginal gel is a nucleotide reverse transcriptase inhibitor-based topical microbicide candidate with significant promise as a safe and effective means of prevention of HIV transmission.

2.2 Tenofovir Gel and the MTN Research Agenda

HPTN 050 assessed the safety and acceptability of tenofovir 1% vaginal gel for vaginal use among sexually abstinent and active women. HPTN 050 also evaluated the pharmacokinetic parameters of this candidate microbicide also known as PMPA gel. An ongoing expanded safety study (HPTN 059) is now underway to determine the safety of tenofovir 1% gel as a vaginal microbicide over 24 weeks of use, and to gain additional information about the product's acceptability. Another protocol (MTN-001) is currently in the final planning stages and will be a Phase 2 study comparing the safety and pharmacokinetics of oral versus vaginal tenofovir. Thus far, tenofovir gel has been shown to be safe and well-tolerated among young sexually active women.[2]

To fulfill an ambitious research agenda that is comprehensive in its approach, the MTN agenda also encompasses proactive investigations of safe candidate microbicides into pregnancy. This protocol will be the first investigation of a candidate microbicide among gravid women.

2.3 Candidate Microbicides and Pregnancy

The study of candidate microbicides in pregnancy is compelling for many practical and scientific reasons:

1. Microbicides are intended to be used among reproductive-age sexually active women to minimize and/or eliminate the risk of transmission of HIV and other STIs. One of the common occurrences in this target population is pregnancy, both intended and unintended.

2. Among pregnant and post-partum women, sexual activity is common, including sexual activity with multiple partners.

3. Recent data suggests that pregnancy represents a time of potential heightened risk for the sexual acquisition of HIV.

4. In practical terms, if microbicides become widely available, pregnant women will likely use them with or without evidence of safety. In the absence of safety data there could be recommendations to perform a pregnancy test prior to each use, which would provide a logistical barrier to widespread use.

5. Anti-HIV microbicides potentially might be used for the prevention of maternal-to-child transmission of HIV; therefore precise pharmacokinetic information and investigation are needed.

Pregnancy is a common occurrence in young sexually active women of childbearing age, yet precise rates are difficult to calculate. Rough estimates indicate that the pregnancy rate among young sexually active women not using contraception in the United States/1,000 women/year is 100, with the peak ages being in the 20-29 age range.[3] Pregnancy is estimated to occur in approximately 85% of non-contracepting sexually-active females per year. It is important to remember that this number may be drastically different in other less-developed parts of the world 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.[4,5] The projected use of microbicides among the target population is to be frequent and widespread, and therefore, pregnancy will become a common occurrence for the typical users.

Among pregnant and post-partum women, numerous investigators have demonstrated that sexual activity is common, and sometimes encompasses multiple partners, which increases the risk of HIV and STI transmission. Solberg et al., using puerperal recollection of sexuality during the previous pregnancy, demonstrated that although the majority of women decreased coital frequency as pregnancy progressed, the large majority continued sexual activity, with 90% sexually active in the first trimester and greater than 50% sexually active in the 3rd trimester.[6] 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.[7]

A subsequent publication by Read and Klebanoff using the Vaginal Infections in Prematurity study 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.[8] Lastly, Rowland et al. using post-partum survey data demonstrated that within six weeks postpartum, nearly 50% of women had

resumed sexual activity.[9] These studies taken together highlight the fact that pregnancy is a time of frequent sexual activity with an ongoing risk for HIV acquisition.

Preliminary studies have suggested an increased risk of HIV seroconversion in pregnancy, yet were not directly set up to assess that specific question.[10,11] 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, HIV acquisition was investigated over a five year period (1994-1999). The study identified 2625 women who began a pregnancy with a negative HIV serology study, 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. These finding have significant implications in terms of maternal to child transmission of HIV given the high viral loads that accompany new HIV infection and the documented importance of viral load on maternal-child transmission.[13] These findings compel the medical community to improve HIV prevention strategies in this potentially vulnerable physiologic time period of pregnancy.

A potential role exists for microbicides in decreasing maternal-child intrapartum transmission. If microbicides were demonstrated to effectively decrease lower 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. In addition, 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. Investigation in these regards is necessary to realize this full potential.

2.4 Tenofovir Gel

Tenofovir 1% vaginal gel (tenofovir gel) was chosen as a high priority microbicide candidate for a number of reasons, including its activity in target cells for HIV infection (Langerhans dendritic cells; monocyte/macrophages, and T cells) of the vagina and cervix and the low frequency of local and systemic toxicity observed in the HPTN 050 Phase I study of tenofovir 1% gel. In addition, animal studies have demonstrated that tenofovir gel prevents establishment of systemic infection in macaques when administered prior to or following intravaginal challenge with simian immunodeficiency virus (SIV) and that it inhibits vaginal transmission of SIV in macaques.[14]

The tenofovir gel formulation is a novel nucleotide analog belonging to the class of acyclic phosphonomethylether nucleotides with potent activity against retroviruses. The most recent clinical data of the tenofovir gel comes from a Phase I safety and tolerability study (HPTN 050), in which 84 low risk (60 HIV-uninfected and 24 HIV-infected) women applied either 0.3% or 1% tenofovir gel once or twice daily. Both formulations were well

tolerated in both HIV-uninfected and HIV-infected women. The adverse event (AE) and safety profile in HPTN 050 was reviewed recently by the FDA which subsequently allowed the initiation of the HPTN-059 extended safety protocol for vaginal tenofovir.

Tenofovir gel has been chosen as the first microbicide to be tested in pregnancy for several reasons. Tenofovir gel is the agent farthest along in clinical testing of the topical antiretroviral agents being developed and evaluated as microbicides. As such, it is expected to enter large scale Phase IIb/III trials within the next one to two years. Parallel evaluations in pregnant women during Phase I testing and demonstrations of safety would potentially allow continuation of use during pregnancy for women enrolled in Phase III trials. Oral tenofovir disoproxil fumarate is classified by the FDA as a pregnancy category B drug.

In addition, oral tenofovir is being studied for use in prevention of peripartum maternal-to-child transmission of HIV-1 in late pregnancy. Data on the first cohort of 15 women enrolled to Pediatric AIDS Clinical Trials Group (PACTG) Protocol 394 have been presented.[15] 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 pharmacokinetics and safety in the mother and infant were evaluated. No significant adverse events 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 blood/maternal 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. The study is continuing with a dose of 900 mg to the mother at onset of labor or before cesarean delivery. Another study, HPTN 057 is also evaluating maternal intrapartum and neonatal pharmacokinetics and safety of tenofovir. Both of these studies are in preparation for a large Phase III trial (expected sample size of approximately 2000 pregnant women) evaluating the use of oral tenofovir with emtricitabine and nevirapine for prevention of perinatal transmission and development of resistance of HIV-1. This selection is based on the expected effectiveness and safety of oral tenofovir in pregnancy. Given the low levels of tenofovir detected in the cord blood after a maternal oral dose of 600 mg, levels after intravaginal exposure to 40 mg would be expected to be lower, but must be evaluated.

Additional safety data regarding the use of tenofovir in pregnancy are available from the Antiretroviral Pregnancy Registry (APR). Through April 1, 2007, 266 cases of first trimester exposure and 208 cases with exposure to tenofovir later in pregnancy have been reported with no increase in birth defects or unusual pattern of defects detected (first trimester: 7 of 266 (2.6%); later in pregnancy: 3 of 208 (1.4%)).[16]

2.5 Mechanism of Action

Tenofovir disoproxil fumarate (TDF) is an acyclic nucleoside phosphonate diester analog of adenosine monophosphate. TDF requires initial diester hydrolysis for conversion to tenofovir and subsequent phosphorylations by cellular enzymes to form

tenofovir diphosphate. Tenofovir diphosphate inhibits the activity of HIV-1 reverse transcriptase by competing with the natural substrate deoxyadenosine 5'-triphosphate and, after incorporation into DNA, by DNA chain termination. Tenofovir diphosphate is a weak inhibitor of mammalian DNA polymerases α, β, and mitochondrial DNA polymerase γ.[17]

2.6 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 EC_{50} (50% effective concentration) values for tenofovir were in the range of 0.04 μM to 8.5 μM. In drug combination studies of tenofovir with nucleoside reverse transcriptase inhibitors (amprenavir, indinavir, nelfinavir, ritonavir, saquinavir), additive synergistic effects were observed. Tenofovir displayed antiviral activity in vitro against HIV-1 clades A, B, C, D, E, F, G, and O (EC_{50} values ranged from 1.6 μM to 4.9 μM).

2.7 Animal Studies

Pharmacokinetics

Single-dose pharmacokinetics of vaginal administration in female rabbits has been previously examined (0.5 mL, 1% w/v tenofovir, 5 mg per animal, 50 μCi/kg).[14] Plasma concentrations of radioactivity were highest at the first sample time point (0.5 hr) and below the level of quantification at 24 hours. Pharmacokinetic parameters including the proportion of dose absorbed systemically could not be estimated, due to the very low plasma concentrations.

In a tissue distribution study using the same tenofovir vaginal gel formulation, dose and strength as the above study, eighteen female rabbits were administered an intravaginal dose using a gavage needle.[14] 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.

The pharmacokinetics, excretion and tissue distribution of ^{14}C-PMPA (radiolabeled tenofovir) were evaluated in rats following intravaginal administration of an earlier formulation of tenofovir gel containing propylene glycol.[18] 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. The apparent maximum concentration (C_{max}) for tenofovir occurred at the earliest time point (15 minute), 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 $AUC_{(0\text{-}24)}$ with

historical AUC data for an intravenous dose of 10 mg/kg tenofovir in rats (9.71 µg hr/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 ^{14}C-PMPA (10 mg/kg, 100 µCi/kg) administered as an 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 pharmacokinetics of radiolabeled tenofovir were evaluated via plasma and vaginal biopsies collected from four rhesus monkeys following single-dose intravaginal administration of tenofovir 1% vaginal gel. 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 pre-clinical toxicity of tenofovir gel has been evaluated in 14-day rat and 10-day rabbit vaginal irritation and toxicity studies.[19,20] 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).

Tenofovir and TDF administered in toxicology studies to rats, dogs, and monkeys at exposures (based on AUCs) greater than or equal to six fold those observed in humans caused bone toxicity. In monkeys the bone toxicity was diagnosed as osteomalacia. Osteomalacia observed in monkeys appeared to be reversible upon dose reduction or discontinuation of tenofovir. In rats and dogs, the bone toxicity manifested as reduced bone mineral density. The mechanism(s) underlying bone toxicity is unknown.

Evidence of renal toxicity was noted in four animal species.[17] Increases in serum creatinine, BUN, glycosuria, proteinuria, phosphaturia, and/or calciuria and decreases in serum phosphate were observed to varying degrees in these animals. These toxicities were noted at exposures (based on AUCs) 2–20 times higher than those observed in humans. The relationship of the renal abnormalities, particularly the phosphaturia, to the bone toxicity is not known.

Long-term oral carcinogenicity studies of TDF in mice and rats were carried out at exposures up to approximately 16 times (mice) and five times (rats) those observed in humans at the therapeutic dose for HIV infection.[17] At the high dose in female mice, liver adenomas were increased at exposures 16 times that observed in humans. In rats, the study was negative for carcinogenic findings at exposures up to five times that observed in humans at the therapeutic dose. TDF was mutagenic in the in vitro mouse lymphoma assay and negative in an in vitro bacterial mutagenicity test (Ames test). In an in vivo mouse micronucleus assay, TDF was negative when administered to male mice.

There were no effects on fertility, mating performance or early embryonic development when TDF was administered to male rats at a dose equivalent to 10 times the human dose based on body surface area comparisons for 28 days prior to mating and to female rats for 15 days prior to mating through day seven of gestation. There was, however, an alteration of the estrous cycle in female rats.

Reproduction studies have been performed in rats and rabbits at doses up to 14 and 19 times the human dose based on body surface area comparisons and revealed no evidence of impaired fertility or harm to the fetus due to tenofovir.

2.8 Safety in Pregnancy

Tenofovir gel has not been studied in pregnancy in humans to date. Oral tenofovir, however, has been used in pregnancy and limited data exist. The Antiretroviral Pregnancy Registry has collected data on greater than 5,900 live births that have been exposed to one or more antiretroviral agent. Of that cohort, 474 of the fetuses were exposed to tenofovir. Overall ten birth defects have been recorded. The registry concluded that there was no pattern of defects linked to tenofovir exposure given the overall low rate of 2.1% (within accepted background rate of defects in the population of around 3%).

Tenofovir has been studied in gravid rhesus monkeys and does cross the placenta following subcutaneous administration in this simian model.[21] Based on the low plasma protein binding in pharmacologic studies, it is expected that tenofovir will cross the human placenta with systemic dosing.

In particular, the preliminary data from PACTG Protocol 394 suggest that the single dose administration of the tenofovir gel formulation would not pose undue risk to participants and their fetuses. As stated previously, no significant adverse events 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 low and all levels were below the level of quantitation in the infants at 12, 24, and 36 hours of age. The study is continuing with a dose of 900 mg to the mother at onset of labor or before cesarean delivery. Given the low neonatal levels detected after oral administration in PACTG 394, we would not expect detectable levels in the neonates born to mothers receiving single-dose vaginal product in MTN-002. Likewise, given the low systemic levels noted with vaginal administration in HPTN 050, we would not expect significant concentration of tenofovir in the breast milk of MTN-002 participants.

The single vaginal dosing in this study in addition to the term status of the fetus makes any significant effect on the fetus highly unlikely, assuming placental transfer does take place. In addition, systemic absorption of tenofovir from the vaginal gel appears to be minimal, (approximately 1% of the therapeutic oral dose) and the perceived risk of an effect on bone mineral density is extremely low from a single-dose vaginal study, if existent at all.

2.9 Clinical Studies

HPTN 050, also known as Phase I Safety and Acceptability Study of the Vaginal Microbicide Agent PMPA Gel is the most recently published study of tenofovir vaginal gel. Eighty-four (60 HIV-uninfected and 24 HIV-infected) women applied either 0.3% or 1% tenofovir gel once or twice daily. Both of the tenofovir gel formulations were well tolerated in both HIV-uninfected and HIV-infected women. Although 91% reported at least one AE, the majority (88%) were mild and limited to the GU tract. Product concentration, sexual activity and HIV status were not associated with a specific AE pattern. No clinically significant systemic toxicity was observed. Tenofovir gel showed no negative effect on vaginal microflora in this study. No new resistance mutations were detected in plasma or cervicovaginal lavage after 14 days of tenofovir gel use but three women had plasma mutations associated with low level tenofovir resistance at day 0 and 14 (M41L, L210M, ±T215I/Y). Systemic absorption was limited (maximum serum levels 3.1-25.8 ng/ml). The AE and safety profile in HPTN 050 was reviewed recently by the FDA during their review of the HPTN-059 extended safety protocol for vaginal tenofovir.

A male tolerance study of tenofovir 1% vaginal gel (CONRAD A04-099) was recently completed, and is currently in the analysis stage.

Several other tenofovir 1% vaginal gel studies are ongoing or in development. These include:

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

- CONRAD A04-095: Single Dose and 14-Day Once or Twice-daily Pharmacokinetic Study of the Vaginal Microbicide Agent 1% Tenofovir Gel

- CAPRISA 004: Safety and Effectiveness Study of a Candidate Vaginal Microbicide for Prevention of HIV (Phase IIb, two-arm, double-blinded, randomized, placebo controlled trial comparing coitally dependent 1% tenofovir gel with a placebo gel)

- MTN-001: Phase II Adherence and Pharmacokinetics Study of Oral and Vaginal Preparations of Tenofovir

- MTN-003: Phase 2B Safety and Effectiveness Study of Tenofovir 1% Gel, Tenofovir Disoproxil Fumarate (DF) Tablet and Tenofovir DF-Emtricitabine Tablet for the Prevention of HIV-1 Infection in Women

- MTN-007: Phase I Rectal Safety of Tenofovir Gel

2.10 Vaginal Gel Pharmacokinetics

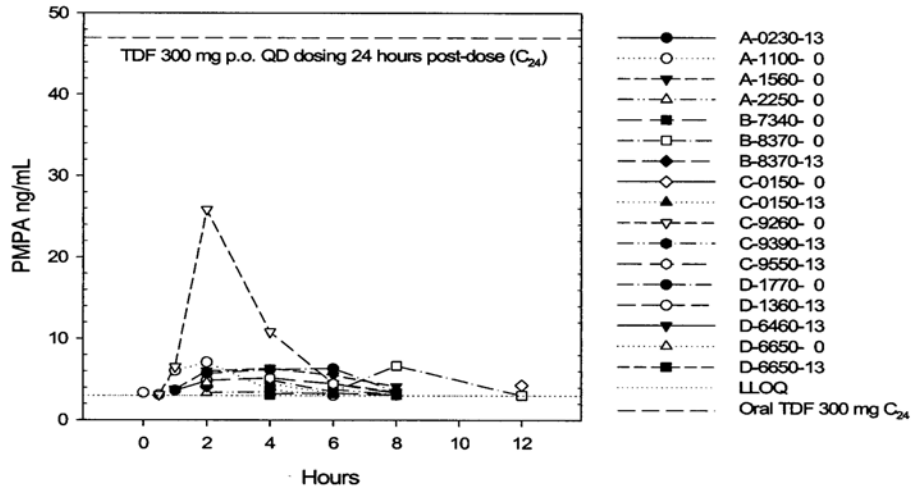
Limited vaginal pharmacokinetic data in primates and humans demonstrate that tenofovir gel is broadly distributed in vaginal tissues following vaginal application and can penetrate to epithelial tissues.[22] The amount of tenofovir administered by intravaginal application of 4 grams of a 1% dose is 40 mg. This dose of tenofovir is slightly more than half the amount absorbed following oral ingestion (approximately 25% or 75 mg) of a 300 mg tablet of TDF.

Tenofovir (0.3% and 1%) gel was recently tested in the HPTN 050 Phase I study. In this trial, tenofovir gel was administered intravaginally in four groups of women: sexually abstinent HIV-uninfected and HIV infected women, and sexually active HIV-uninfected and HIV-infected women. The women and their male partners (in the sexually active cohorts) were also asked to assess the acceptability of the product. Results from the HPTN 050 Phase I study have shown tenofovir 1% gel to be safe and acceptable.[2] In light of the favorable findings, an expanded safety and tolerability study of tenofovir gel is underway (HPTN 059).

HPTN 050 also addressed pharmacokinetics of vaginally administered tenofovir gel. Fourteen of 25 women (56%) with pharmacokinetic results had low, but detectable, serum tenofovir levels (limit of quantitation: 3.0 ng/mL) at some point in the 12 hours after dosing on either Day 0 (following the first dose) or on Day 13 (after daily dosing); three of the 14 had detectable levels on both days. The maximum tenofovir concentrations (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 to10.9 ng/mL at four 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 limit of quantitation [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.[23] Findings from CONRAD A04-095 are expected to further inform the pharmacokinetic data on vaginally administered tenofovir gel.

Figure 1 presented 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; lower limit of quantitation (LLOQ) approximately 3.0 ng/mL [dotted line]). Legend indicates “cohort” – “ID” – “study day”. For reference the tenofovir level associated with the median 24 hour post-dose blood concentration following an oral 300 mg tenofovir dose is indicated with dashed line.

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



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

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

The current study will expand the ongoing evaluation of tenofovir gel into the term pregnant population. It is conceivable that the absorption and pharmacokinetics of tenofovir gel in the 3rd trimester of pregnancy may differ from the non-pregnant data above, given increased blood flow to the pelvic tissues and engorgement of the pelvic vessels. With hypothesized increased absorption from the pelvic tissues, the potential for placental transfer exists. This protocol will also evaluate that endpoint.

The validated LCMS (liquid chromatography mass spectrometry) method planned for MTN-002 has a limit of quantitation (LOQ) of 5 ng/mL, but uses only 50 microliters of fluid. Previous methods have had a LOQ of 3 ng/mL but used 250 microliters. Since assay sensitivity is largely determined by mass in the sample, not concentration, this likely represents a 3 fold improvement in sensitivity if we use a similar volume as in previous studies. To improve even further, we are planning to dedicate 1 mL serum for the blood tenofovir assay to further increase the sensitivity, possibly below 1 ng/mL. This may increase the number of samples quantifiable in the blood assuming similar pharmacokinetics as in HPTN 050.

No PK analysis is planned beyond description 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, area under the concentration time curve (AUC), and terminal elimination slope. Since 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, data from this study cannot be used to estimate initial absorption, AUC, or terminal elimination slope.

2.11 Study Hypothesis

We hypothesize that:

- Plasma absorption in participants will be detectable in a percentage of women similar to that seen in HPTN 050 (approximately 33%)

- Of women with detectable levels of tenofovir, a small fraction will have detectable levels in endometrium, cord blood, placental tissue, and amniotic fluid

2.12 Justification of Dosing

Choice of the tenofovir 1% gel concentration for MTN-002 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 I study, the Phase I dose ranging study of tenofovir gel (0.3% once daily, then 1% once daily, then 0.3% twice daily followed by 1% twice daily). In this study, of the two doses and frequencies studied in the dose finding cohort, the 1% gel applied intravaginally twice daily for 14 days was well tolerated and was identified as the highest practical dose and frequency for further study in subsequent cohorts.

The second line of evidence is from vaginal transmission inhibition studies performed in non-human primates. Six separate studies provided evidence for efficacy of the gel over a range of tenofovir concentrations of 0.3% to 10%. Although the total data are limited and a powered statistical determination as to the efficacy of 1% tenofovir gel versus 0.3% and 10% cannot be made, empirical examination of the efficacy data identifies 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 $\log_{10}$ copies/mL after daily administration for 21 days. Comparison of the predicted cervicovaginal concentrations of tenofovir gel delivered to those achieved systemically at the standard treatment dose of 300 mg oral TDF, and tenofovir's characteristic prolonged intracellular half-life (diphosphate form, nine to 50 hours depending upon cell type), suggest that an initial and potentially durable barrier to HIV transmission may be possible. In terms of weighing potential risks and benefits, the 1% tenofovir gel minimizes the potential risks of vaginal epithelial toxicity while providing the potential benefit of delivering sufficient tenofovir to achieve an initial and possibly durable barrier to infection.

3 OBJECTIVES

3.1 Primary Objectives

- Assess term pregnancy maternal single-dose pharmacokinetics of tenofovir 1% vaginal gel

3.2 Secondary Objectives

- Characterize the systemic safety profile of single-dose tenofovir 1% vaginal gel in term gravidas
- Compare 3rd trimester absorption of tenofovir 1% vaginal gel to absorption in non-pregnant recent historic controls[2]
- Assess amniotic fluid, cord blood, endometrial tissue and placental tissue levels following single-dose tenofovir 1% vaginal gel

4 STUDY DESIGN

4.1 Identification of Study Design

MTN-002 will be a Phase I, single-site, open label investigation into the single-dose pharmacokinetic parameters and placental transfer of tenofovir gel when administered to term gravidas scheduled for elective cesarean section. We will enroll 16 healthy HIV-uninfected women, obtain baseline blood samples, place the gel in the vagina with a target administration time approximately two hours prior to the expected time of cesarean section (optimally a minimum of one hour prior to the collection of cord blood), and then obtain blood samples at the following times: 1, 2, 4, 6, 8, 12 and 24 hours following gel placement. In addition, amniotic fluid, placenta, and endometrial specimens will be collected.

4.2 Summary of Major Endpoints

- Maternal 3rd trimester pharmacokinetic measures (AUC, C_{max},)
- Endometrial tenofovir levels
- Placental transfer (cord blood tenofovir levels, placental tissue tenofovir levels, amniotic fluid tenofovir levels)

4.3 Description of Study Population

The study population will consist of 16 pregnant women aged 18-45 who are healthy, non-HIV infected and at term. The gravidas will be expected to be scheduled for elective cesarean section (CS) at ≥37 and <42 completed gestational weeks and will lack evidence of placental disease (no hypertension, diabetes mellitus, collagen vascular disease, placental abruption, etc.) as described in Section 5.3.

4.4 Time to Complete Enrollment

Time to complete study enrollment is expected to be approximately eighteen months.

4.5 Study Group

A single study arm is planned. A total of approximately 16 women will be enrolled. Additional participants will be enrolled to ensure that a total of 16 evaluable participants complete the study.

The following types of participants will be replaced:

- Participants who are enrolled but do not receive study gel
- Participants who receive study gel but whose time of cesarean delivery is greater than 8 hours following the time of study gel administration

4.6 Sequence and Duration 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 around the target date/time.

The time of cesarean section may be changed for clinical, administrative, or other reasons outside the control of the study staff. Gel administration that occurs outside the specified window due to such changes will not be considered a protocol violation.

Table 1: Visit Windows

Visit	Screening and Enrollment	Gel Administration	0-12 hr PK	24 Hour Evaluation	Two Week Phone Call
Window	Up to four weeks prior to expected date of gel administration (Day 0) and with reasonable allowance for turn-around-time of laboratory analyses drawn at Screening and Enrollment	Up to four hours prior to the expected time of cesarean section, with an optimal minimum of one hour prior to the expected time of cord blood collection	15 minutes +/- scheduled blood draw	22 – 26 hours	Day 10 – Day 18
Target	No target	Defined as Day 0, Time 0 2 hours prior to expected time of cesarean delivery	1, 2, 4, 6, 8, 12 hours following gel placement	24 hours following gel placement	Day 14

4.7 Expected Duration of Participation

The expected duration of participation for individual enrolled participants will depend on how long they enroll prior to the date of cesarean section, but may range from approximately three to six weeks. Participants who have Adverse Events (AEs) which are not resolved at the Two Week Phone Call will be followed beyond this point using Unscheduled Visits as necessary until a clinically acceptable resolution of the AE(s) (at the discretion of the Site Principal Investigator (PI) or NIH (Medical Monitors) has been documented, including resolution date, if possible. No further study data (for purposes of data analysis) will be collected for these participants after the Two Week Phone Call.

4.8 Site

A single site is planned for this trial: University of Pittsburgh, Pittsburgh, USA

5 STUDY POPULATION

5.1 Selection of the Study Population

5.1.1 Composition

It is anticipated that the study population will be primarily composed of Caucasian and African-American women consistent with the primary racial and ethnic composition of patients at Magee-Womens Hospital of UPMC. Women of other racial and ethnic backgrounds will not be excluded. As this study will be assessing the pharmacokinetics of a vaginally applied product among pregnant women, only female participants will be enrolled.

5.1.2 Recruitment

Members of the research team including the Site PI will recruit potential participants during the antenatal period using IRB-approved materials.

5.2 Inclusion Criteria

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

1. Age 18-45 years at screening and enrollment, inclusive, and verified per site standard operating procedure (SOP).

2. Willing and able to provide written informed consent for screening and enrollment

3. General good health as determined by the site Investigator of Record (IoR) or designee at Screening and Enrollment Visit

4. HIV-uninfected (per HIV Testing Algorithm, Appendix II)

5. HBsAg negative at Screening and Enrollment Visit or documented negative during this pregnancy

6. Pregnancy with the following characteristics:

    - Viable
    - Singleton
    - Without ultrasound evidence of significant fetal congenital anomaly (in the opinion of the IoR or designee)
    - Term (37 0/7 to 41 6/7 weeks, inclusive, with gestational dating criteria per SOP) at the time of planned cesarean section
    - Planned cesarean section

7. Normal Pap (or completed evaluation of abnormal Pap) in the 12 calendar months prior to screening per SOP

8. Willing to:
    - abstain from vaginal sex, anal sex, and receptive oral sex for at least two weeks after gel placement
    - abstain from intravaginal products and practices (including douching) during study participation
    - not participate in other drug or device study during study participation
    - participate as required by protocol, including study product administration, assessments and follow-up schedule

5.3 Exclusion Criteria

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

1. Maternal or fetal condition that necessitates urgent cesarean section (e.g. active labor, non-reassuring fetal heart tracing)

2. Documented rupture of the amniotic membranes, as defined in the SOP

3. Known maternal disease with predictable negative affect on placental function (e.g. hypertension, diabetes mellitus, collagen vascular disease, clinically significant maternal anemia)

4. Known placental/fetal abnormalities that could affect placental transfer (e.g. placental abruption, placenta previa, placenta accreta, intrauterine growth restriction, two vessel cord, etc.)

5. Serum creatinine at Screening and Enrollment Visit greater than 1.0 mg/dL

6. AST and/or ALT at screening greater than 1.5 ULN (upper limit of normal)

7. Current or recent (within 48 hours) use of vaginal medications at the Screening and Enrollment visit (per participant report)

8. Untreated sexually transmitted infection or (as applicable) exposure to partner's infection, including chlamydia, gonorrhea, trichomoniasis, non-gonococcal urethritis *Note: women diagnosed with an STI during screening or in the process of enrollment will be offered or referred for treatment in accordance with CDC guidelines. These participants will be eligible for enrollment once they have completed treatment(s) and are asymptomatic for the STI(s).*

9. Symptomatic vaginitis, including bacterial vaginosis and vulvovaginal candidiasis (participants with asymptomatic signs of bacterial vaginosis and/or yeast are still eligible for enrollment)

10. Participation in any other investigational drug or device trial within 30 days prior to enrollment visit

11. At screening or enrollment, 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

6 STUDY PRODUCT

6.1 Regimen

All study participants will receive a single vaginal dose of 4 grams of tenofovir 1% gel followed by 24 hours of intensive PK sampling.

6.2 Administration

Four grams of tenofovir 1% gel will be administered vaginally, using the vaginal applicator provided, by the authorized clinician, approximately two hours prior to the expected time of cesarean section (optimally at least one hour prior to the collection of cord blood).

6.3 Study Product Formulation and Preparation

Tenofovir gel is a clear, transparent viscous gel packaged in epoxy inner-lined aluminum tubes with white polyethylene screw caps equipped with a puncture tip. Each single-dose tube contains nominally 6 grams of tenofovir gel at a concentration of 1% (weight per weight) formulated in purified water with edetate disodium, citric acid, glycerin, methylparaben, propylparaben, and hydroxyethylcellulose, with pH adjusted between 4.0 and 5.0. The study gel is applied with a polyethylene applicator capable of administering a 4 gram dose. The product must be stored at 25°C (77°F) with excursions permitted to 15-30°C (59-86°F).

Immediately prior to application, the authorized clinician will fill the vaginal applicator by removing the cap from the tube of study gel, puncturing the metal seal on the tube with the pointed tip of the cap, screwing the end of the applicator onto the tube and slowly squeezing gel out of the tube and into the applicator. The plunger will stop when the applicator is full (contains the 4 grams of study product).

6.4 Study Product Supply and Accountability

6.4.1 Study Product Supply

Tenofovir 1% gel and applicators will be provided by CONRAD (Arlington, VA). Tenofovir 1% gel is manufactured, packaged, labeled, analyzed and released by Gilead Sciences (Foster City, CA) under current good manufacturing practices (cGMP), 21 Code of Federal Regulation, conditions.

6.4.2 Study Product Acquisition

Tenofovir 1% gel and vaginal applicators will be available through the DAIDS Clinical Research Products Management Center. The Pharmacist of Record can obtain the

study product for this protocol by following the instructions in the *Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks*.

6.4.3 Dispensing

The tenofovir 1% gel tube for a study participant and a vaginal applicator will be dispensed only upon receipt of a written prescription from an authorized prescriber. The study product should be kept in the pharmacy until the day of the planned cesarean section.

6.4.4 Accountability

The Pharmacist of Record is required to maintain complete records of all study products received from the DAIDS Clinical Research Products Management Center and subsequently dispensed. All unused study products must be returned to the DAIDS Clinical Research Products Management Center after the study is completed or terminated. The procedures to be followed are provided in the *Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks*.

6.4.5 Retrieval of Unused Study Products

Physician investigators and authorized study site staff must return any unused study product tubes and unadministered study product in applicators to the pharmacy.

6.5 Concomitant Medications and Procedures

With the exception of those not permitted under inclusion/exclusion criteria, concomitant medications will be permitted. These include both prescription and non-prescription medications.

All concomitant medications reported throughout the course of the study will be recorded on the participant's chart on forms designated for that purpose. Inpatient medications (including medications for anesthesia, intravenous fluids, and others), prescription medications, over-the-counter preparations, vitamins and nutritional supplements, and herbal preparations will all be recorded as concomitant medications. Forms will include a place to record the time of administration for all medications up until 24 hours following the administration of the study gel.

6.5.1 Prohibited Medications and Practices

Vaginal douching will not be permitted to protect the integrity of the lower genital tract and reduce the possibility of adverse events due to agents other than the study products. However, in the event that prohibited practices are reported following

administration of study gel, the protocol-specified visit schedule will continue for such participants for safety assessment through study exit. All concomitant medications will be recorded on Concomitant Medication Records.

Vaginal, anal, and receptive oral sex will not be permitted for at least two weeks following gel administration. Women will be advised to follow the instructions of their surgeon.

7 STUDY PROCEDURES

The following should take place for study participants:

- Screening and Enrollment Visit
- Pharmacokinetic Measures (includes gel administration)
- 24-hour Evaluation
- Two Week Phone Call

7.1 Screening and Enrollment Visit

The Screening and Enrollment Visit will occur approximately one to four weeks prior to the participant's scheduled C/S, but no more than four weeks before the expected date of cesarean section. This will allow the receipt of results for laboratory measures employed as components of screening.

Screening and Enrollment procedures are outlined below in Table 2: Screening and Enrollment Visit. If necessary, components of the Screening and Enrollment Visit may occur on different days. If necessary, components of the Screening and Enrollment Visit may take place at a location affiliated with the study site (for example, a satellite clinic).

With the permission of the participant, a physician investigator or designee will be available to answer questions of the primary obstetrician or other clinical care staff regarding the requirements of study participation. Appropriate written materials for clinical care staff members describing study procedures and supporting the correct interpretation of the study protocol will be placed in the participant's prenatal chart after written informed consent has been obtained from the participant.

Participants will be contacted with results of laboratory tests done at the Screening and Enrollment Visit once results are available; all results are likely to be available within approximately one week. Results other than HIV testing may be provided by phone.

Table 2: Screening and Enrollment Visit

Screening and Enrollment Visit	
Component	**Procedure/Analysis**
Administrative	• Obtain written informed consent • Collect locator information • Administer eligibility assessment • Assign Participant ID • Obtain signed records release • Record demographics • Plan for Pharmacokinetic Measures Visit (Date of Planned C/S) • Provide reimbursement for study visit
Clinical	• Review prenatal record • Review ultrasound report(s) • Record medical history • Record concomitant medications • Perform targeted physical exam • Perform pelvic exam
Laboratory	• Collect pelvic specimens o Trichomonas culture o *Wet prep and vaginal pH o *Herpes Culture • Collect urine specimen o Urine SDA for chlamydia and gonorrhea • Collect blood specimen o Serum creatinine o AST and ALT o Rapid HIV test with pre- and post-test counseling o *Confirmatory Testing for HIV o *HBsAg o *RPR o *Confirmatory Testing for Syphilis

*As clinically appropriate

7.2 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. 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.

7.3 Pharmacokinetic Measures

For the purposes of scheduling subsequent evaluation and follow-up, the date of gel administration will be considered Day 0. The time of gel administration will be considered Time 0. Timed pharmacokinetic measures are timed by hours passed since gel administration, not cesarean section.

A physician investigator will not be the primary or first assistant surgeon for the cesarean section. A physician investigator will be responsible for collection of study-related specimens in the operating room.

Flow cytometry for CD38 and CD95 will be obtained pre-dose on Day 0. This will be required as a surrogate for cell activation and proliferation markers, respectively, to serve as covariates in the intracellular model building.

If not already in place, a saline lock (or similar device) will be inserted to facilitate the collection of multiple blood specimens. This device may be replaced as needed in the event of a non-functioning device. Specimens will not be drawn from the site of an ongoing intravenous infusion.

Surgery and anesthesia may contribute to a temporary increase in intravascular volume, though this is often a function of the duration of surgery. Accordingly, there may be an increase in volume of distribution, especially for water soluble drugs, that would transiently reduce drug concentrations in the blood. Drugs used in anesthesia (including their time of administration) and operative time are routinely recorded and these data will be captured.

As the majority of these evaluations are likely to occur in the pre-operative area, operating room, and post-operative areas of the hospital, participants will not receive reimbursement for this study visit in these settings, which are expected to be relatively short-term. Rather, reimbursement will be provided for all study participation that occurs in the inpatient setting at the 24 Hour Evaluation or other setting as needed.

Table 3: Pharmacokinetic Measures: Gel Administration Day (Day 0)

Pharmacokinetic Measures		
Component		**Procedure/Analysis**
Pre-Gel	**Admin.**	• Update locator information • Place copy of consent in inpatient chart • Review inpatient chart • Administer eligibility assessment/Confirm eligibility • Schedule next study evaluation
	Clinical	• Update medical history • Update concomitant medications • Update adverse events • Perform targeted physical exam • Perform pelvic exam

	Lab	• Insert/Replace saline lock or similar (if not already inserted)
		• Draw blood for:
		  o maternal plasma tenofovir level
		  o flow cytometry

Gel		• Gel administration by study physician
Post-Gel	**C/S**	• Amniotic fluid collection • Endometrial tissue collection
	Clinical	• Collect adverse events at each PK time point • Review inpatient chart (during 1-12 hours post-gel)

	Lab	• Insert/Replace saline lock as needed
		• Draw blood for maternal plasma tenofovir level
		  o 1, 2, 4, 6, 8, and 12 hour time points
		  ▪ *Note: the above time points are after gel administration.*
		  ▪ *Some time points may occur before cesarean section.*
		  ▪ *The allowable window for each blood draw is +/-15 minutes.*
		• Cord blood collection
		  o may also be collected during cesarean section if possible
		• Placental tissue collection
		  o may also be collected during cesarean section if possible

7.4 24 Hour Evaluation

The 24 Hour Evaluation will occur between 22 and 26 hours following product administration.

Table 4: 24 Hour Evaluation

24 Hour Evaluation	
Component	**Procedure/Analysis**
Administrative	• Update locator information • Schedule Two Week Phone Call • Provide reimbursement
Clinical	• Review inpatient chart • Record/Update concomitant medications • Record/Update adverse events • *Perform targeted physical exam • *Perform pelvic exam
Laboratory	• Draw blood for maternal plasma tenofovir level

*only as clinically indicated

7.5 Two Week Phone Call

The Two Week Phone Call will collect data on any new or outstanding adverse events, as well as other procedures as outlined in Table 5. The window for the phone call is Day 10 to Day 18.

Table 5: Two Week Phone Call

Two Week Phone Call	
Component	**Procedure/Analysis**
Administrative	• Update locator information • Update demographics • *Review inpatient chart • *Review outpatient chart • Trigger staff to send reimbursement for phone call • *Schedule next visit
Clinical	• Update concomitant medications • Update adverse events

*as clinically indicated

7.6 Unscheduled Visit

An Unscheduled Visit may occur at any time during study participation, and may be triggered by participant report of an adverse event that is unresolved at the 24 Hour Evaluation, or that is reported following the 24 Hour Evaluation.

Table 6: Unscheduled Visit

Safety Visit	
Component	**Procedure/Analysis**
Administrative	• Update locator information • *Sign records release • *Schedule next visit/call
Clinical	• Update concomitant medications • Update adverse events • Perform targeted physical exam • *Perform pelvic exam • *Review inpatient chart • *Review outpatient chart
Laboratory	• *Serum creatinine • *ALT • *AST • *RPR • *Confirmatory testing for syphilis • *Rapid HIV Test • *Confirmatory testing for HIV with pre-and post-test counseling • *HBsAg • *Maternal plasma tenofovir level • *Urine SDA for CT and GC • *Urinalysis • *Urine culture and sensitivity • *Wet prep and vaginal pH • *Trichomonas culture • *Herpes culture • *Other laboratory tests deemed clinically appropriate, when possible after consultation with the PSRT

*as clinically indicated

7.7 Final Contact

In most cases the final contact will be the Two Week Phone Call. If necessary, the study site may complete the final contact visit(s), including the script for the Two Week Phone Call, at the study site or at community based locations, depending on site

capacities and site and participant preferences. All final contacts must be documented in participant study records.

7.8 Clinical Evaluations and Procedures

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

7.9 Laboratory Evaluations

7.9.1 Local Laboratory

The local laboratory, site investigator, or designee will run the following, as indicated:

- Serum creatinine
- AST
- ALT
- Rapid HIV test
- Confirmatory testing for HIV
- Hepatitis B surface antigen
- Rapid plasma reagin
- Confirmatory testing for syphilis
- Wet preparation slide
- Vaginal pH
- Herpes culture
- Urinalysis
- Urine culture and sensitivity
- Flow cytometry

7.9.2 Network Laboratory

The Network Laboratory will run the following:

- Urine SDA for chlamydia and gonorrhea
- Trichomonas culture
- Pharmacokinetic analyses (at NL Pharmacology Core)

The following specimens will be collected for pharmacokinetic analyses of tenofovir according to guidelines outlined in the Site SOPs.

- Maternal blood
- Amniotic fluid
- Cord blood
- Placental tissue

- Endometrial tissue

Based on HPTN 050, 64% of measurable samples fell in the 2 to 6 hour sampling window which was the basis for selection of sampling times to maximize capture of peak levels in the blood. Measurable levels were spread almost evenly among the 2, 4, and 6 hour sample times. Subjects' peak tenofovir concentrations occurred throughout this window. In HPTN 050, 38% of measurable levels were after 4 hours so they are somewhat less likely to be measurable, but will be nearly as informative as the 4 hour sample.

MTN-002 will use a more sensitive tenofovir assay than HPTN 050. Our validated liquid chromatography mass spectrometry (LCMS) method has a limit of quantitation (LOQ) of 5 ng/mL, but uses only 50 microliters of fluid. Previous methods have had a LOQ of 3 ng/mL but used 250 microliters. Since assay sensitivity is largely determined by mass in the sample, not concentration, this likely represents a 3 fold improvement in sensitivity if we use a similar volume as in previous studies. To improve even further, we are planning to dedicate 1 mL serum for the blood tenofovir assay to further increase the sensitivity, possibly below 1 ng/mL. This may increase the number of samples quantifiable in the blood assuming similar pharmacokinetics as in HPTN 050.

As stated above, a validated assay for tenofovir in plasma is currently available. Validated assays for tenofovir in endometrial tissue, amniotic fluid and placental tissue have not yet been developed, but are expected to be ready in 2007. Pharmacokinetic samples may be batch shipped from the NL to the NL Pharmacology Core for assay.

The principal parameter of interest after intravaginal dosing will be the AUC. Plasma will also be analyzed for routine pharmacokinetic parameters (C_{max}, T_{max}).

7.10 Specimen Collection and Processing

Each study site will adhere to the standards of good clinical laboratory practice, the HPTN-MTN Network Laboratory Manual, DAIDS Laboratory Requirements, and site standard operating procedures for proper collection, processing, labeling, transport, and storage of specimens at the local laboratory. Specimen collection, testing, and storage 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 as outlined in the site SOPs.

7.11 Specimen Handling

Specimens will be handled in accordance with Requirements for DAIDS Sponsored and/or Funded Laboratories in Clinical Trials.

7.12 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. Biohazardous waste will be contained according to institutional, transportation/carrier, and all other applicable regulations.

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, Medical Officers, SDMC Clinical Affairs Research Nurse, and Protocol Statistician, will serve as the 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 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. Adverse event 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. 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 expertise in the fields of microbicides, biostatistics, HIV transmission and medical ethics may be invited to join the PSRT safety review. A decision to stop the trial may be made by the PSRT at this time or at any such time that the team agrees that an unacceptable type and/or frequency of AEs has been observed.

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 US FDA and the site CRS 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 tenofovir gel.

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

Study site staff will document on study CRFs all AEs reported by or observed in enrolled study participants from the time of enrollment until study termination, regardless of severity and presumed relationship to study product. The Protocol Specific Toxicity

Table (Appendix V) will be the primary tool for grading adverse events for this protocol, with the exception of asymptomatic bacterial vaginosis which will not be a reportable AE. Adverse events not included in that table will be graded by the DAIDS AE Grading Table Version 1.0, December 2004. In cases where an AE is covered in both tables, the Protocol Specific Toxicity Table will be the grading scale utilized.

8.3.2 Serious Adverse Event

Serious adverse events (SAEs) will be defined per 21 CFR 312.32 guidelines as AEs occurring at any dose that:

- Result in death
- Are life-threatening adverse events
- Require Inpatient hospitalization or prolongation of existing hospitalization
- Result in persistent or significant disability/incapacity, or
- Are congenital anomalies/birth defects

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant or 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. Degrees of relatedness will be categorized as one of the following.

- *Definitely related*: adverse event and administration of study agent are related in time, and a direct association can be demonstrated with the study agent.

- *Probably related*: adverse event and administration of study agent are reasonably related in time, and the adverse event is more likely explained by the study agent than by other causes.

- *Possibly related*: adverse event and administration of study agent are reasonably related in time, and the adverse event can be explained equally well by causes other than the study agent.

- *Probably not related*: a potential relationship between administration of study agent and adverse event could exist, but is unlikely, and the adverse event is most likely explained by causes other than the study agent.

- *Not related*: the adverse event is clearly explained by another cause unrelated to administration of the study agent. Reportable events must have documentation to support the determination of "not related".

8.4 Expedited Adverse Event Reporting Requirements

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

AEs reported on an expedited basis must be documented on the DAIDS Expedited Adverse Event Reporting Form (EAE Reporting Form) available on the RCC website: http://rcc.tech-res-intl.com. DAIDS EAE forms should be submitted to DAIDS through the Regulatory Compliance Center (RCC) Safety Office (rccsafetyoffice@tech-res.com) or call 1-800-537-9979 or 301-897-1709 or fax 1-800-275-7619 or 301-897-1710.

EAE Reporting Requirements for this Study
EAE Reporting Level
This study uses the Intensive Level of expedited AE reporting as defined in the DAIDS EAE Manual.

Study Agents for Expedited Reporting to DAIDS
The study agents that must be considered in determining relationships of AEs requiring expedited reporting to DAIDS are the tenofovir 1% vaginal gel and study gel applicator.

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

EAE Reporting Periods
AEs must be reported on an expedited basis at the Intensive Level during the Protocol-defined EAE Reporting Period, which is the entire study duration for an individual subject (from study enrollment until study termination).

After the end of the Protocol-defined EAE Reporting Period stated above, 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.

8.5 Local Regulatory Requirements

Site investigators will submit AE information in accordance with local regulatory agencies' or other local authorities' requirements. This reporting will include site IRB-mandated reporting of AEs, SAEs, and other relevant safety information.

8.6 Social Harms Reporting

Although social harms are not expected in this study, the study site will make every effort to protect participant privacy and confidentiality, it is possible that participants' involvement in the study could become known to others, and that social harms may result. Social harms that are judged by the Investigator of Record to be serious or unexpected will be reported to the responsible site IRB at least annually, or according to their individual requirements. In the event that a participant reports social harm, every effort will be made by study staff to provide appropriate care and counseling to the participant, and/or referral to appropriate resources for the safety of the participant as needed.

9 CLINICAL MANAGEMENT

9.1 Toxicity Management

Based on results from previous clinical trials, significant toxicity in study participants is not expected in this trial of a single dose administration with minimal expected systemic absorption of 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 any patient with known risk factors for liver disease; however, cases have also been reported in patients with no known risk factors.

In the event of an expedited adverse event that occurs at the study site and that is judged to be definitely, probably, possibly, or probably not related to the study gel or applicator, product exposure will be minimized to the extent possible via cervicovaginal lavage (if clinically appropriate) and suspected toxicity will be managed according to Site SOPs by a physician on the study team at the discretion of the site investigator. Unless the participant withdraws her consent, she will remain in the study to complete all non-product related evaluations unless clinically contraindicated according to Appendix I.

9.2 Criteria for Withholding Study Product

Participants may decline administration of study product. Physician investigators may decide not to administer study product to participants to protect their safety.

The withholding of study product will occur only under certain criteria. The criteria for an individual participant are:

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

At least one of the above criteria must be met for the participant to not receive study product.

9.3 Criteria for Early Termination of Study Participation

Participants may voluntarily withdraw from the study for any reason at any time. The principal investigators may, with the approval of the PSRT, withdraw participants to protect their safety, and/or if participants are unable or unwilling to comply with study procedures.

Early (premature) termination of study participants will occur only under certain criteria. The criteria for early termination from the study for an individual participant are:

- Request by participant to withdraw
- Request by the principal investigator to protect the participant's safety and/or if the participant is unable or unwilling to comply with study procedures

At least one of the above criteria must be met for the participant to be terminated early from the study.

10 STATISTICAL CONSIDERATIONS

10.1 Overview and General Design

This is a single-dose, single-site, open label study. All the enrolled women will be administered tenofovir 1% vaginal gel. The planned total length of follow-up is 2 weeks.

10.2 Study Major Endpoints

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

- Maternal 3rd trimester pharmacokinetic measures: AUC, C_{max}
- Endometrial tenofovir levels
- Placental transfer : cord blood tenofovir levels, placental tissue tenofovir levels, and amniotic fluid tenofovir levels

10.3 Study Hypothesis

We hypothesize:

- Study hypothesis 1: Plasma absorption in participants will be detectable in a percentage of women similar to that seen in HPTN 050 (approximately 33%)
- Study hypothesis 2: Of women with detectable levels of tenofovir, a small fraction will have detectable levels in endometrium, cord blood, placental tissue, and amniotic fluid

10.4 Sample Size

The power of the study can be characterized as follows: if the overall absorption rate (defined as the proportion of women with detectable levels of PMPA in plasma, endometrium, cord blood, placental tissue, and/or amniotic fluid) was expected to be 33%, 16 women would provide 72% power to exclude absorption rate > 60%. In addition, the lower and upper bounds of the exact 95% confidence interval (CI) around the absorption rate are 13% and 57%, respectively, if the observed number of absorption in a cohort of 16 women was 5 (31%: 5 out 16).

For endpoints where no or very low absorption rates are expected (e.g., endometrium, cord blood, placental tissue, and amniotic fluid), 16 women would provide 80% power to exclude absorption rate > 25% assuming the true rate of absorption is 5%. In addition, the upper bounds of the exact 95% confidence interval (CI) around the absorption rate are 20% and 30% if the observed number of absorption in a cohort of 16 women was 0 or 1, respectively.

Additional participants may enroll in the study, at the discretion of the protocol team, to replace currently enrolled participants who receive study gel but whose time of cesarean delivery is greater than 8 hours following the time of study gel administration Thus, in the event that participants are replaced for this purpose, the total sample size at the end of the study may slightly exceed 16 participants who received study gel.

A sample size of 16 women is insufficient to provide a formal statistical assessment for the comparison between the plasma absorption level observed in HPTN 050 and the one that will be observed in this study.

10.5 Participant Accrual, Follow-up, and Retention

The accrual period will be 18 months. The study site will recruit and enroll a total of 16 participants. Each participant will be followed for two weeks following gel administration. Once a participant has enrolled in the study, the study site will make every reasonable effort to retain her for the entire study period.

10.6 Data and Safety Monitoring and Analysis

10.6.1 Study Monitoring Committee (SMC)

No Data and Safety Monitoring Board oversight is planned for this study. 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 four to six months, 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.

10.6.2 Primary Analysis

Descriptive statistics to assess women characteristics will be used: for categorical variables, the number and percent in each category; for continuous variables, the mean, median, standard deviation, quartiles and range (minimum, maximum). Similarly, descriptive statistics for continuous variables will be use to describe levels of tenofovir in levels in plasma, in endometrium, in cord blood, in placental tissue, and in amniotic fluid. If a substantial number of women are below the limit of detection of the assay, descriptive statistics for continuous variables will not be used. Rather, proportion of women with detectable levels will be computed along with an exact 95% confidence interval based on the Clopper-Pearson method.

Blood plasma pharmacokinetics of tenofovir will be evaluated after vaginal administration. Pharmacokinetic parameter estimates will include peak concentration (C_{max}), time to peak concentration (T_{max}), and area under the concentration time curve (AUC) for tenofovir in the blood. Descriptive statistics will be used to summarize these PK parameters in the cohort. Cord blood, amniotic fluid, placental tissue, and endometrial tissue tenofovir levels will also be summarized using descriptive statistics. The ratio of concentrations of tenofovir in maternal blood relative to temporally matched cord blood, amniotic fluid, and endometrial tissue concentrations, 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.

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

Source documents and access to source data/documents will be maintained in accordance with the Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials. The investigator will maintain, and store securely, complete, accurate and current study records throughout the study. In accordance with U.S. regulations, the investigator will retain all study records on site for at least two years after study closure. Study records will not be destroyed prior to receiving approval for record destruction from DAIDS. Applicable records include source documents, site registration documents and reports, correspondence, informed consent forms, and notations of all contacts with the participant.

11.3 Quality Control and Quality Assurance

Quality control and quality assurance procedures for MTN-002 will be performed in accordance with Requirements for Clinical Quality Management Plans at DAIDS Funded and/or Supported Clinical Research Sites.

11.4 Study Coordination

DAIDS holds the IND application for this study (#55690). Assignment of all sponsor responsibilities for this study will be specified in a Clinical Trials Agreement 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 Family Health International, the Statistical Center for HIV/AIDS Research & Prevention (SCHARP), and the MTN Network Laboratory.

12 CLINICAL SITE MONITORING

Study monitoring will be carried out by PPD (Wilmington, NC). On-site study monitoring will be performed in accordance with Requirements for On-Site Monitoring of DAIDS Funded and/or Sponsored Clinical Trials. Site monitoring visits will be conducted to assess compliance with Health and Human Services (HHS) Regulations 45 Code of Federal Regulations (CFR) Part 46 and 21 CFR Parts 50, 56, and 312. Study monitors will visit the site to:

- Verify compliance with human subjects and other research regulations and guidelines, including confidentiality procedures, informed consent process, and regulatory documentation

- Assess adherence to the study protocol, site SOPs, and local counseling practices

- Confirm the quality and accuracy of information collected at the study site and entered into the study database, including the validation of data reported on case report and DataFax forms

- Assess the resolution of any past or ongoing issues identified at previous monitoring visits

Site investigators will allow study monitors to inspect study facilities and documentation (e.g., informed consent forms, clinic and laboratory records, other source documents, case report forms), as well as observe the performance of study procedures. Investigators also will allow inspection of all study-related documentation by authorized representatives of the MTN CORE, MTN Network Laboratory, Family Health International, SCHARP, NIAID, and US regulatory authorities. A site visit log will be maintained at the study site to document all visits.

13 HUMAN SUBJECTS PROTECTIONS

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

13.1 Institutional Review Board

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 Institutional Review Board (IRB) prior to implementation of the protocol. Any amendments to the protocol and/or informed consents must be approved by the IRB and DAIDS prior to implementation.

13.2 Protocol Registration

The study site will complete protocol registration with the DAIDS RCC Protocol Registration Office. 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. Protocol registration material can be sent electronically to epr@tech-res.com. For questions regarding protocol registration, please call (301) 897-1707. 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 Chair and NIAID Medical Officer. All protocol amendments must be submitted to and approved by the IRB and the RCC prior to implementing the amendment.

13.3 Risk Benefit Statement

13.3.1 Risks

Phlebotomy may lead to discomfort, feelings of dizziness or faintness, and/or bruising, swelling and/or infection. Pelvic examination may cause mild discomfort and/or vaginal bleeding or spotting. Participation in clinical research includes the risks of loss of confidentiality and discomfort with personal nature of questions.

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.

Some of the possible side effects of the study gel are 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 I study resulted in minimal local irritation and little or no systemic adverse effects were identified. 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. The risks associated with tenofovir gel are believed to be less than those identified for systemic use. In the HPTN 050 Phase I study of tenofovir gel, 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 I 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.

This is a single-dose study and maternal plasma 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.

There are currently no data on the passage of tenofovir into breast milk following administration of the oral or vaginal formulation of tenofovir. The molecular weight and low plasma protein binding suggest that the drug will be excreted into human breast milk, though the expected concentration in this single-dose study of a vaginal product would be negligible. The effects of exposure on a nursing infant are unknown.

No risks are associated with a collection of a small portion of amniotic fluid immediately prior to rupture of the membranes during cesarean section. Minimal extra time (< 30 seconds) will be added to the surgery because of amniotic fluid collection.

No extra risk to either the mother or fetus is associated with the collection of one aliquot of umbilical cord blood after the delivery of the baby. This is often part of the normal process to determine blood incompatibility between mother and neonate, and is commonly done without consequence. Minimal extra time (< 30 seconds) will be added to the surgery because of cord blood collection; this is not expected to pose a clinically significant risk to participants.

Study participants will experience no additional discomfort from the endometrial sampling since they will be adequately anesthetized for the primary surgical procedure. Additional bleeding may be encountered in the removal of the endometrial sample. This is not anticipated to be clinically important as the routine closure of the uterine incision will proceed immediately after the sample is obtained. Because the material will be of

low volume, it should not affect that participant's future ability to have a vaginal birth after cesarean (VBAC). Prolongation of surgical and anesthetic time should also be minimal. No unforeseen complications have arisen to date in numerous studies employing both endometrial and myometrial sampling at the time of cesarean, and less bleeding is expected with endometrial sampling given more superficial sampling.

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 Good Clinical Practices (GCP) and to the ethical principles that have their origin in the Declaration of Helsinki. Study staff must document the informed consent process in accordance with the Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials. 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 Appendix VI 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/EC's written approval/favorable opinion of the protocol, informed consent forms, and any other study-related information to be provided to participants. This study does not plan to enroll children under 18.

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. 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.

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 file cabinets in areas with access limited to study staff. Data collection, process, and administrative forms, laboratory specimens, and other reports will be identified by a coded number only to maintain participant confidentiality. All records that contain names or other personal identifiers, such as locator forms and informed consent forms, will be stored separately from study records identified by code number. All local databases will be secured with password-protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link participant ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access. Participants' study information will not be released without their written permission, except as necessary for monitoring (see Section 12).

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

The Allegheny County Health Department (Pittsburgh, PA) mandates the reporting of certain sexually transmitted infections, including chlamydia, gonorrhea, syphilis and HIV. Participants will be informed of this when written informed consent is obtained for the Screening and Enrollment Visit.

13.6 Special Populations

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

13.6.1 Pregnant Women

Pregnant women are the target population of this protocol. During the informed consent process, women will be informed that the collected data on oral TDF in pregnancy thus far does not suggest any deleterious effects. Oral tenofovir disoproxil fumarate is classified by the FDA as a pregnancy category B drug. Animal studies have failed to demonstrate a risk to the fetus. Preliminary data from Pediatric AIDS Clinical Trials Group (PACTG) 394, which investigated fourteen mother-infant pairs using a single oral dose of TDF 600 mg, failed to demonstrate any significant neonatal effects. In addition, tenofovir concentrations in infants were all below the detectable levels at twelve hours of age, and were only detectable earlier in less than half of the infants.

13.6.2 Children

The NIH has mandated that children be included in research trials when appropriate. This study meets "Justifications for Exclusion" criteria for younger children as set forth by the NIH. Specifically, "insufficient data are available in adults to judge potential risk in children" and "children should not be the initial group to be involved in research studies." This protocol will include children aged 18 to 21 years old.

13.6.3 Prisoners

MTN-002 does not meet the criteria for prisoner participation per US 45 Code of Federal Regulations (CFR) 46.306 (a)(2)(D). MTN-002 is not suitable for further reviews by local IRBs for the inclusion of prisoners.

13.7 Incentives

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

13.8 Communicable Disease Reporting

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

13.9 Access to HIV-related Care

13.9.1 HIV Counseling and Testing

HIV pretest and post-test counseling will be provided to all potential study participants who consent to undergo HIV screening to determine their eligibility for this study. 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 NICHD, NIAID, the MTN, CONRAD, the US FDA, the Office of Human Research Protections (OHRP), other government or regulatory authorities, or the site IRB.

14 PUBLICATION POLICY

DAIDS and MTN policies and a Clinical Trial Agreement (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.

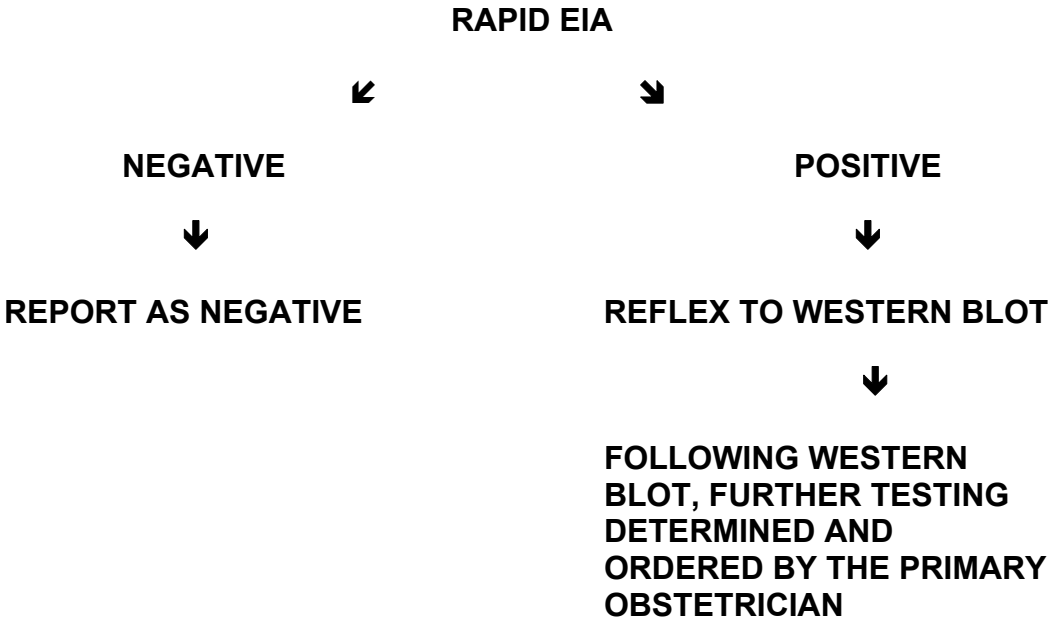
15 APPENDICES

APPENDIX I: SCHEDULE OF STUDY VISITS AND EVALUATIONS

	SCREENING AND ENROLLMENT	GEL ADMIN. (DAY OF C/S)	1-12 HOUR PHARMACOKINETIC MEASURES (1, 2, 4, 6, 8, 12 HOURS)	24 HOUR EVALUATION	TWO WEEK PHONE CALL	UNSCHEDULED VISIT
ADMINISTRATIVE						
Informed Consent	x					
Place Copy of Consent in Inpatient Chart		x (pre-gel)				
Administer Elig. Assessment/Confirm Eligibility	x	x (pre-gel)				
Obtain Signed Records Release◆	x					▲
Assign Participant ID	x					
Record/Update Locator Information	x	x (pre-gel)		x	x	x
Record/Update Demographics	x				x	
Plan/Schedule Next Visit/Call	x	x (pre-gel)		x	▲	▲
Reimbursement	x			x	x	
CLINICAL						
Review Prenatal Record	x					
Review Ultrasound Report(s)	x					
Review Inpatient Chart	▲	x (pre-gel)	▲ (post-gel)	x	▲	▲
Record/Update Medical History	x	x (pre-gel)				
Record/Update Concomitant Medications	x	x (pre-gel)		x	x	x
Pelvic Exam	x	x (pre-gel)		▲		▲
Targeted Physical Exam	x	x (pre-gel)		▲		x
Record/Update Adverse Events		x (pre- and post-gel)	x (post-gel, at each time point)	x	x	x
Insert/Replace Saline Lock or Similar		x (pre-gel)	▲ (post-gel)			
LABORATORY						
Serum Creatinine	x					▲
AST/ALT	x					▲
Rapid HIV Test with Pre- and Post-test Counseling	x					▲
Confirmatory Testing for HIV	▲					▲
HBsAg	▲					▲
Urine SDA for CT and GC	x					▲
Urinalysis						▲
Urine Culture and Sensitivity						▲
Wet Prep and Vaginal pH	▲					▲
Trichomonas Culture	x					▲
Herpes Culture	▲					▲
RPR	▲					▲
Confirmatory Testing for Syphilis	▲					▲
Maternal Plasma Tenofovir Level		x (pre-gel)	x (at each time point)	x		▲
Flow Cytometry		x (pre-gel)				
Collect Amniotic Fluid		x (dur. C/S)				
Collect Endometrial Tissue		x (dur. C/S)				
Collect Cord Blood		x (during or after C/S)				
Collect Placental Tissue		x (during or after C/S)				
GEL ADMINISTRATION						
Gel Administration by Study Physician		x				

X = protocol-defined procedure; ▲= performed as indicated, ◆ = Obtain again on day of discharge from hospital, if possible
At Unscheduled Visit, investigator(s) may order other laboratory tests deemed clinically appropriate, when possible after consultation with the PSRT. The target time for gel administration is 2 hours prior to the time of expected C/S. Pelvic exams following the Screening and Enrollment Visit should include components as clinically indicated.

APPENDIX II: HIV TESTING ALGORITHM



APPENDIX III: COMPONENTS OF EXAMINATIONS

Targeted Physical Exam

- Vital signs (may be transcribed from chart if taken in past hour)
    - Temperature
    - Pulse
    - Blood pressure
- General appearance
- Abdomen
- 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
- Pelvic exams following the Screening and Enrollment Visit should include components as clinically indicated

APPENDIX IV: DAIDS AE GRADING TABLE

Quick Reference

The Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events ("DAIDS AE grading table") is a descriptive terminology which can be utilized for Adverse Event (AE) reporting. A grading (severity) scale is provided for each AE term.

General Instructions

Estimating Severity Grade

If the need arises to grade a clinical AE that is not identified in the DAIDS AE grading table, use the category "Estimating Severity Grade" located at the top of Page 3. For AEs that are not listed in the table but will be collected systematically for a study/trial, protocol teams are highly encouraged to define study-specific severity scales within the protocol or an appendix to the protocol. (Please see "Template Wording for the Expedited Adverse Event Reporting Section of DAIDS-sponsored Protocols".) This is particularly important for laboratory values because the "Estimating Severity Grade" category only applies to clinical symptoms.

Grading Adult and Pediatric AEs

The DAIDS AE grading table includes parameters for grading both Adult and Pediatric AEs. When a single set of parameters is not appropriate for grading specific types of AEs for both Adult and Pediatric populations, separate sets of parameters for Adult and/or Pediatric populations (with specified respective age ranges) are given in the table. If there is no distinction in the table between Adult and Pediatric values for a type of AE, then the single set of parameters listed is to be used for grading the severity of both Adult and Pediatric events of that type.

Determining Severity Grade

If the severity of an AE could fall under either one of two grades (e.g., the severity of an AE could be either Grade 2 or Grade 3), select the higher of the two grades for the AE.

Definitions

Basic Self-care Functions	Adult Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding. Young Children Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).
LLN	Lower limit of normal
Medical Intervention	Use of pharmacologic or biologic agent(s) for treatment of an AE.
NA	Not Applicable

Operative Intervention	Surgical OR other invasive mechanical procedures.
ULN	Upper limit of normal
Usual Social & Functional Activities	Adult Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc. Young Children Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

Contents

CLINICAL				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
ESTIMATING SEVERITY GRADE				
Clinical adverse event NOT identified elsewhere in this DAIDS AE grading table	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions OR Medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death
SYSTEMIC				
Acute systemic allergic reaction	Localized urticaria (wheals) with no medical intervention indicated	Localized urticaria with medical intervention indicated OR Mild angioedema with no medical intervention indicated	Generalized urticaria OR Angioedema with medical intervention indicated OR Symptomatic mild bronchospasm	Acute anaphylaxis OR Life-threatening bronchospasm OR laryngeal edema
Chills	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	NA
Fatigue Malaise	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Incapacitating fatigue/ malaise symptoms causing inability to perform basic self-care functions
Fever (nonaxillary)	37.7 – 38.6°C	38.7 – 39.3°C	39.4 – 40.5°C	> 40.5°C
Pain (indicate body site) DO NOT use for pain due to injection (See Injection Site Reactions: Injection site pain) See also Headache, Arthralgia, and Myalgia	Pain causing no or minimal interference with usual social & functional activities	Pain causing greater than minimal interference with usual social & functional activities	Pain causing inability to perform usual social & functional activities	Disabling pain causing inability to perform basic self-care functions OR Hospitalization (other than emergency room visit) indicated

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

CLINICAL				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Unintentional weight loss	NA	5 – 9% loss in body weight from baseline	10 – 19% loss in body weight from baseline	≥ 20% loss in body weight from baseline OR Aggressive intervention indicated [e.g., tube feeding or total parenteral nutrition (TPN)]
INFECTION				
Infection (any other than HIV infection)	Localized, no systemic antimicrobial treatment indicated AND Symptoms causing no or minimal interference with usual social & functional activities	Systemic antimicrobial treatment indicated OR Symptoms causing greater than minimal interference with usual social & functional activities	Systemic antimicrobial treatment indicated AND Symptoms causing inability to perform usual social & functional activities OR Operative intervention (other than simple incision and drainage) indicated	Life-threatening consequences (e.g., septic shock)
INJECTION SITE REACTIONS				
Injection site pain (pain without touching) Or Tenderness (pain when area is touched)	Pain/tenderness causing no or minimal limitation of use of limb	Pain/tenderness limiting use of limb OR Pain/tenderness causing greater than minimal interference with usual social & functional activities	Pain/tenderness causing inability to perform usual social & functional activities	Pain/tenderness causing inability to perform basic self-care function OR Hospitalization (other than emergency room visit) indicated for management of pain/tenderness
Injection site reaction (localized)				
Adult > 15 years	Erythema OR Induration of 5x5 cm – 9x9 cm (or 25 cm^2 – 81cm^2)	Erythema OR Induration OR Edema > 9 cm any diameter (or > 81 cm^2)	Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage	Necrosis (involving dermis and deeper tissue)
Pediatric ≤ 15 years	Erythema OR Induration OR Edema present but ≤ 2.5 cm diameter	Erythema OR Induration OR Edema > 2.5 cm diameter but < 50% surface area of the extremity segment (e.g., upper arm/thigh)	Erythema OR Induration OR Edema involving ≥ 50% surface area of the extremity segment (e.g., upper arm/thigh) OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage	Necrosis (involving dermis and deeper tissue)

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

CLINICAL				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Pruritis associated with injection See also Skin: Pruritis (itching - no skin lesions)	Itching localized to injection site AND Relieved spontaneously or with < 48 hours treatment	Itching beyond the injection site but not generalized OR Itching localized to injection site requiring ≥ 48 hours treatment	Generalized itching causing inability to perform usual social & functional activities	NA
SKIN – DERMATOLOGICAL				
Alopecia	Thinning detectable by study participant (or by caregiver for young children and disabled adults)	Thinning or patchy hair loss detectable by health care provider	Complete hair loss	NA
Cutaneous reaction – rash	Localized macular rash	Diffuse macular, maculopapular, or morbilliform rash OR Target lesions	Diffuse macular, maculopapular, or morbilliform rash with vesicles or limited number of bullae OR Superficial ulcerations of mucous membrane limited to one site	Extensive or generalized bullous lesions OR Stevens-Johnson syndrome OR Ulceration of mucous membrane involving two or more distinct mucosal sites OR Toxic epidermal necrolysis (TEN)
Hyperpigmentation	Slight or localized	Marked or generalized	NA	NA
Hypopigmentation	Slight or localized	Marked or generalized	NA	NA
Pruritis (itching – no skin lesions) (See also Injection Site Reactions: Pruritis associated with injection)	Itching causing no or minimal interference with usual social & functional activities	Itching causing greater than minimal interference with usual social & functional activities	Itching causing inability to perform usual social & functional activities	NA
CARDIOVASCULAR				
Cardiac arrhythmia (general) (By ECG or physical exam)	Asymptomatic AND No intervention indicated	Asymptomatic AND Non-urgent medical intervention indicated	Symptomatic, non-life-threatening AND Non-urgent medical intervention indicated	Life-threatening arrhythmia OR Urgent intervention indicated
Cardiac-ischemia/infarction	NA	NA	Symptomatic ischemia (stable angina) OR Testing consistent with ischemia	Unstable angina OR Acute myocardial infarction

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

CLINICAL				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Hemorrhage (significant acute blood loss)	NA	Symptomatic AND No transfusion indicated	Symptomatic AND Transfusion of ≤ 2 units packed RBCs (for children ≤ 10 cc/kg) indicated	Life-threatening hypotension OR Transfusion of > 2 units packed RBCs (for children > 10 cc/kg) indicated
Hypertension				
Adult > 17 years (with repeat testing at same visit)	> 140 – 159 mmHg systolic OR > 90 – 99 mmHg diastolic	> 160 – 179 mmHg systolic OR > 100 – 109 mmHg diastolic	> 180 mmHg systolic OR > 110 mmHg diastolic	Life-threatening consequences (e.g., malignant hypertension) OR Hospitalization indicated (other than emergency room visit)
Pediatric ≤ 17 years (with repeat testing at same visit)	NA	91st – 94th percentile adjusted for age, height, and gender (systolic and/or diastolic)	≥ 95th percentile adjusted for age, height, and gender (systolic and/or diastolic)	Life-threatening consequences (e.g., malignant hypertension) OR Hospitalization indicated (other than emergency room visit)
Hypotension	NA	Symptomatic, corrected with oral fluid replacement	Symptomatic, IV fluids indicated	Shock requiring use of vasopressors or mechanical assistance to maintain blood pressure
Pericardial effusion	Asymptomatic, small effusion requiring no intervention	Asymptomatic, moderate or larger effusion requiring no intervention	Effusion with non-life threatening physiologic consequences OR Effusion with non-urgent intervention indicated	Life-threatening consequences (e.g., tamponade) OR Urgent intervention indicated
Prolonged PR interval				
Adult > 16 years	PR interval 0.21 – 0.25 sec	PR interval > 0.25 sec	Type II 2nd degree AV block OR Ventricular pause > 3.0 sec	Complete AV block
Pediatric ≤ 16 years	1st degree AV block (PR > normal for age and rate)	Type I 2nd degree AV block	Type II 2nd degree AV block	Complete AV block

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

CLINICAL				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Prolonged QTc				
Adult > 16 years	Asymptomatic, QTc interval 0.45 – 0.47 sec OR Increase interval < 0.03 sec above baseline	Asymptomatic, QTc interval 0.48 – 0.49 sec OR Increase in interval 0.03 – 0.05 sec above baseline	Asymptomatic, QTc interval ≥ 0.50 sec OR Increase in interval ≥ 0.06 sec above baseline	Life-threatening consequences, e.g. Torsade de pointes or other associated serious ventricular dysrhythmia
Pediatric ≤ 16 years	Asymptomatic, QTc interval 0.450 – 0.464 sec	Asymptomatic, QTc interval 0.465 – 0.479 sec	Asymptomatic, QTc interval ≥ 0.480 sec	Life-threatening consequences, e.g. Torsade de pointes or other associated serious ventricular dysrhythmia
Thrombosis/embolism	NA	Deep vein thrombosis AND No intervention indicated (e.g., anticoagulation, lysis filter, invasive procedure)	Deep vein thrombosis AND Intervention indicated (e.g., anticoagulation, lysis filter, invasive procedure)	Embolic event (e.g., pulmonary embolism, life-threatening thrombus)
Vasovagal episode (associated with a procedure of any kind)	Present without loss of consciousness	Present with transient loss of consciousness	NA	NA
Ventricular dysfunction (congestive heart failure)	NA	Asymptomatic diagnostic finding AND intervention indicated	New onset with symptoms OR Worsening symptomatic congestive heart failure	Life-threatening congestive heart failure
GASTROINTESTINAL				
Anorexia	Loss of appetite without decreased oral intake	Loss of appetite associated with decreased oral intake without significant weight loss	Loss of appetite associated with significant weight loss	Life-threatening consequences OR Aggressive intervention indicated [e.g., tube feeding or total parenteral nutrition (TPN)]
Ascites	Asymptomatic	Symptomatic AND Intervention indicated (e.g., diuretics or therapeutic paracentesis)	Symptomatic despite intervention	Life-threatening consequences
Cholecystitis	NA	Symptomatic AND Medical intervention indicated	Radiologic, endoscopic, or operative intervention indicated	Life-threatening consequences (e.g., sepsis or perforation)

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

CLINICAL				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Constipation	NA	Persistent constipation requiring regular use of dietary modifications, laxatives, or enemas	Obstipation with manual evacuation indicated	Life-threatening consequences (e.g., obstruction)
Diarrhea				
Adult and Pediatric ≥ 1 year	Transient or intermittent episodes of unformed stools OR Increase of ≤ 3 stools over baseline per 24-hour period	Persistent episodes of unformed to watery stools OR Increase of 4 – 6 stools over baseline per 24-hour period	Bloody diarrhea OR Increase of ≥ 7 stools per 24-hour period OR IV fluid replacement indicated	Life-threatening consequences (e.g., hypotensive shock)
Pediatric < 1 year	Liquid stools (more unformed than usual) but usual number of stools	Liquid stools with increased number of stools OR Mild dehydration	Liquid stools with moderate dehydration	Liquid stools resulting in severe dehydration with aggressive rehydration indicated OR Hypotensive shock
Dysphagia-Odynophagia	Symptomatic but able to eat usual diet	Symptoms causing altered dietary intake without medical intervention indicated	Symptoms causing severely altered dietary intake with medical intervention indicated	Life-threatening reduction in oral intake
Mucositis/stomatitis (clinical exam) Indicate site (e.g., larynx, oral) See Genitourinary for Vulvovaginitis See also Dysphagia-Odynophagia and Proctitis	Erythema of the mucosa	Patchy pseudomembranes or ulcerations	Confluent pseudomembranes or ulcerations OR Mucosal bleeding with minor trauma	Tissue necrosis OR Diffuse spontaneous mucosal bleeding OR Life-threatening consequences (e.g., aspiration, choking)
Nausea	Transient (< 24 hours) or intermittent nausea with no or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24 – 48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Pancreatitis	NA	Symptomatic AND Hospitalization not indicated (other than emergency room visit)	Symptomatic AND Hospitalization indicated (other than emergency room visit)	Life-threatening consequences (e.g., circulatory failure, hemorrhage, sepsis)

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

CLINICAL				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Proctitis (functional-symptomatic) Also see Mucositis/stomatitis for clinical exam	Rectal discomfort AND No intervention indicated	Symptoms causing greater than minimal interference with usual social & functional activities OR Medical intervention indicated	Symptoms causing inability to perform usual social & functional activities OR Operative intervention indicated	Life-threatening consequences (e.g., perforation)
Vomiting	Transient or intermittent vomiting with no or minimal interference with oral intake	Frequent episodes of vomiting with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
NEUROLOGIC				
Alteration in personality-behavior or in mood (e.g., agitation, anxiety, depression, mania, psychosis)	Alteration causing no or minimal interference with usual social & functional activities	Alteration causing greater than minimal interference with usual social & functional activities	Alteration causing inability to perform usual social & functional activities	Behavior potentially harmful to self or others (e.g., suicidal and homicidal ideation or attempt, acute psychosis) OR Causing inability to perform basic self-care functions
Altered Mental Status For Dementia, see Cognitive and behavioral/attentional disturbance (including dementia and attention deficit disorder)	Changes causing no or minimal interference with usual social & functional activities	Mild lethargy or somnolence causing greater than minimal interference with usual social & functional activities	Confusion, memory impairment, lethargy, or somnolence causing inability to perform usual social & functional activities	Delirium OR obtundation, OR coma
Ataxia	Asymptomatic ataxia detectable on exam OR Minimal ataxia causing no or minimal interference with usual social & functional activities	Symptomatic ataxia causing greater than minimal interference with usual social & functional activities	Symptomatic ataxia causing inability to perform usual social & functional activities	Disabling ataxia causing inability to perform basic self-care functions

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

CLINICAL				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Cognitive and behavioral/attentional disturbance (including dementia and attention deficit disorder)	Disability causing no or minimal interference with usual social & functional activities OR Specialized resources not indicated	Disability causing greater than minimal interference with usual social & functional activities OR Specialized resources on part-time basis indicated	Disability causing inability to perform usual social & functional activities OR Specialized resources on a full-time basis indicated	Disability causing inability to perform basic self-care functions OR Institutionalization indicated
CNS ischemia (acute)	NA	NA	Transient ischemic attack	Cerebral vascular accident (CVA, stroke) with neurological deficit
Developmental delay – **Pediatric ≤ 16 years**	Mild developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Moderate developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Severe developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Developmental regression, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting
Headache	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions OR Hospitalization indicated (other than emergency room visit) OR Headache with significant impairment of alertness or other neurologic function
Insomnia	NA	Difficulty sleeping causing greater than minimal interference with usual social & functional activities	Difficulty sleeping causing inability to perform usual social & functional activities	Disabling insomnia causing inability to perform basic self-care functions
Neuromuscular weakness (including myopathy & neuropathy)	Asymptomatic with decreased strength on exam OR Minimal muscle weakness causing no or minimal interference with usual social & functional activities	Muscle weakness causing greater than minimal interference with usual social & functional activities	Muscle weakness causing inability to perform usual social & functional activities	Disabling muscle weakness causing inability to perform basic self-care functions OR Respiratory muscle weakness impairing ventilation

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

CLINICAL				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Neurosensory alteration (including paresthesia and painful neuropathy)	Asymptomatic with sensory alteration on exam or minimal paresthesia causing no or minimal interference with usual social & functional activities	Sensory alteration or paresthesia causing greater than minimal interference with usual social & functional activities	Sensory alteration or paresthesia causing inability to perform usual social & functional activities	Disabling sensory alteration or paresthesia causing inability to perform basic self-care functions
Seizure: (new onset) **– Adult ≥ 18 years** See also Seizure: (known pre-existing seizure disorder)	NA	1 seizure	2 **–** 4 seizures	Seizures of any kind which are prolonged, repetitive (e.g., status epilepticus), or difficult to control (e.g., refractory epilepsy)
Seizure: (known pre-existing seizure disorder) **– Adult ≥ 18 years** For worsening of existing epilepsy the grades should be based on an increase from previous level of control to any of these levels.	NA	Increased frequency of pre-existing seizures (non-repetitive) without change in seizure character OR Infrequent break-through seizures while on stable medication in a previously controlled seizure disorder	Change in seizure character from baseline either in duration or quality (e.g., severity or focality)	Seizures of any kind which are prolonged, repetitive (e.g., status epilepticus), or difficult to control (e.g., refractory epilepsy)
Seizure **– Pediatric < 18 years**	Seizure, generalized onset with or without secondary generalization, lasting < 5 minutes with < 24 hours post ictal state	Seizure, generalized onset with or without secondary generalization, lasting 5 – 20 minutes with < 24 hours post ictal state	Seizure, generalized onset with or without secondary generalization, lasting > 20 minutes	Seizure, generalized onset with or without secondary generalization, requiring intubation and sedation
Syncope (not associated with a procedure)	NA	Present	NA	NA
Vertigo	Vertigo causing no or minimal interference with usual social & functional activities	Vertigo causing greater than minimal interference with usual social & functional activities	Vertigo causing inability to perform usual social & functional activities	Disabling vertigo causing inability to perform basic self-care functions

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

CLINICAL				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
RESPIRATORY				
Bronchospasm (acute)	FEV1 or peak flow reduced to 70 – 80%	FEV1 or peak flow 50 – 69%	FEV1 or peak flow 25 – 49%	Cyanosis OR FEV1 or peak flow < 25% OR Intubation
Dyspnea or respiratory distress				
Adult ≥ 14 years	Dyspnea on exertion with no or minimal interference with usual social & functional activities	Dyspnea on exertion causing greater than minimal interference with usual social & functional activities	Dyspnea at rest causing inability to perform usual social & functional activities	Respiratory failure with ventilatory support indicated
Pediatric < 14 years	Wheezing OR minimal increase in respiratory rate for age	Nasal flaring OR Intercostal retractions OR Pulse oximetry 90 – 95%	Dyspnea at rest causing inability to perform usual social & functional activities OR Pulse oximetry < 90%	Respiratory failure with ventilatory support indicated
MUSCULOSKELETAL				
Arthralgia See also Arthritis	Joint pain causing no or minimal interference with usual social & functional activities	Joint pain causing greater than minimal interference with usual social & functional activities	Joint pain causing inability to perform usual social & functional activities	Disabling joint pain causing inability to perform basic self-care functions
Arthritis See also Arthralgia	Stiffness or joint swelling causing no or minimal interference with usual social & functional activities	Stiffness or joint swelling causing greater than minimal interference with usual social & functional activities	Stiffness or joint swelling causing inability to perform usual social & functional activities	Disabling joint stiffness or swelling causing inability to perform basic self-care functions
Bone Mineral Loss				
Adult ≥ 21 years	BMD t-score -2.5 to -1.0	BMD t-score < -2.5	Pathological fracture (including loss of vertebral height)	Pathologic fracture causing life-threatening consequences
Pediatric < 21 years	BMD z-score -2.5 to -1.0	BMD z-score < -2.5	Pathological fracture (including loss of vertebral height)	Pathologic fracture causing life-threatening consequences
Myalgia (non-injection site)	Muscle pain causing no or minimal interference with usual social & functional activities	Muscle pain causing greater than minimal interference with usual social & functional activities	Muscle pain causing inability to perform usual social & functional activities	Disabling muscle pain causing inability to perform basic self-care functions

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

CLINICAL				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Osteonecrosis	NA	Asymptomatic with radiographic findings AND No operative intervention indicated	Symptomatic bone pain with radiographic findings OR Operative intervention indicated	Disabling bone pain with radiographic findings causing inability to perform basic self-care functions
GENITOURINARY				
Cervicitis (symptoms) (For use in studies evaluating topical study agents) For other cervicitis see Infection: Infection (any other than HIV infection)	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions
Cervicitis (clinical exam) (For use in studies evaluating topical study agents) For other cervicitis see Infection: Infection (any other than HIV infection)	Minimal cervical abnormalities on examination (erythema, mucopurulent discharge, or friability) OR Epithelial disruption < 25% of total surface	Moderate cervical abnormalities on examination (erythema, mucopurulent discharge, or friability) OR Epithelial disruption of 25 – 49% total surface	Severe cervical abnormalities on examination (erythema, mucopurulent discharge, or friability) OR Epithelial disruption 50 – 75% total surface	Epithelial disruption > 75% total surface
Inter-menstrual bleeding (IMB)	Spotting observed by participant OR Minimal blood observed during clinical or colposcopic examination	Inter-menstrual bleeding not greater in duration or amount than usual menstrual cycle	Inter-menstrual bleeding greater in duration or amount than usual menstrual cycle	Hemorrhage with life-threatening hypotension OR Operative intervention indicated
Urinary tract obstruction (e.g., stone)	NA	Signs or symptoms of urinary tract obstruction without hydronephrosis or renal dysfunction	Signs or symptoms of urinary tract obstruction with hydronephrosis or renal dysfunction	Obstruction causing life-threatening consequences

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

CLINICAL				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Vulvovaginitis (symptoms) (Use in studies evaluating topical study agents) For other vulvovaginitis see Infection: Infection (any other than HIV infection)	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions
Vulvovaginitis (clinical exam) (Use in studies evaluating topical study agents) For other vulvovaginitis see Infection: Infection (any other than HIV infection)	Minimal vaginal abnormalities on examination OR Epithelial disruption < 25% of total surface	Moderate vaginal abnormalities on examination OR Epithelial disruption of 25 - 49% total surface	Severe vaginal abnormalities on examination OR Epithelial disruption 50 - 75% total surface	Vaginal perforation OR Epithelial disruption > 75% total surface
OCULAR/VISUAL				
Uveitis	Asymptomatic but detectable on exam	Symptomatic anterior uveitis OR Medical intervention indicated	Posterior or pan-uveitis OR Operative intervention indicated	Disabling visual loss in affected eye(s)
Visual changes (from baseline)	Visual changes causing no or minimal interference with usual social & functional activities	Visual changes causing greater than minimal interference with usual social & functional activities	Visual changes causing inability to perform usual social & functional activities	Disabling visual loss in affected eye(s)
ENDOCRINE/METABOLIC				
Abnormal fat accumulation (e.g., back of neck, breasts, abdomen)	Detectable by study participant (or by caregiver for young children and disabled adults)	Detectable on physical exam by health care provider	Disfiguring OR Obvious changes on casual visual inspection	NA
Diabetes mellitus	NA	New onset without need to initiate medication OR Modification of current medications to regain glucose control	New onset with initiation of medication indicated OR Diabetes uncontrolled despite treatment modification	Life-threatening consequences (e.g., ketoacidosis, hyperosmolar non-ketotic coma)

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

CLINICAL				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Gynecomastia	Detectable by study participant or caregiver (for young children and disabled adults)	Detectable on physical exam by health care provider	Disfiguring OR Obvious on casual visual inspection	NA
Hyperthyroidism	Asymptomatic	Symptomatic causing greater than minimal interference with usual social & functional activities OR Thyroid suppression therapy indicated	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment modification	Life-threatening consequences (e.g., thyroid storm)
Hypothyroidism	Asymptomatic	Symptomatic causing greater than minimal interference with usual social & functional activities OR Thyroid replacement therapy indicated	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment modification	Life-threatening consequences (e.g., myxedema coma)
Lipoatrophy (e.g., fat loss from the face, extremities, buttocks)	Detectable by study participant (or by caregiver for young children and disabled adults)	Detectable on physical exam by health care provider	Disfiguring OR Obvious on casual visual inspection	NA

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
HEMATOLOGY	***Standard International Units are listed in italics***			
Absolute CD4+ count **– Adult and Pediatric > 13 years** (HIV NEGATIVE ONLY)	300 – 400/mm^3 *300 – 400/µL*	200 – 299/mm^3 *200 – 299/µL*	100 – 199/mm^3 *100 – 199/µL*	< 100/mm^3 *< 100/µL*
Absolute lymphocyte count **– Adult and Pediatric > 13 years** (HIV NEGATIVE ONLY)	600 – 650/mm^3 *0.600 x 10^9 – 0.650 x 10^9/L*	500 – 599/mm^3 *0.500 x 10^9 – 0.599 x 10^9/L*	350 – 499/mm^3 *0.350 x 10^9 – 0.499 x 10^9/L*	< 350/mm^3 *< 0.350 x 10^9/L*
Absolute neutrophil count (ANC)				
Adult and Pediatric, > 7 days	1,000 – 1,300/mm^3 *1.000 x 10^9 – 1.300 x 10^9/L*	750 – 999/mm^3 *0.750 x 10^9 – 0.999 x 10^9/L*	500 – 749/mm^3 *0.500 x 10^9 – 0.749 x 10^9/L*	< 500/mm^3 *< 0.500 x 10^9/L*
Infant*†, 2 – ≤ 7 days	1,250 – 1,500/mm^3 *1.250 x 10^9 – 1.500 x 10^9/L*	1,000 – 1,249/mm^3 *1.000 x 10^9 – 1.249 x 10^9/L*	750 – 999/mm^3 *0.750 x 10^9 – 0.999 x 10^9/L*	< 750/mm^3 *< 0.750 x 10^9/L*
Infant*†, 1 day	4,000 – 5,000/mm^3 *4.000 x 10^9 – 5.000 x 10^9/L*	3,000 – 3,999/mm^3 *3.000 x 10^9 – 3.999 x10^9/L*	1,500 – 2,999/mm^3 *1.500 x 10^9 – 2.999 x 10^9/L*	< 1,500/mm^3 *< 1.500 x 10^9/L*
Fibrinogen, decreased	100 – 200 mg/dL *1.00 – 2.00 g/L* OR 0.75 – 0.99 x LLN	75 – 99 mg/dL *0.75 – 0.99 g/L* OR 0.50 – 0.74 x LLN	50 – 74 mg/dL *0.50 – 0.74 g/L* OR 0.25 – 0.49 x LLN	< 50 mg/dL *< 0.50 g/L* OR < 0.25 x LLN OR Associated with gross bleeding
Hemoglobin (Hgb)				
Adult and Pediatric ≥ 57 days (HIV POSITIVE ONLY)	8.5 – 10.0 g/dL *1.32 – 1.55 mmol/L*	7.5 – 8.4 g/dL *1.16 – 1.31 mmol/L*	6.50 – 7.4 g/dL *1.01 – 1.15 mmol/L*	< 6.5 g/dL *< 1.01 mmol/L*
Adult and Pediatric ≥ 57 days (HIV NEGATIVE ONLY)	10.0 – 10.9 g/dL *1.55 – 1.69 mmol/L* OR Any decrease 2.5 – 3.4 g/dL *0.39 – 0.53 mmol/L*	9.0 – 9.9 g/dL *1.40 – 1.54 mmol/L* OR Any decrease 3.5 – 4.4 g/dL *0.54 – 0.68 mmol/L*	7.0 – 8.9 g/dL *1.09 – 1.39 mmol/L* OR Any decrease ≥ 4.5 g/dL *≥ 0.69 mmol/L*	< 7.0 g/dL *< 1.09 mmol/L*
Infant*†, 36 – 56 days (HIV POSITIVE OR NEGATIVE)	8.5 – 9.4 g/dL *1.32 – 1.46 mmol/L*	7.0 – 8.4 g/dL *1.09 – 1.31 mmol/L*	6.0 – 6.9 g/dL *0.93 – 1.08 mmol/L*	< 6.00 g/dL *< 0.93 mmol/L*
Infant*†, 22 – 35 days (HIV POSITIVE OR NEGATIVE)	9.5 – 10.5 g/dL *1.47 – 1.63 mmol/L*	8.0 – 9.4 g/dL *1.24 – 1.46 mmol/L*	7.0 – 7.9 g/dL *1.09 – 1.23 mmol/L*	< 7.00 g/dL *< 1.09 mmol/L*

LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Infant*†, 1 – 21 days (HIV POSITIVE OR NEGATIVE)	12.0 – 13.0 g/dL *1.86 – 2.02 mmol/L*	10.0 – 11.9 g/dL *1.55 – 1.85 mmol/L*	9.0 – 9.9 g/dL *1.40 – 1.54 mmol/L*	< 9.0 g/dL *< 1.40 mmol/L*
International Normalized Ratio of prothrombin time (INR)	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 3.0 x ULN	> 3.0 x ULN
Methemoglobin	5.0 – 10.0%	10.1 – 15.0%	15.1 – 20.0%	> 20.0%
Prothrombin Time (PT)	1.1 – 1.25 x ULN	1.26 – 1.50 x ULN	1.51 – 3.00 x ULN	> 3.00 x ULN
Partial Thromboplastin Time (PTT)	1.1 – 1.66 x ULN	1.67 – 2.33 x ULN	2.34 – 3.00 x ULN	> 3.00 x ULN
Platelets, decreased	100,000 – 124,999/mm^3 *100.000 x 10^9 – 124.999 x 10^9/L*	50,000 – 99,999/mm^3 *50.000 x 10^9 – 99.999 x 10^9/L*	25,000 – 49,999/mm^3 *25.000 x 10^9 – 49.999 x 10^9/L*	< 25,000/mm^3 *< 25.000 x 10^9/L*
WBC, decreased	2,000 – 2,500/mm^3 *2.000 x 10^9 – 2.500 x 10^9/L*	1,500 – 1,999/mm^3 *1.500 x 10^9 – 1.999 x 10^9/L*	1,000 – 1,499/mm^3 *1.000 x 10^9 – 1.499 x 10^9/L*	< 1,000/mm^3 *< 1.000 x 10^9/L*
CHEMISTRIES	***Standard International Units are listed in italics***			
Acidosis	NA	pH < normal, but ≥ 7.3	pH < 7.3 without life-threatening consequences	pH < 7.3 with life-threatening consequences
Albumin, serum, low	3.0 g/dL – < LLN *30 g/L – < LLN*	2.0 – 2.9 g/dL *20 – 29 g/L*	< 2.0 g/dL *< 20 g/L*	NA
Alkaline Phosphatase	1.25 – 2.5 x ULN†	2.6 – 5.0 x ULN†	5.1 – 10.0 x ULN†	> 10.0 x ULN†
Alkalosis	NA	pH > normal, but ≤ 7.5	pH > 7.5 without life-threatening consequences	pH > 7.5 with life-threatening consequences
ALT (SGPT)	1.25 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10.0 x ULN	> 10.0 x ULN
AST (SGOT)	1.25 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10.0 x ULN	> 10.0 x ULN
Bicarbonate, serum, low	16.0 mEq/L – < LLN *16.0 mmol/L – < LLN*	11.0 – 15.9 mEq/L *11.0 – 15.9 mmol/L*	8.0 – 10.9 mEq/L *8.0 – 10.9 mmol/L*	< 8.0 mEq/L *< 8.0 mmol/L*
Bilirubin (Total)				
Adult and Pediatric > 14 days	1.1 – 1.5 x ULN	1.6 – 2.5 x ULN	2.6 – 5.0 x ULN	> 5.0 x ULN
Infant*†, ≤ 14 days (non-hemolytic)	NA	20.0 – 25.0 mg/dL *342 – 428 µmol/L*	25.1 – 30.0 mg/dL *429 – 513 µmol/L*	> 30.0 mg/dL *> 513.0 µmol/L*
Infant*†, ≤ 14 days (hemolytic)	NA	NA	20.0 – 25.0 mg/dL *342 – 428 µmol/L*	> 25.0 mg/dL *> 428 µmol/L*
Calcium, serum, high (corrected for albumin)				

LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Adult and Pediatric ≥ 7 days	10.6 – 11.5 mg/dL *2.65 – 2.88 mmol/L*	11.6 – 12.5 mg/dL *2.89 – 3.13 mmol/L*	12.6 – 13.5 mg/dL *3.14 – 3.38 mmol/L*	> 13.5 mg/dL *> 3.38 mmol/L*
Infant*†, < 7 days	11.5 – 12.4 mg/dL *2.88 – 3.10 mmol/L*	12.5 – 12.9 mg/dL *3.11 – 3.23 mmol/L*	13.0 – 13.5 mg/dL *3.245 – 3.38 mmol/L*	> 13.5 mg/dL *> 3.38 mmol/L*
Calcium, serum, low (corrected for albumin)				
Adult and Pediatric ≥ 7 days	7.8 – 8.4 mg/dL *1.95 – 2.10 mmol/L*	7.0 – 7.7 mg/dL *1.75 – 1.94 mmol/L*	6.1 – 6.9 mg/dL *1.53 – 1.74 mmol/L*	< 6.1 mg/dL *< 1.53 mmol/L*
Infant*†, < 7 days	6.5 – 7.5 mg/dL *1.63 – 1.88 mmol/L*	6.0 – 6.4 mg/dL *1.50 – 1.62 mmol/L*	5.50 – 5.90 mg/dL *1.38 – 1.51 mmol/L*	< 5.50 mg/dL *< 1.38 mmol/L*
Cardiac troponin I (cTnI)	NA	NA	NA	Levels consistent with myocardial infarction or unstable angina as defined by the manufacturer
Cardiac troponin T (cTnT)	NA	NA	NA	≥ 0.20 ng/mL OR Levels consistent with myocardial infarction or unstable angina as defined by the manufacturer
Cholesterol (fasting)				
Adult ≥ 18 years	200 – 239 mg/dL *5.18 – 6.19 mmol/L*	240 – 300 mg/dL *6.20 – 7.77 mmol/L*	> 300 mg/dL *> 7.77 mmol/L*	NA
Pediatric < 18 years	170 – 199 mg/dL *4.40 – 5.15 mmol/L*	200 – 300 mg/dL *5.16 – 7.77 mmol/L*	> 300 mg/dL *> 7.77 mmol/L*	NA
Creatine Kinase	3.0 – 5.9 x ULN†	6.0 – 9.9 x ULN†	10.0 – 19.9 x ULN†	≥ 20.0 x ULN†
Creatinine	1.1 – 1.3 x ULN†	1.4 – 1.8 x ULN†	1.9 – 3.4 x ULN†	≥ 3.5 x ULN†
Glucose, serum, high				
Nonfasting	116 – 160 mg/dL *6.44 – 8.88 mmol/L*	161 – 250 mg/dL *8.89 – 13.88 mmol/L*	251 – 500 mg/dL *13.89 – 27.75 mmol/L*	> 500 mg/dL *> 27.75 mmol/L*
Fasting	110 – 125 mg/dL *6.11 – 6.94 mmol/L*	126 – 250 mg/dL *6.95 – 13.88 mmol/L*	251 – 500 mg/dL *13.89 – 27.75 mmol/L*	> 500 mg/dL *> 27.75 mmol/L*
Glucose, serum, low				
Adult and Pediatric ≥ 1 month	55 – 64 mg/dL *3.05 – 3.55 mmol/L*	40 – 54 mg/dL *2.22 – 3.06 mmol/L*	30 – 39 mg/dL *1.67 – 2.23 mmol/L*	< 30 mg/dL *< 1.67 mmol/L*
Infant*†, < 1 month	50 – 54 mg/dL *2.78 – 3.00 mmol/L*	40 – 49 mg/dL *2.22 – 2.77 mmol/L*	30 – 39 mg/dL *1.67 – 2.21 mmol/L*	< 30 mg/dL *< 1.67 mmol/L*

LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Lactate	< 2.0 x ULN without acidosis	≥ 2.0 x ULN without acidosis	Increased lactate with pH < 7.3 without life-threatening consequences	Increased lactate with pH < 7.3 with life-threatening consequences
LDL cholesterol (fasting)				
Adult ≥ 18 years	130 – 159 mg/dL *3.37 – 4.12 mmol/L*	160 – 190 mg/dL *4.13 – 4.90 mmol/L*	≥ 190 mg/dL *≥ 4.91 mmol/L*	NA
Pediatric > 2 - < 18 years	110 – 129 mg/dL *2.85 – 3.34 mmol/L*	130 – 189 mg/dL *3.35 – 4.90 mmol/L*	≥ 190 mg/dL *≥ 4.91 mmol/L*	NA
Lipase	1.1 – 1.5 x ULN	1.6 – 3.0 x ULN	3.1 – 5.0 x ULN	> 5.0 x ULN
Magnesium, serum, low	1.2 – 1.4 mEq/L *0.60 – 0.70 mmol/L*	0.9 – 1.1 mEq/L *0.45 – 0.59 mmol/L*	0.6 – 0.8 mEq/L *0.30 – 0.44 mmol/L*	< 0.60 mEq/L *< 0.30 mmol/L*
Pancreatic amylase	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	> 5.0 x ULN
Phosphate, serum, low				
Adult and Pediatric > 14 years	2.5 mg/dL – < LLN *0.81 mmol/L – < LLN*	2.0 – 2.4 mg/dL *0.65 – 0.80 mmol/L*	1.0 – 1.9 mg/dL *0.32 – 0.64 mmol/L*	< 1.00 mg/dL *< 0.32 mmol/L*
Pediatric 1 year – 14 years	3.0 – 3.5 mg/dL *0.97 – 1.13 mmol/L*	2.5 – 2.9 mg/dL *0.81 – 0.96 mmol/L*	1.5 – 2.4 mg/dL *0.48 – 0.80 mmol/L*	< 1.50 mg/dL *< 0.48 mmol/L*
Pediatric < 1 year	3.5 – 4.5 mg/dL *1.13 – 1.45 mmol/L*	2.5 – 3.4 mg/dL *0.81 – 1.12 mmol/L*	1.5 – 2.4 mg/dL *0.48 – 0.80 mmol/L*	< 1.50 mg/dL *< 0.48 mmol/L*
Potassium, serum, high	5.6 – 6.0 mEq/L *5.6 – 6.0 mmol/L*	6.1 – 6.5 mEq/L *6.1 – 6.5 mmol/L*	6.6 – 7.0 mEq/L *6.6 – 7.0 mmol/L*	> 7.0 mEq/L *> 7.0 mmol/L*
Potassium, serum, low	3.0 – 3.4 mEq/L *3.0 – 3.4 mmol/L*	2.5 – 2.9 mEq/L *2.5 – 2.9 mmol/L*	2.0 – 2.4 mEq/L *2.0 – 2.4 mmol/L*	< 2.0 mEq/L *< 2.0 mmol/L*
Sodium, serum, high	146 – 150 mEq/L *146 – 150 mmol/L*	151 – 154 mEq/L *151 – 154 mmol/L*	155 – 159 mEq/L *155 – 159 mmol/L*	≥ 160 mEq/L *≥ 160 mmol/L*
Sodium, serum, low	130 – 135 mEq/L *130 – 135 mmol/L*	125 – 129 mEq/L *125 – 129 mmol/L*	121 – 124 mEq/L *121 – 124 mmol/L*	≤ 120 mEq/L *≤ 120 mmol/L*
Triglycerides (fasting)	NA	500 – 750 mg/dL *5.65 – 8.48 mmol/L*	751 – 1,200 mg/dL *8.49 – 13.56 mmol/L*	> 1,200 mg/dL *> 13.56 mmol/L*
Uric acid	7.5 – 10.0 mg/dL *0.45 – 0.59 mmol/L*	10.1 – 12.0 mg/dL *0.60 – 0.71 mmol/L*	12.1 – 15.0 mg/dL *0.72 – 0.89 mmol/L*	> 15.0 mg/dL *> 0.89 mmol/L*
URINALYSIS	***Standard International Units are listed in italics***			
Hematuria (microscopic)	6 – 10 RBC/HPF	> 10 RBC/HPF	Gross, with or without clots OR with RBC casts	Transfusion indicated
Proteinuria, random collection	1 +	2 – 3 +	4 +	NA
Proteinuria, 24 hour collection				

LABORATORY					
PARAMETER		**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
	Adult and Pediatric ≥ 10 years	200 – 999 mg/24 h *0.200 – 0.999 g/d*	1,000 – 1,999 mg/24 h *1.000 – 1.999 g/d*	2,000 – 3,500 mg/24 h *2.000 – 3.500 g/d*	> 3,500 mg/24 h *> 3.500 g/d*
	Pediatric > 3 mo - < 10 years	201 – 499 mg/m^2/24 h *0.201 – 0.499 g/d*	500 – 799 mg/m^2/24 h *0.500 – 0.799 g/d*	800 – 1,000 mg/m^2/24 h *0.800 – 1.000 g/d*	> 1,000 mg/ m^2/24 h *> 1.000 g/d*

APPENDIX V: PROTOCOL SPECIFIC TOXICITY TABLE

Female Genital Grading Table for Use in Microbicide Studies

INDIVIDUAL SIGNS/SYMPTOMS					
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
GENERAL					
Odor	No complaint	Mild-moderate unpleasant odor	Severe unpleasant odor	NA	NA
PAIN AND TENDERNESS **(Specify Area: Vulvar/Perineum, Vagina, Cervix (including cervical motion tenderness), Uterus, Adnexae, Pelvic/Lower Abdominal, or Ovulatory)** ***Note – if both pain and tenderness are present, only report the one with the most severe grade**					
Pain* [1]	None	Pain causing no or minimal interference with usual social & functional activities	Pain causing greater than minimal interference with usual social & functional activities or the need for non-narcotic medication	Pain causing inability to perform usual social & functional activities or the need for narcotic medication	Disabling pain causing inability to perform basic self-care functions OR hospitalization (other than emergency room visit) indicated
Tenderness* [1]	None	Mild tenderness	Moderate tenderness	Severe tenderness	NA
Dyspareunia (pain with sexual activity)	None	Pain causing no or minimal interference with sexual function	Pain causing greater than minimal interference with sexual function	NA	NA
Dysmenorrhea/cramping with menses	None	Pain causing no or minimal interference with usual social & functional activities	Pain causing greater than minimal interference with usual social & functional activities or the need for non-narcotic medication	Pain causing inability to perform usual social or functional activities or the need for narcotic medication	NA

[1] If pain or tenderness is included in the grading of another category (e.g., PID), it should not be graded again in the pain or tenderness category.

Female Genital Grading Table for Use in Microbicide Studies

INDIVIDUAL SIGNS/SYMPTOMS					
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
GENITOURINARY SIGNS/SYMPTOMS – VULVA					
Vulvar/vaginal itching	None	Itching causing no, mild, or moderate interference with usual social & functional activities	Itching causing inability to perform usual social & functional activities; may require intervention such as antihistamine or bathing to provide relief	NA	NA
Vulvar edema	None	Mild, non-pitting edema	Moderate, 1-2+ pitting edema	3+ pitting edema, severe enough to require urinary drainage, or weeping edema ± skin breakdown	NA
Vulvar erythema	None	Erythema covering < 50% of vulvar surface	Erythema covering ≥ 50% of vulvar surface	NA	NA
Vulvar lesions (findings seen only by colposcopy should not be included here)	Normal variants including skin tags, moles, scars, etc.	Blisters, ulcerations, or pustules - no treatment indicated	Blisters, ulcerations or pustules, with treatment indicated	Severe epithelial disruption with hospitalization indicated	NA
Vulvar rash	None	Rash covering < 50% of vulvar surface	Rash covering ≥ 50% of vulvar surface	Severe epithelial disruption with hospitalization indicated	NA
Bartholin's or Skene's gland	No findings	Cyst with no inflammation	Cyst or abscess with outpatient intervention indicated	Cyst or abscess with hospitalization indicated	Necrotizing fasciitis from Bartholin's abscess
GENITOURINARY SIGNS/SYMPTOMS – VAGINA **** Note – if vaginal discharge is present both by history and on examination, only report the one with the most severe grade**					
Vaginal edema	None	Mild-moderate engorgement	Loss of ruggae and friability	NA	NA

Female Genital Grading Table for Use in Microbicide Studies

INDIVIDUAL SIGNS/SYMPTOMS					
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Vaginal erythema	None	Erythema covering < 50% of vaginal surface	Erythema covering ≥ 50% of vaginal surface	NA	NA
Vaginal dryness	No complaint	Dryness causing no or minimal interference with usual sexual, social, & functional activities	Dryness causing greater than minimal interference with usual sexual, social, & functional activities	NA	NA
Vaginal discharge by participant report **	Participant's usual amount of discharge, regardless of color or quantity	Mild-moderate increase in amount above participant baseline - no sanitary protection required	Profuse increase in discharge requiring pad use or other hygienic intervention	NA	NA
Vaginal discharge as observed by clinician ** (red or brown discharge should be reported under bleeding, not discharge)	Slight amount of discharge, any color	Mild-moderate increase in amount	Significant increase in amount with pooling in vagina on examination	NA	NA
Vaginal abrasions or lacerations (including probable applicator injuries)	None	Superficial disruptions and disruptions extending through the mucosa with minimal impact on life	Large disruptions extending through the mucosa or large superficial disruptions, hospitalization not indicated	Large disruptions extending through the mucosa or large superficial disruptions, hospitalization indicated	Lacerations extending into the peritoneal cavity, bladder, or rectum
Vaginal lesions (findings seen only by colposcopy should not be included here)	Normal variants including skin tags, moles, scars, etc.	Blisters, ulcerations, or pustules, no treatment indicated	Blisters, ulcerations, or pustules with treatment indicated	Severe epithelial disruption requiring hospitalization	NA
Vaginal and Cervical masses (polyps, myomas, or possible malignancy)	None or normal variants such as Nabothian cyst or Gartner duct cyst	Polyp or myoma or undiagnosed mass without symptoms	Polyp, myoma, or undiagnosed mass causing mild symptoms, e.g., bleeding/pain not requiring more than mild analgesia	Polyp, myoma, or undiagnosed mass causing severe symptoms, e.g., bleeding/pain affecting bladder and bowel function	Visible cervical cancer

Female Genital Grading Table for Use in Microbicide Studies

INDIVIDUAL SIGNS/SYMPTOMS					
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
GENITOURINARY SIGNS/SYMPTOMS – CERVIX					
Cervical edema and friability	None	Edema without friability	Friable cervix	NA	NA
Cervical erythema	None	Erythema covering < 50% of cervix	Erythema covering ≥ 50% of cervix	NA	NA
Cervical discharge	White or clear discharge	Small amount of purulent discharge at os	Purulent discharge extending onto cervix or vagina	NA	NA
Visible cervical lesions (findings seen only by colposcopy should not be included here)	Normal variants including skin tags, moles, scars, etc.	Blisters, ulcerations, or pustules, no treatment indicated	Blisters, ulcerations, or pustules with treatment indicated	NA	NA
GENITOURINARY SIGNS/SYMPTOMS – UTERUS					
Uterine masses/enlargement based on bimanual examination	Normal to 8 week size, no palpable myomas	Enlarged uterus and mild symptoms, e.g., bleeding/pain requiring mild analgesics	Enlarged uterus/myoma with moderate pain or symptoms, e.g., bleeding	Mass causing severe bleeding/pain or with impact on bowel/bladder function	Uterine mass that requires transfusion or surgery
Polyp, submucosal fibroid, or thickened endometrium detected by transvaginal ultrasound (new or increasing in size from prior exam)	None or unchanged/reduced in size from prior exam	New myomas < 6 cm diameter (single or multiple) or diameter increased < 6 cm since prior exam	New myomas ≥ 6 cm diameter (single or multiple) or diameter increased ≥ 6 cm since prior exam	Hospitalization and/or surgery indicated	NA
GENITOURINARY SIGNS/SYMPTOMS – ADNEXA					
Not pregnancy- or infection-related adnexal masses based on bimanual exam (use if no ultrasound done; if ultrasound done, use ultrasound categories below)	None, ≤ 4 cm, normal size ovary	> 4 cm with minimal or no symptoms	> 4 cm with severe symptoms, e.g., pain, but hospitalization not indicated (see footnote #1)	> 4 cm with severe symptoms, e.g., pain and hospitalization indicated (see footnote #1)	NA

Female Genital Grading Table for Use in Microbicide Studies

INDIVIDUAL SIGNS/SYMPTOMS					
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Hydrosalpinx based on ultrasound	None	Asymptomatic, suspected hydrosalpinx	Hydrosalpinx with pain, but without evidence of infection or ectopic pregnancy	Signs/symptoms of infection with hospitalization and/or surgery indicated	NA
Adnexal mass based on ultrasound	None	Simple cyst, asymptomatic	Simple cyst, symptomatic	Mass suspicious for malignancy	Malignant mass
GENITOURINARY SIGNS/SYMPTOMS – ABDOMEN					
Abdominal mass not palpable on pelvic exam of unknown diagnosis	None or known (pre-existing) mass unchanged in size	New mass or increased size of known mass requiring mild analgesia with minimal impact	New mass or increased size of known mass with moderate symptoms	Mass causing severe bleeding/ pain with impact on bladder/bowel function or with hospitalization indicated	Malignancy
GENITOURINARY SIGNS/SYMPTOMS – URINARY TRACT					
Urinary frequency	None	Up to 2 times participant's normal frequency	> 2 times participant's normal frequency	NA	NA
Dysuria	None	Superficial only	Deep ± superficial	Inability to void due to pain	NA
Hematuria	None	Microscopic, no intervention indicated (beyond evaluation for infection)	Gross blood in urine or medical intervention/ evaluation indicated (beyond evaluation for infection)	Persistent bleeding with transfusion, hospitalization or intervention indicated to obtain hemostasis (endoscopy, interventional radiology, or operative)	Profuse hemorrhage with shock or orthostatic dizziness

Female Genital Grading Table for Use in Microbicide Studies

COMPOSITE SIGNS/SYMPTOMS (Use instead of individual categories if 2 or more signs/symptoms are present)					
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD** (Use if all signs/ symptoms would individually be Grade 0 or 1)	**GRADE 2 MODERATE** (Use if one or more signs/symptoms would individually be Grade 2 and all others Grade 0 or 1)	**GRADE 3 SEVERE** (Use if one or more signs/symptoms would individually be Grade 3)	**GRADE 4 POTENTIALLY LIFE-THREATENING**
NO ORGANISM IDENTIFIED BUT INADEQUATE TESTING PERFORMED					
Vulvovaginitis (combinations of pain, itching, erythema, edema, rash, tenderness, or discharge)	None	Mild signs/ symptoms	Moderate signs/ symptoms	Severe signs/ symptoms	NA
Cervicitis (combinations of dyspareunia, erythema, edema, tenderness, and discharge)	None	Mild signs/ symptoms	Moderate signs/ symptoms	Severe signs/ symptoms	NA
PID (if Gonorrhea or Chlamydia identified use that category)	None	NA	Cervicitis with mild uterine tenderness, ± mild cervical motion tenderness, no signs of peritoneal irritation	More diffuse tenderness, any signs of peritoneal irritation, or indications for hospitalization	Tubo-ovarian abscess or surgery required for resolution
NO ORGANISM IDENTIFIED AFTER APPROPRIATE TESTING PERFORMED					
Vulvovaginitis (combinations of pain, itching, erythema, edema, rash, tenderness, or discharge)	None	Mild signs/ symptoms	Moderate signs/ symptoms	Severe signs/ symptoms	NA
Cervicitis (combinations of dyspareunia, erythema, edema, tenderness, and discharge)	None	Mild signs/ symptoms	Moderate signs/ symptoms	Severe signs/ symptoms	NA
PID (if Gonorrhea or Chlamydia identified use that category)	None	NA	Cervicitis with mild uterine tenderness, ± mild cervical motion tenderness, no signs of peritoneal irritation	More diffuse tenderness, any signs of peritoneal irritation, or indications for hospitalization	Tubo-ovarian abscess or surgery required for resolution

Female Genital Grading Table for Use in Microbicide Studies

INFECTIONS AND DYSPLASIA					
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
GENITOURINARY INFECTIONS					
Genital herpes	No lesions	Characteristic ulcerative or vesicular lesions confirmed by culture, PCR, Tzanck prep or other diagnostic test of lesion or previous type-specific serology, covering < 25% of vulva, vagina, or cervix	Same criteria as mild but covering 25-50% of vulvar, vaginal, or cervical surface	Same criteria as mild but covering > 50% of vulvar, vaginal, or cervical surface	Symptoms of significant systemic involvement, e.g., encephalitis, hepatitis
Candida	Absence of symptoms regardless of candida test results	Positive culture, wet mount, or other laboratory test for yeast, with mild symptoms	Positive culture, wet mount, or other laboratory test for yeast, with moderate to severe symptoms	NA	NA
Trichomonas	Negative	NA	Positive wet mount, culture, PCR or other licensed test, excluding pap smear, showing T. vaginalis, regardless of symptoms	NA	NA
Bacterial Vaginosis (BV)	Negative	Asymptomatic BV diagnosed by Amsel criteria, wet mount, Gram stain, or licensed diagnostic test	Symptomatic confirmed by wet mount, Gram stain, or any licensed diagnostic test	NA	NA

Female Genital Grading Table for Use in Microbicide Studies

INFECTIONS AND DYSPLASIA					
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Chlamydia	Negative	NA	Positive culture or other diagnostic test for Chlamydia, asymptomatic or with mild uterine or cervical motion tenderness (no signs of peritoneal irritation)	Positive test for Chlamydia with abdominal or uterine or adnexal tenderness on examination, with or without adnexal mass, diffuse tenderness, any signs of peritoneal irritation, or indications for hospitalization	Tubo-ovarian abscess or surgery required for resolution
Gonorrhea	Negative	NA	Positive culture or other diagnostic test for Gonorrhea, asymptomatic or with mild uterine or cervical motion tenderness (no signs of peritoneal irritation)	Positive test for Gonorrhea with abdominal or uterine or adnexal tenderness on examination, with or without adnexal mass, diffuse tenderness, any signs of peritoneal irritation, or indications for hospitalization	Tubo-ovarian abscess or surgery required for resolution or disseminated gonococcal infection
Urinary tract infection (by urinalysis and urine culture)	Negative	5-10 WBC/hpf on urinalysis with a negative culture per protocol definition (with or without symptoms)	> 10 WBC/hpf on urinalysis OR a positive culture per protocol definition (with or without symptoms)	Pyelonephritis	Sepsis (septicemia) due to urinary tract infection

Female Genital Grading Table for Use in Microbicide Studies

INFECTIONS AND DYSPLASIA					
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Syphilis	Negative treponemal or non-treponemal test or both positive with known treatment and stable titers (< 4 fold increase)	NA	Syphilis diagnosed by a positive treponemal test along with a positive non-treponemal test and no previous treatment or a four-fold rise in titer on the non-treponemal test after previous treatment regardless of symptoms or non-oral lesions positive by darkfield exam for treponemes	Criteria for Grade 2 Syphilis in the presence of neurologic symptoms or a positive CSF VDRL or FTA-ABS	NA
GENITAL DYSPLASIA					
Condyloma (specify site: cervical, vaginal, vulvar, perianal)	None	Condylomata causing no or mild interference with daily function	Condylomata causing moderate interference with daily function	Condylomata causing severe interference with daily function, secondary infection, or hospitalization indicated	NA
Intraepithelial Neoplasia by biopsy (VIN, CIN, VAIN)	None	Intraepithelial Neoplasia 1 (IN1)	Intraepithelial Neoplasia 2 (IN2)	Carcinoma in situ (CIS)	Invasive carcinoma
Pap (use this category only if treatment performed without diagnostic testing, otherwise use biopsy category above)	nl PAP	ASCUS or LSIL	HSIL	Carcinoma in situ or Carcinoma	NA

Female Genital Grading Table for Use in Microbicide Studies

UTERINE BLEEDING AND PREGNANCY COMPLICATIONS					
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
ABNORMAL UTERINE BLEEDING UNRELATED TO PREGNANCY					
Menorrhagia [2] (prolonged and/or heavy menstrual bleeding)	Participant report of normal bleeding relative to her baseline	Increase from usual with no or minimal interference with usual social & functional activities (including sexual functioning)	Increase from usual with moderate interference with usual social & functional activities (including sexual)	Incapacitating or severe interference with usual social & functional activities (including sexual functioning), transfusion indicated	Life threatening hemorrhage with or without shock
Metrorrhagia [2] (intermenstrual or frequent bleeding)	None or any expected nonmenstrual bleeding	Increase from usual with no or minimal interference with usual social & functional activities (including sexual functioning)	Increase from usual with moderate interference with usual social & functional activities (including sexual)	Incapacitating or severe interference with usual social & functional activities (including sexual functioning), transfusion indicated	Life threatening hemorrhage with or without shock
Unexplained infrequent bleeding (excludes expected absence of menses due to hormonal contraception or pregnancy/postpartum)	Participant report of normal or expected bleeding frequency	No menses for 1-3 months (missed menses)	No menses for > 3 months (oligomenorrhea/ amenorrhea)	NA	NA
Postcoital bleeding	None	Occasional (< 25% of coital acts) OR Increase from usual with no or minimal interference with usual social functioning (including sexual functioning)	Frequent (25-75% of coital acts) OR Increase from usual with moderate interference with usual social functioning (including sexual)	Consistent (> 75% of coital acts) OR Incapacitating or severe interference with usual social functioning (including sexual functioning), transfusion indicated	Life threatening hemorrhage with or without shock

[2] If both Menorrhagia and Metrorrhagia are present, a single adverse event should be reported as "Menometrorrhagia" and graded per the Menorrhagia grading scale.

Female Genital Grading Table for Use in Microbicide Studies

UTERINE BLEEDING AND PREGNANCY COMPLICATIONS					
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
COMPLICATIONS OF PREGNANCY					
First trimester bleeding	None	Spotting or bleeding less than menses with continuation of pregnancy	Bleeding like menses or heavier with continuation of pregnancy	Spontaneous abortion, or profuse bleeding with dizziness or orthostatic hypotension, transfusion indicated	Spontaneous abortion with profuse bleeding and/or shock
Postabortal endometritis/salpingitis	None	Low grade fever and uterine tenderness, resolved with oral antibiotics	Moderate symptoms, requiring ≤ 3 days of parenteral antibiotics	Severe symptoms requiring > 3 days of IV antibiotics or development of tubo-ovarian abscess	Ruptured TOA or diffuse peritonitis or severe uterine infection for which operative intervention indicated
Postpartum hemorrhage	EBL < 500 cc for vaginal delivery or < 1000 cc after CS or reported as normal	EBL 500-1000 for vaginal delivery or 1000-1500 for CS or reported as slightly increased	EBL > 1000 for vaginal delivery or > 1500 for CS, with or without mild dizziness, no transfusion required	Hemorrhage at a level for which transfusion of 1-2 units of packed cells, but no other blood products indicated	Hemorrhage with shock or coagulopathy, for which transfusion of > 2 units of packed cells or any amount of other blood components is indicated
Postpartum endometritis	None	Low grade fever and uterine tenderness, resolved with oral antibiotics	Moderate symptoms, treated by ≤ 3 days of parenteral antibiotics	Severe symptoms treated with > 3 days of IV antibiotics or addition of heparin	Severe infection or infection for which operative intervention is indicated
Chorioamnionitis	None	Fever (38°C – 38.4°C or 100.4°F – 100.9°F) with two or more: FHR > 160 BPM, maternal HR > 120, uterine tenderness between contractions or purulent AF or preterm labor	Same as Grade 1 plus fever 38.5°C – 40°C or 101°F – 104°F	Criteria for Grade 2 plus fetal distress or fever > 40°C or 104°F	Criteria for Grade 3 plus either fetal demise or maternal symptoms of shock

Female Genital Grading Table for Use in Microbicide Studies

UTERINE BLEEDING AND PREGNANCY COMPLICATIONS					
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Episiotomy infection	None	Mild erythema, edema, and tenderness of wound	Fever > 38°C or 100.4°F with erythema, edema, and tenderness of wound	Fever with wound dehiscence or debridement required	Fever with signs of wound infection and shock or necrotizing fasciitis
Second/third trimester bleeding	None	Bleeding less than menses	Bleeding like menses or greater, but not requiring intervention	Bleeding requiring delivery or other intervention, e.g., transfusion	Bleeding with fetal demise or coagulopathy
Preterm rupture of membranes	None	NA	Preterm rupture with hospitalization but not resulting in delivery at less than 37 weeks' gestation	Delivery at 33-36 weeks' gestation or 1501-2500 grams birth weight	Delivery < 33 weeks' gestation or ≤ 1500 grams birth weight
Preterm contractions	None	Preterm contractions which resolve without medical intervention	Preterm contractions with cervical change which result in medical intervention but not resulting in preterm delivery	Delivery at 33-36 weeks' gestation or 1501-2500 grams birth weight	Delivery < 33 weeks' gestation or ≤ 1500 grams birth weight
Poor fetal growth	At or above 10th percentile	Fetal growth < 10th percentile but ≥ 3rd percentile for gestational age by ultrasound or newborn exam	NA	Fetal growth < 3rd percentile for gestational age by ultrasound or newborn exam	NA

APPENDIX VI: SAMPLE INFORMED CONSENT FORM (SCREENING AND ENROLLMENT)

MTN-002
Phase I Study of the Maternal Single-dose Pharmacokinetics and Placental Transfer of Tenofovir 1% Vaginal Gel among Healthy Term Gravidas

Final Version 1.0
August 29, 2007

PRINCIPAL INVESTIGATOR: **Richard H. Beigi MD, MSc**
PHONE: **412-641-5403**
Short Title for the Study: **Maternal Tenofovir Gel PK Study**

Introduction

This is a study of pregnant women who have normal healthy pregnancies. We are testing a drug called tenofovir gel that in the future may be used to prevent the spread of human immunodeficiency virus (HIV), but this is not yet proven. HIV is the virus that causes AIDS. We are studying this gel in pregnant women to see if it is safe and to see where the drug that is in the gel goes (is found) in the body. You are being asked to be in this study because you have a healthy pregnancy and are planning to have cesarean delivery. This study is being paid for by the United States National Institutes of Health. The person in charge of this study is Richard H. Beigi, MD, MSc.

Before you decide whether to be in this study, we want to explain the purpose of the study, the risks and benefits, and what is expected of you. This consent form gives information that the study staff will discuss with you. You are free to ask questions at any time. If you agree to be in this study, you will be asked to sign this form. You will be offered a copy to keep.

Why Is This Study Being Done?

The main purpose of this study is to see if one dose (about one teaspoon) of study gel placed in the vagina gets absorbed into a pregnant woman's blood, placenta, uterus, baby's umbilical cord blood, and the amniotic fluid around the baby. This is important information to know because pregnant women may be at higher risk to get HIV if they are exposed to it and pregnant women may also want to use tenofovir gel if it is approved for use.

What Do I Have To Do If I Am In This Study?

The visits you will have in this study are described in more detail in other sections below. If you decide to be in this study, you will be asked to participate today and then at the time of your scheduled cesarean (no more than four weeks from now) and for 14 days after. On the day of your cesarean right before the surgery, you will have a single dose of the study gel placed in your vagina and will have your blood drawn. Then you will have seven more blood samples taken over the next 24 hours. During your

cesarean, you will also have a small sample of the amniotic fluid taken and a small sample of the lining of your womb taken to test to see if tenofovir is there. Blood from the umbilical cord and a piece of the placenta will be collected after the cord has been clamped and the placenta delivered. A study physician will examine you about 24 hours after you receive the study gel. You will then be called two weeks after your delivery to see how you are doing. We will also ask you to sign permission forms so that we can get copies of any hospital records for the time that you are in the study. We will also ask that you do not have any vaginal, anal or receptive oral sex for 2 weeks after the gel placement on the day of your cesarean. We also will ask that you do not place anything into your vagina during the study and that you do not take part in any other drug or device study during your participation in this study

Screening and Enrollment Visit:

This visit will continue today after you read, discuss and sign this form. It will take about 90 minutes. The study clinician will review your medical history, including your prenatal chart and ultrasound report or reports, to make sure you meet the requirements for this study. Then you will be asked a few more questions. The questions will be about you, where you live, your health, and the medicines you take. You also will:

- Give urine to test for gonorrhea, and chlamydia. Gonorrhea and chlamydia are infections passed during sex.
- Have a brief physical exam, including an exam of your genital area and inside your vagina using a speculum (like for a Pap smear—a Pap smear is a test for cervical cancer). During the exam a test for Trichomonas (another infection passed during sex) will be done. If you have symptoms of a vaginal infection, like itching or odor, you will get a test for vaginal infections using a swab that will collect a few drops of vaginal discharge. If you have signs of herpes infection, a herpes culture test would be done.
- Give about 6 tablespoons of blood. This blood will be used for:
  - Tests to check on the overall health of your liver and kidneys
  - Tests for HIV in your blood
  - Test for Hepatitis B if you did not have one during this pregnancy
  - Test or tests for syphilis if you have signs of syphilis today

The HIV test results will take about one hour and we will give you your results today. We will give you your other results when they are available (usually less than a week).

Study staff will explain all exam and test results to you. If the tests show that you have any infections passed during sex, study staff will give you treatment for these infections, free of charge. You can also bring your partner here for testing and treatment for these infections, free of charge.

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).

Main Study Visit [The Day of Your Cesarean and for 24 Hours After Gel]:
This visit will take place on the day of your cesarean from the time right before the cesarean until about 24 hours after you receive the study gel. During this time we will:

- Talk to you about any new medical problems since we saw you last
- Ask you about any medications you are taking or have taken since the last time we saw you
- Review your medical chart
- Talk to other medical and nursing staff members who are taking care of you
- Put a copy of this consent form in your medical chart
- Ask if there are any changes in the ways that we can get in contact with you (such as new phone numbers or addresses) and make notes on these
- Do a brief physical exam and pelvic exam (including a check of your cervix)
- Insert a saline lock in your hand or arm (a saline lock is a device that will allow us to draw blood samples without inserting a needle through your skin every time).
- Place 4 grams of study gel into your vagina using a special applicator similar to a tampon.
- Draw 1 teaspoon of blood (before inserting the study gel), and then over the next 24 hours we will draw 7 more teaspoons of blood from you at separate times, through the saline lock. These teaspoons of blood will tell us whether or not the drug has gotten into your blood. The first sample of blood will also be checked with a test called flow cytometry. This test helps scientists understand why medicines do or don't get absorbed into the bloodstream.

During your cesarean, we will also collect about 1 tablespoon of amniotic fluid and a small amount (about half an inch in diameter) of tissue from your womb (the same tissue that comes out with your monthly period) to look for the drug. Amniotic fluid is the liquid inside the water bag in the womb. During or after your cesarean, we will collect 1 teaspoon of the blood from the baby's umbilical cord after it has been separated from the baby and a piece of the placenta after it has been delivered (about half an inch in diameter).

Twenty-four hours after you receive the study gel, you will:

- Have your blood drawn to check for tenofovir in the blood
- Tell the study staff about any problems that you may have
- Tell the study staff about any medications that you may have taken
- Have a brief physical exam by a doctor
- Have your medical chart reviewed by study staff

Final Contact:
We will contact you by phone two weeks after your cesarean to ask how you are doing. If you have a problem that may be related to being in the study we might ask you to come in for a visit or visits. Otherwise, you are done with the study at this point.

Any Time During The Study:
Please tell the study staff about any medical problems you have during the study. You can contact the study staff between regular visits to report these problems. The study staff will check you as needed and either give or refer you for medical care. At each study visit, the study staff will update information on where you live and how to keep in touch with you.

If your participation in the study ended early, we would still ask you to come in for study visits and have the safety check-ups that are talked about in this form. If you start the study but do not get the study gel for some reason, we would not do the tests on your blood that are regularly scheduled for after the study gel.

How Many Women Will Be In this Study?
About sixteen women will be in this study.

How Long Will I be In This Study?
We expect that you will be in this study for six weeks at the most.

Can the Doctor Take Me Off This Study Early?
The study doctor may take you off the study early without your permission if:

- The study is stopped or canceled.
- Staying in the study would be harmful to you.
- Other reasons that may prevent you from completing the study successfully.

What are the risks of this study?

Risks of Blood Draws:
When your blood is taken, and when you have a saline lock inserted, you may feel discomfort or pain. You may feel dizzy or lightheaded. You may have a bruise, swelling, or infection where the needle goes into your arm.

Risks of Genital Exams:
You may feel discomfort or pressure during exams of your genital area and inside your vagina. You may have mild vaginal bleeding (spotting) after the exam. If this happens, it will usually stop quickly after the exam.

Risks Related to the Study Drug:
The study gel used in this study may have side effects, some of which are listed below. Please note that these lists do not include all the side effects seen with these drugs. These lists include the more serious or common side effects with a known or possible relationship. If you have questions concerning additional study drug side effects please ask the study staff.

Some of the effects of the tenofovir gel are still unknown. Some possible effects are dryness, itching, burning, or pain in the genital area. You may also have discharge if the

study gel comes out of the vagina. In about half of the women tested before, there was a small amount of irritation in the genital area. It is possible that tenofovir gel could be absorbed from the vagina into the blood.

Based on the earlier study of tenofovir gel, a small amount of tenofovir gel from the vagina was absorbed into the blood in about half of the women tested (about 1% of the amount that is absorbed when the oral pill is taken). If the gel is absorbed into the blood, it is not known whether this will cause any bad effects.

There are other side effects in patients taking the oral form (a pill) of tenofovir which is absorbed into the blood. However, these side effects may have been because of other medicines that patients were taking or because of the HIV itself.

The following side effects have been associated with the use of oral (by mouth) tenofovir pills:

- Upset stomach, vomiting, gas, loose or watery stools.
- Dizziness.
- Abdominal pain.
- Lack of energy.
- Kidney damage or failure.
- Inflammation or swelling and possible damage to the pancreas.
- Shortness of breath.
- Rash.
- Low phosphate, a chemical in the blood.
- Increased liver function tests in children
- Allergic reaction, which may include fever, rash, upset stomach, vomiting, loose or watery stools, abdominal pain, achiness, shortness of breath or a general feeling of illness.
- Changes in bone growth and strength were seen in study animals given tenofovir. It is unknown if long term use of topical tenofovir will cause bone abnormalities in adults. Bone thinning has been seen in adults and children taking oral tenofovir.

Laboratory tests have shown changes in the bones of patients treated with the pill form of tenofovir. An earlier study has shown that only a small amount of tenofovir gets into the blood with gel use. For that reason and because there will be only one dose of the study gel, the risk of changes to the bones when using the gel is very low.

If you are HIV-infected you should not receive the study gel. This is because it is not known what effect tenofovir gel could have on the HIV virus. There is a small possibility that tenofovir could change the virus. If the virus changes normal treatment for HIV may not work on the virus.

If you have a positive test for hepatitis B you should not receive the study gel. It is not known what effect tenofovir gel could have on the hepatitis B virus. There may be a risk that tenofovir will change the hepatitis B virus. If the virus changes normal treatment for

hepatitis B may not work on the virus. It is not known what effect tenofovir gel could have on the disease condition in people with hepatitis B virus.

Risks Related To Pregnancy

We believe that there is little risk to your pregnancy from this study because you are only getting one dose of the study gel right before you deliver, and we expect only very small amounts of tenofovir to get into your blood, if any. We are not sure if the drug goes into the placenta, the fluid around your baby or your baby's blood and those are some of the reasons we are doing the study. Nearly 500 women have been exposed to multiple doses of the oral form of this drug in pregnancy and there have not been more birth defects in their babies compared to other babies whose mothers never took tenofovir. A recent study found very low amounts of tenofovir in the babies of mothers who took oral tenofovir during pregnancy.

We do not expect any significant risk to your baby in this study. This is because you will only get one dose of the study gel, and we do not expect that much if any of the gel will pass into the baby's blood. Another study found that babies whose mothers took the oral form of tenofovir did not have problems. If your baby's blood did absorb some tenofovir, possible side effects 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.

Risks to Breastfeeding

We do not know if tenofovir passes into breast milk after women receive the oral or vaginal form of tenofovir. If tenofovir passed into your breast milk, it is not expected that the amount from one dose of the study gel 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.

Risks from Procedures During your Surgery:

No risks are associated with a collection of a small portion of amniotic fluid (about one tablespoon) immediately before delivery of the baby during cesarean section. Minimal extra time (approximately less than 30 seconds) is expected to be added to the surgery because of amniotic fluid collection.

There is no extra risk to either you or your baby from collecting the umbilical cord blood after the delivery of the baby. Any other samples of cord blood that may be drawn as part of your baby's medical care will be drawn first. Collection of cord blood is often done after delivery as part of the normal care. Minimal extra time (approximately less than 30 seconds) is expected to be added to the surgery because of cord blood collection.

You will not have any additional discomfort from the sampling of tissue from your womb because you should already be numb for the surgery. When we take a piece of tissue from your womb you may have a small amount of extra bleeding, but we would expect this bleeding to stop quickly on its own or to be easy to stop. This specimen collection is not expected to affect your ability to try to have a vaginal birth after cesarean (VBAC) in your next pregnancy. This also is not expected to add much additional time to the surgery (approximately less than 30 seconds).

Other Possible Risks:
You may become embarrassed, worried, or nervous when discussing sexual behaviors, HIV, and other infections passed during sex. You may feel worried or anxious while waiting for your test results. Trained staff members are available to help you deal with any feelings or questions you have.

Possible Risks to Your Privacy
We will make every effort to protect your privacy while you are in this study. Your visits here will take place in private. However, it is possible that others may learn of your participation here and, because of this, may treat you unfairly. For example, you could have problems getting or keeping a job or being accepted by your family or community. There also is a risk to your privacy if someone else taking part in this study knows you.

What are the Benefits of This Study?
You and your baby will get no direct benefit from being in this study. However, you will receive a number of services while taking part in this study, including:

- Information, counseling and testing related to HIV and other infections passed during sex.
- Treatment for infections passed during sex if you have any (including treatment for your partners).
- Referrals to medical care and other services you may need.

Your or others may benefit in the future from information learned in this study. Knowledge gained from this study may help in the development of microbicides for the prevention of HIV infection. You may also get some personal satisfaction from being part of research on preventing HIV.

What Other Choices Do I Have Besides This Study?
You do not have to participate in this study. The decision to not be in this study will not affect your care in any way.

What About Confidentiality?
We will do everything we can to protect your privacy. In addition to the efforts of the study staff to help keep your personal information private, we have applied for a Certificate of Confidentiality from the U.S. Federal Government. This certificate means that researchers cannot be forced to tell people who are not connected with this study,

such as the court system, about your participation. Also, any scientific publication about this study will not use your name or identify you personally.

People who may review your records include: the U.S. Food and Drug Administration (FDA), (insert Name of Site) IRB, National Institutes of Health (NIH), study staff, study monitors, drug companies supporting this study, and their designees. The Certificate of Confidentiality does not prevent you from releasing information about yourself and your participation in the study to anyone you choose.

Even with the Certificate of Confidentiality, if the study staff learns of possible child abuse and/or neglect or a risk of harm to yourself or others, we will be required to tell the proper authorities.

If you test positive for gonorrhea, chlamydia, syphilis, or HIV, the Commonwealth of Pennsylvania requires that your name be given to the Allegheny County Health Department. You may be contacted and asked questions about your sexual partners.

What Are The Costs To Me?

There is no cost to you for study visits, exams, laboratory tests, or other procedures. This study will not provide prenatal care, delivery, postpartum, or routine newborn care.

Will I Receive Any Payment?

You will receive payment for your time and effort in this study. You will also receive payment for activities affected by your participation in this study.

What Happens If I Am Injured?

If you are injured as a result of being in this study, you will be given immediate treatment for your injuries. If your infant is injured as a result of being in this study, he or she will be given immediate treatment for injuries as well. However, you or your insurance company (or the infant's insurance company) may have to pay for this care. This institution or the United States National Institutes of Health does not have a program to provide money for your or your infant's injuries. You will not be giving up any of your legal rights by signing this consent form.

What Are My Rights?

Being in this study is completely voluntary. You may choose not to be in or to leave the study at any time. You will be treated the same no matter what you decide. If you choose not to be in or to leave the study, you will not lose the benefit of services to which you would otherwise be entitled at this clinic. Study staff will tell you about any new information from this or other studies that may affect your health, welfare, or willingness to stay in this study. At the end of the study, you will be told when study results may be available and how to learn about them.

What Do I Do If I Have Problems or Questions?

For questions about this study or a research-related injury, contact:

Richard H. Beigi, MD, MSc
Magee-Womens Hospital of UPMC
300 Halket Street
Pittsburgh, PA 15213
(412) 641-5403

For questions about your rights as a research participant, contact:
I understand that I may contact the Human Subjects Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668) to discuss problems, concerns, and questions; obtain information; offer input; or discuss situations in the event that the research team is unavailable.

SIGNATURES
The above information has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions about any aspect of this research study during the course of this study, and that such future questions will be answered by a qualified individual or by the investigator(s) listed on the first page of this consent document at the telephone number(s) given. I understand that I may always request that my questions, concerns or complaints be addressed by a listed investigator

If you have read the informed consent, or had it read and explained to you, and all your questions have been answered, and you agree to be in this study, please sign your name or make your mark below.

Participant's Name (print)	Participant's Signature and Date
Study Staff Conducting Consent Discussion (print)	Study Staff Signature and Date
Witness' Name (print) (As appropriate)	Witness's Signature and Date

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