HIV PREVENTION TRIALS NETWORK

HPTN 059, Phase II Expanded Safety and Acceptability Study of the Vaginal Microbicide 1% Tenofovir Gel, Version 1.0, dated 17 March 2005.

AMENDMENT #1
Final Version 2.0
13 March 2006
IND 55, 690

Rationale for Modifications

The primary rationale for the modifications included in this protocol amendment is to add University of Alabama at Birmingham (UAB) as an additional domestic site, and, to address questions and comments from protocol team members since the approval of HPTN 059 Version 1.0 dated March 17th 2005. UAB has been added as a domestic site to aid in achieving the targeted enrollment numbers in a timely manner. Additionally, ongoing efforts to prepare for study implementation have identified certain aspects of the protocol that require updating, correction, or clarification to ensure consistent interpretation of, and adherence to, protocol specifications and DAIDS and HPTN policies and procedures across study sites.

Summary of Revisions

1. Updated the Protocol Team Roster, Schema, Study Design, Study Population, Statistical Section, and Informed Consents to reflect the addition of University of Alabama UAB as an additional domestic site

2. Replaced all references to New York with Bronx-Lebanon Hospital Center (BLHC)

3. Updated Safety (Section 1.3.4) and Risk (Section 8.3) Sections to match DAIDS Drug Risk List for Tenofovir Disoproxil Fumarate (Tenofovir DF, Viread®, and, Viread® Package Insert)

4. Updated protocol and informed consents per FDA suggested changes:
    - Included explanation in informed consent forms for why participants with chronic hepatitis B virus (CHBV) will be followed an additional three months
    - Clarified in Protocol Section 1.3.4 and informed consent forms that using topical tenofovir may cause bone thinning in adults and children.

5. Revised directions and guidelines for handling study gel in Section 4 “Study Treatment, Product and Intervention”

6. Revised Inclusion and Exclusion Criteria and the following study procedures:
    - Updated inclusion criteria to have HIV testing at screening
    - Clarified exclusion criteria for pelvic exam findings
    - Updated AE/EAE reporting for cervicitis/vaginitis and added Table 2: Severity Grading for Vulvovaginitis and Cervicitis

- Revised the language for participants who become pregnant during the study, so that pregnant participants can resume study gel
- Revised the guidelines for resuming study drug in subjects who voluntarily discontinue study gel
- Revised and Updated Table 1 “Use of Topical Tenofovir to Prevent Vaginal Transmission of SIV”
- Updated Appendix II “Outcomes, Diagnostics, and Follow up Evaluations” to add permanent discontinuation for Grade 4 EAEs
- Updated Appendix III “HPTN HIV Antibody Algorithm”

7. Clarified when douching history and use will be captured on CRFs

8. Included definition and details for the PSRT and revised meeting timeline in Section 6, Safety Monitoring and Adverse Event Reporting

9. Clarified the scope of AE reporting in Section 6, Safety Monitoring and Adverse Event Reporting.

10. Randomization procedures have been updated

11. Provided Final Contact procedures for CHBV participants

12. Revised Section 2.4.1 “Study Visits and Evaluations”, Section 5 “Study Procedures”, and, Section 7.6.2 “Analysis of PK data” to clarify instructions for collecting Weeks 4, 12 and 20 PK specimens

13. Participants Who Voluntarily Discontinue Study Gel Use or Miss One or More Follow up Visits has been revised to read “Participants Discontinue Study Gel Use”

14. Instructions for managing Grade 4 EAEs have been added to Appendix II.

15. Updated References. Refer to Items 9, 10 and 37 for package insert updates, and Items 7, 8 and 36 for updates to HPTN 050 data updates.

16. Updated the protocol version number, date and table of contents. Other administrative and typographical clarifications and corrections have been incorporated throughout the protocol and sample informed consent forms as needed

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Implementation of Modifications

Prior to implementing the procedures described below, HPTN 059 study sites will submit this amendment, the corresponding protocol Version 2.0, and updated site-specific informed consent forms to all relevant regulatory authorities and Institutional Review Boards and Ethics Committees (IRBs/ECs). The Division of AIDS Regulatory Affairs Branch will submit this amendment to the United States Food and Drug Administration for inclusion in Investigational New Drug (IND) application #55, 690.

Upon receipt of all required regulatory and IRB/EC approvals, completion of protocol registration procedures with the DAIDS Regulatory Compliance Center, and activation by the HPTN Coordinating and Operations Center, the protocol modifications listed below will be implemented. Detailed modifications of the protocol text are indicated by ~~strikethrough~~ (for deletions) and **bold** (for additions).

Detailed Listing of Modifications

1. On the Cover Page, added new Investigator:

Protocol Co-Chair
Craig Hoesley, MD
University of Alabama at Birmingham
Birmingham, Alabama, USA

2. In the list of Abbreviations and Acronyms, added the following acronyms:

BLHC	**Bronx-Lebanon Hospital Center**
CBO	**community based organization**
IFA	**Immuno-fluorescent antibody**
UAB	**University of Alabama at Birmingham (site)**

3. In the Protocol Team Roster:

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4. In the Protocol Schema:

Design:	Phase II, four arm, ~~two~~ **three** site, randomized, double blind, controlled trial comparing tenofovir 1 % vaginal gel or placebo gel used once daily and tenofovir 1% vaginal gel or placebo gel used prior to intercourse, with 24 weeks of product exposure and follow up. Participants with chronic hepatitis B virus (CHBV) will have an additional 12 weeks follow up.
Study Size:	Approximately 100 participants **will be enrolled** ~~per site~~ **at the Pune** ~~India~~ **site**~~,~~ and approximately 100 **participants will be enrolled between the Birmingham site and the Bronx Site**, for a total of 200 women. **The US sites will competitively enroll the 100 participants.**
Study Duration:	Accrual will require approximately ~~10~~ **six** calendar months. Each participant will be followed for up to 24 weeks. CHBV infected participants will additionally return to site at 4, 8 and 12 weeks after completion of study gel use. Therefore the entire study should be completed within ~~19~~**15** calendar months.

Study Sites:

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

5. In Protocol Section 1.3, Rationale, second paragraph, first sentence, revised to:

   The goal of this ~~two~~ **three**-center phase II study is to determine the safety of tenofovir 1% gel as a vaginal microbicide over 24 weeks of use, and to gain additional information about the product's acceptability.

6. In Protocol Section 1.3.1, revised Table 1 to delete Virus column, revised note at the bottom of the table, and other minor grammatical changes:

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

~~Study~~	~~Virus~~	~~Number of Exposures~~	~~Treatment~~	~~Time of administration~~	~~Number Infected~~	~~Progesterone Pretreatment~~
~~1~~	~~SIVmac251*~~	~~2~~	~~1 mL vehicle~~	~~-24 h, 0 h, 24 h, 48 h~~	~~2 of 2~~	~~No**~~
			~~10% tenofovir~~	~~-24 h, 0 h, 24 h, 48 h~~	~~0 of 4~~	
~~2~~	~~SIVmac251*~~	~~1~~	~~untreated control~~	~~N/A~~	~~5 of 5~~	~~No**~~
			~~10% tenofovir~~	~~-24 h , -15 m, +24 h~~	~~1 of 5~~	
			~~1% tenofovir~~	~~-24 h , -15 m, +24 h~~	~~1 of 5~~	
			~~1% tenofovir~~	~~-15 m~~	~~2 of 5~~	
~~3~~	~~SIVmac251*~~	~~1~~	~~untreated control~~	~~N/A~~	~~2 of 5~~	~~No**~~
			~~vehicle~~	~~-15 m~~	~~1 of 5~~	
			~~1% tenofovir~~	~~-15 m~~	~~1 of 5~~	
			~~1% tenofovir~~	~~- 2 h~~	~~3 of 5~~	
			~~1% tenofovir~~	~~- 8 h~~	~~1 of 5~~	
~~4~~	~~SIVmac251*~~	~~1~~	~~untreated control~~	~~N/A~~	~~4 of 5~~	~~No**~~
			~~vehicle~~	~~-15 m~~	~~2 of 5~~	
			~~1 % tenofovir~~	~~-15 m~~	~~1 of 5~~	
			~~1 % tenofovir~~	~~- 2 h~~	~~1 of 5~~	
			~~1 % tenofovir~~	~~- 8 h~~	~~2 of 5~~	
~~5~~	~~SIVmac251*~~	~~1~~	~~untreated control~~	~~N/A~~	~~2 of 5~~	~~No**~~
			~~vehicle~~	~~- 2 h~~	~~2 of 5~~	
			~~1 % tenofovir~~	~~- 2 h~~	~~0 of 5~~	
~~6~~	~~Sivmac251~~	~~1~~	~~untreated control~~	~~N/A~~	~~8 of 8~~	~~Yes***~~
			~~Vehicle~~	~~12 h prior~~	~~8 of 8~~	
			~~1% PMPA~~	~~12 h prior~~	~~5 of 8~~	
			~~Vehicle~~	~~24 h prior~~	~~8 of 8~~	
			~~1% PMPA~~	~~24 h prior~~	~~8 of 8~~	
			~~1% PMPA~~	~~72, 48, 24 h prior~~	~~6 of 8~~	

~~* All studies were performed with the SIVmac251 isolate of SIV, and female rhesus macaques were inoculated intravaginally. Infections were performed without progesterone pretreatment, except for study six. The indicated studies were performed by 3 independent investigators with studies 2, 3, 4, and 5 being performed by the same laboratory.~~

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

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

7. In Protocol Section 1.3.2, Clinical Research, second paragraph, fourth sentence, revised to match updated references:

   Although 92% reported at least one adverse event (AE) the majority of these events were mild (87%) and limited to the genitourinary tract (~~77~~**70**%).[6]

8. In Protocol Section 1.3.2, Clinical Research, fourth paragraph, revised to match updated references:

Fourteen of 25 women (56%) with PK results had low, but detectable, serum tenofovir levels (limit of quantitation: 3.0 ng/mL) at some point in the 12 hours after dosing on either Day 0 (following the first dose) or on Day 13 (after daily dosing); three of the 14 had detectable levels on both days. The maximum tenofovir concentrations (Cmax) ranged from 3.~~1~~**0** to 25.8 ng/mL, with no clear dose-concentration relationship identified. For the woman with the 25.8 ng/mL level, this peak level occurred 2 hours following the dose; the level rapidly declined to 10.~~8~~**9** ng/mL at 4 hours and was undetectable at 12 hours following the dose. Besides the outlier with the highest tenofovir level, the next highest Cmax was 7.1 ng/mL. Considering all women in the PK cohort, the median tenofovir Cmax was 3.4 ng/mL (interquartile range: below limit of quantitation [3.0 ng/mL] to 4.7 ng/mL)[6]. The median Cmax for all subjects (3.4 ng/mL) corresponds to approximately 1% of the maximum (Cmax, ss) and 7% of the minimum (C_{24} single dose) blood concentrations at steady-state with 300 mg daily oral tenofovir dosing[~~6~~**7**].

9. In Protocol Section 1.3.4 "Safety", fourth paragraph:

Changes in bone growth and strength were seen in study animals given tenofovir. Tenofovir and its oral form ~~(TDF)~~ administered in systemic dose toxicology studies to rats, dogs and monkeys at exposures (based on areas under the plasma concentration curve (AUC)) greater than or equal to ~~6~~ **six** fold those observed in humans caused bone toxicity. In monkeys the bone toxicity was diagnosed as osteomalacia. Osteomalacia observed in monkeys appeared to be reversible upon dose reduction or discontinuation of tenofovir. In rats and dogs, the bone toxicity manifested as reduced bone mineral density (BMD). The mechanism(s) underlying bone toxicity is unknown. However, systemic absorption of tenofovir from the vaginal gel appears to be minimal, (approximately 1% of the therapeutic oral dose) and the perceived risk of an effect on BMD is low.

10. In Protocol Section 1.3.7.2, Hepatitis B Virus Rebound, first paragraph, first sentence, deleted lamivudine:

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

11. In Protocol Section 2.4, Study Design, first paragraph:

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

12. In Protocol Section 2.4.1, Study Visits and Evaluations, seventh paragraph, revised to:

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

13. In Protocol Section 2.4.1, Study Visits and Evaluations, eighth paragraph, deleted:

    During this visit, non-CHBV participants will complete a study burden assessment~~, and all participants will complete a final acceptability assessment.~~

14. In Protocol Section 3.0, first paragraph, third and fourth sentences:

    There are ~~two~~ **three** sites, Pune, India; and, **UAB** and ~~New York, USA~~ **BLHC** in USA. The **Pune** site~~s~~ will enroll approximately 100 participants ~~each~~, and**, the UAB and BLHC sites will enroll 100 participants between the two sites, for a total of 200 participants among all three sites**.

15. In Protocol Section 3.1, Inclusion Criteria, fourth bullet:

    - be HIV uninfected (per HPTN HIV Antibody Testing Algorithm, Appendix III) at screening ~~and enrollment~~

16. In Protocol Section 3.2, Exclusion Criteria, eighth bullet, deleted the following:

    Normal laboratory values are based on site specific local laboratory (LL) normal reference ranges. ~~for the New York site; and on kit normal ranges for the India site.~~

17. In Protocol Section 3.2, Exclusion Criteria, 12th bullet:

    - ~~have an abnormal pelvic exam finding (observed by study staff, e.g., vulvar, vaginal, cervical and/or perineal ulcer and/or lesion and/or deep epithelial disruption, at screening or enrollment)~~

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

      Note: Otherwise eligible participants with exclusionary pelvic exam finding(s) may be enrolled and randomized after the exclusionary finding(s) have resolved. If resolution is documented within 56 days of providing informed consent for screening, the participant may be enrolled, without being rescreened.

18. In Protocol Section 3.2, Exclusion Criteria, 13th bullet, deleted third note pertaining to HSV-2 participants, and modified second note:

~~*Note: Women who are HSV-2 seropositive will only be excluded if they have active genital herpes (active herpes lesions). Once lesions are resolved, participant may be rescreened for study entry.*~~

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

19. In Protocol Section 3.3, Recruitment Process, added UAB as another domestic site, and added UAB's Recruitment plan:

The study will be conducted at ~~NARI, and, Bronx-Lebanon Hospital in New York USA~~ Pune, India**, and BLHC and UAB in the US**.

UAB, USA

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

Recruitment will also be conducted via outreach by project staff, and using IRB approved flyers on public kiosks, UAB campus newspaper ads and local radio ads if needed. Women who have responded to previous recruitment efforts or participated in previous studies and meet eligibility requirements will be contacted by phone or by mail with their prior permission. Recruitment information may be sent to medical staff and case managers throughout the medical center as well other local hospitals and clinical practices.

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

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

~~New York~~ **BLHC**, USA

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

20. In Protocol Section 3.6, Participant Withdrawal, fourth paragraph:

> Participants who become pregnant during the study will discontinue ~~gel~~ **product** use while they are pregnant; however they will ~~continue with their follow up visits. For any participants who become pregnant during follow up, site staff will offer counseling on options available to the participant, in accordance with site specific SOPs. If a repeat urine pregnancy test is negative after 42 days, the participant may resume study gel use.~~ **remain in follow-up and may resume product use after birth or pregnancy termination , as evidenced by a negative pregnancy test and normal pelvic exam performed by the study staff.**

21. In Section 4.1.2, the following second paragraph added:

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

22. Section 4.2, the first paragraph modified to:

> Study participants will receive supplies of tenofovir or placebo gel from the study site for use during the study period. All participants will be instructed to start using tenofovir or placebo gel at the time of enrollment and continue using the study gel for 24 weeks~~, however instructions vary according to whether it is used in conjunction with vaginal intercourse (coitally dependent – Arms 1 and 2), or daily (Arms 3 and 4), see Sections 4.2.1 and 4.2.2~~ **Frequency of use will be determined once the participant has been randomized to a specific arm: coitally dependent or daily use**.

23. In Section 4.3, title revised, paragraph added after first paragraph, and, third paragraph added:

> Study ~~Supply~~ **Product Packaging, Labeling, and Study Related Product Supply:**
>
> **Study gel (active or placebo) will be packaged in cartons containing 10 gel tubes and 10 individually wrapped plastic applicators. These study gel cartons will be labeled and securely sealed with tamper-evident tape. A removable portion of the study gel carton will contain unblinded information regarding the identity of the study gel. The Study Pharmacist will remove all unblinded information and record it appropriately, according to instructions in the NIAID Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks (dated September 2002), and, as directed in writing by the HPTN 059 Protocol Pharmacist, prior to dispensation.**
>
> **Study Pharmacists will order and receive study gel cartons from the National Institute of Allergy and Infectious Diseases (NIAID) Clinical Research Product Management Center (CRPMC).**

24. Section 4.3.1, second paragraph deleted:

> ~~Site Pharmacists will obtain study products and applicators from the CRPMC. The study products will be stored in a secure, limited-access area at the site, at controlled room temperature.~~

25. Section 4.4 revised to state:

> ~~The site pharmacists~~**The Study pharmacists** must maintain complete records of all study ~~products (except study provided condoms, panty liners and/or menstrual pads)~~ **gel cartons** received from the CRPMC ~~and subsequently dispensed to study participants.~~ **Additional documentation will also be required by the Pharmacists for study gel re-supply, transfers, chain of custody, returns, destruction (if applicable) and other related issues as outlined in the NIAID Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Network (dated September 2002), and as directed in writing by the HPTN 059 Protocol Pharmacist.**

26. Section 4.5, last paragraph, clarified when douching history will be captured on CRFs:

> Douching history and **any ongoing** use ~~throughout the study~~ will be captured on the Case Report Form (CRFs) **at Weeks 4, 12 and 24.**

27. In Protocol Section 4.7, Concomitant Medications, revised to:

> Any concomitant medications will be permitted for the participant with the exception of those not permitted under criteria for inclusion and exclusion. All concomitant medications will be reported on the study participants' clinical records and recorded on the CRFs for all medications received as of the first Screening visit, and throughout the study. In addition to prescribed and over-the-counter medications, **all** vitamins, herbal remedies, and other traditional preparations will be recorded **on the CRFs as well**. Alcohol and recreational or street drug use will be recorded in clinical progress notes if needed for interpretation/documentation of observed participant health status. Medication used for the treatment of AEs that occur during study participation also will be recorded on applicable ~~study~~ CRFs.
>
> To protect the integrity of the lower genital tract and reduce the possibility of AEs due to agents other than the study gel and applicator, **any** spermicides, diaphragms, ~~and~~ **or** contraceptive vaginal rings should not be used during this study. Participants who report current use of these ~~methods of contraceptive products and devices~~ **contraceptives** during screening or while enrolled in the study will be ~~counseled regarding the use of alternative methods~~ referred to family planning services for **counseling and,** provision of alternative ~~methods~~ **contraceptives** ~~should they enroll in the study~~. ~~Additionally,~~ ~~P~~**p**articipants will not be terminated from the study if they report current use of these contraceptives methods or devices while enrolled in the study. Participants will be encouraged ~~to avoid douching during study participation and~~ to avoid use of vaginally-applied **or inserted** medications/preparations ~~within~~ two hours before and two hours after having vaginal intercourse.

28. In Protocol Section 4.9, Procedures to be Followed in the Event of Pregnancy, fourth paragraph:

> ~~If the Pregnancy test is negative 42 days after the initial positive pregnancy test, the participant may resume study gel use~~. **Participants who become pregnant may resume product use after giving birth or pregnancy termination, as evidenced by a negative pregnancy test and normal pelvic exam performed by study staff. Refer to Section 5.10 on Final Contact procedures for participants who become pregnant during the study.**

29. In Protocol Section 5.4, Weeks 8, 16 and 20 Visits, Note after last bullet:

    Additionally at the Week 20 Visit only:

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

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

30. In Protocol Section 5.9.4, Participants Who Voluntarily Discontinue Study Gel Use, modified title and deleted the note at the bottom of the section:

    Participants Who **Voluntarily Discontinue Study** Gel ~~Use or Miss One or More Follow up Visits~~:

    ~~*Note: participants who return following missed visit or discontinuation of study gel may resume study gel usage at the discretion of the clinician.*~~

31. In Protocol Section 5.10, Final Contact:

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

32. In Protocol Section 6.1, Safety Monitoring, revised paragraph to state:

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

33. In Protocol Section 6.2, Clinical Data Safety Review, third paragraph:

    At the beginning of the study, ~~T~~**t**he PSRT will aim to meet via conference call every two weeks during the period of study implementation, to review clinical and laboratory data reports (blinded by study treatment) generated by the HPTN SDMC.

34. In Protocol Section 6.3, Adverse Event Reporting Requirements, first paragraph, added the following language:

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

35. In Protocol Section 6.3, Adverse Event Reporting Requirements, fifth paragraph:

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

Table 2: Severity Grading for Vulvovaginitis and Cervicitis

	Vulvovaginitis	Cervicitis
Grade 1	**Vulvar and/or vaginal discomfort (including itching or burning), pelvic exam findings indicative of inflammation, and/or other exam findings* (including findings involving epithelial disruption) that do not require medical therapy and that cause no or minimal interference with usual social and functional activities**	**Cervical inflammation or other findings on exam (including erythema, mucopurulent discharge, and/or friability) that do not require medical therapy and that cause no or minimal interference with usual social and functional activities**
Grade 2	**Vulvar and/or vaginal discomfort (including itching or burning), pelvic exam findings indicative of inflammation, and/or other exam findings* (including findings involving epithelial disruption) that require minimal medical therapy (such as a course of topical or oral antibiotics or antifungals) or cause greater than minimal interference with usual social and functional activities**	**Cervical inflammation or other findings on exam (including erythema, mucopurulent discharge, and/or friability) that require minimal medical therapy (such as a course of oral antibiotics) or that cause greater than minimal interference with usual social and functional activities**

	Vulvovaginitis	Cervicitis
Grade 3	**Vulvar and/or vaginal discomfort (including itching or burning), pelvic exam findings indicative of inflammation, and/or other exam findings* (including findings involving epithelial disruption) that result in inability to perform usual social and functional activities and/or require significant medical intervention such as a surgical procedure or hospitalization**	**Cervicitis or other findings on exam (including erythema, mucopurulent discharge, and/or friability) that require significant medical intervention (such as intravenous antibiotics) or that cause inability to perform usual social and functional activities**
Grade 4	**Life threatening — vulvovaginitis with perforation**	**Life threatening**

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

36. In Protocol Section 7.3 "Accrual, Follow up, and Sample Size", revised the accrual plan to include UAB as another domestic site, and included competitive enrollment between both domestic sites:

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

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

37. In Protocol Section 7.4, Random assignment, revised paragraph to:

> Women will be randomized to one of the four arms. ~~Using an unblinded list of product codes and assigned products, the pharmacist at each site will supply each participant with either the active gel or the placebo gel.~~

~~Once randomization has been assigned, study site staff will provide information to women on frequency of use (i.e. daily or coital).~~**The randomization scheme will be generated and maintained by the HPTN SDMC.**
The SDMC will provide each study site with **one set of** randomization envelopes **to be stored and used in the study clinic. Clinic staff will assign these envelopes in sequential order, by envelope number, to eligible participants.**

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

38. In Protocol Section 7.5, Blinding, revised to:

~~Throughout the period of study implementation and data analysis, neither study s~~**S**tudy staff, **with the exception of Study Pharmacy Staff, and**, ~~nor~~ participants will be ~~informed of the participants'~~ **blinded to the** random assignments **of all study participants**. Both study gels will be supplied in identical, single-use tubes, and single use applicators packaged in individual wrappers. ~~Study staff and participants will be unblinded after all study visits and data analyses are completed. Individual exceptions may be considered by the Protocol Chair and Medical Officer in situations where product information may be needed to protect the safety of the participant.~~

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

There are no circumstances under which it is expected that unblinding will be necessary for the provision of medical treatment or to otherwise protect the safety of study participants. As described in Section 4.8, in the event that an Investigator is concerned that a participant might be put at an undue risk by continuing product use, the Investigator may discontinue use by this participant; however, knowledge of the specific product to which the participant was assigned should not be necessary to guide further follow-up and/or treatment. If an Investigator feels that specific product knowledge is necessary to protect participant safety, the Investigator will notify the PSRT to consider and rule upon the request.

39. In Protocol Section 7.6.2 "Analysis of PK Data", clarified instructions for collecting PK specimens at Weeks 4, 12 and 20 visits:

Blood levels of tenofovir will be evaluated after vaginal administration. Specimens from participants receiving placebo gel will not be assayed. A single tenofovir blood level will be drawn at the Week 4, 12 and 20 visits. **For the Weeks 4 and 12 visit specimens the participants will not be instructed to insert the study gel prior to these visits, and the analysis will be performed in an exploratory way by investigation plasma concentrations of tenofovir** during terminal elimination

(~~weeks 4 and 12 approximately~~ **Blood draws are estimated to occur** 12-16 hours post-dose, depending on an individual participant's study gel use). **For the Week 20 visit specimen,** ~~T~~**t**he analysis will be performed in an exploratory way, by investigating plasma concentrations of tenofovir during the peak absorption period following dosing, **however at this visit**, **all participants *will* be instructed to insert the study gel prior to the visit, so that blood will be drawn** approximately 2-6 hours post-dose, the period coinciding with peak concentrations in previous studies [HPTN 050]) ~~.and much later is the dosing interval~~ Tenofovir blood levels will be further investigated by correlating serum tenofovir levels with HSV-2 serostatus, the presence or absence of genital tract inflammation, and frequency of sexual activity.

40. In Protocol Section 8.3, Risks, third paragraph, second sentence, revised to match updated references:

    Although 92% of participants reported at least 1 AE, 87% of those reported AEs were mild, and ~~77~~**70**% of the AEs were limited to the genitourinary tract. Four severe AEs were reported, with only one, lower abdominal pain, thought to be product-related[6]

41. In Protocol Section 8.3, Risks, seventh paragraph, last bullet, and, first sentence of eighth paragraph:

- Changes in bone growth and strength were seen in study animals given tenofovir. It is unknown if long term use of ~~taking~~ tenofovir **gel** ~~for a long time~~ will cause bone abnormalities in adults. ~~In children, some decrease in bone thickness (density has been seen)~~. **Bone thinning has been seen in adults and children taking oral tenofovir**

  Tenofovir and its oral form ~~(TDF)~~ administered in systemic dose toxicology studies to rats, dogs and monkeys at exposures ……

42. In Protocol Section 8.6, Participant Confidentiality, third paragraph, second sentence revised to:

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

43. In Protocol Section 9.1, revised second bullet and added last bullet:

    - blood for HIV, ~~HSV-2~~, syphilis serology and HBsAG
    - **blood for HSV-2 (India only)**

44. In Protocol Section 9.2, added last bullet to:

    - **Blood for HSV-2 (US sites only)**

45. In Protocol Section 11, modified the following references:

6. ~~Mayer Kenneth, Maslankowski L, El-Sadr W, Justman J, Masse B, Hendrix C, Rooney J, Kwiecien A, Soto-Torres L. Abstract - Safety and tolerability of vaginal tenofovir gel (TFV) in HIV uninfected and HIV infected women (HPTN 050). XV International AIDS Conference, Bangkok, July 2004~~

Kenneth H. Mayer et al. Safety and tolerability of tenofovir vaginal gel in abstinent and sexually active HIV-infected and uninfected women. AIDS 2006, 2: 543-551

12. Gilead Sciences Inc. (~~2002~~ **2004**). Full Prescribing Information: Hepsera (adefovir dipivoxil)

15. Gilead Sciences Inc. (~~2004~~ **2005**). Full Prescribing Information: Viread (tenofovir disoproxil fumarate

46. In Protocol Appendix I, Schedule of Study Visits and Evaluations, added * to the following procedures to note that those specimens will be batched:

PERFORM PELVIC EXAM:							
*cervical swab for cytokine and chemokine testing - CL			X	X		X	
*cervical swab for Gram stain – CL			X	X		X	
*vaginal swab for Gram stain assessment – CL	X		X	X		X	
*genital ulcer swab for multiplex PCR - CL	▲		▲	▲	▲	▲	
PERFORM LABORATORY EVALUATIONS:							
*PK sampling – CL				X	X[c]		
*HBV serum archive (if participant provides consent) - LL			●	●[b]		●	●
*HSV-2 serology – LL or CL	X		X	▲	▲	X	
*plasma and serum archive – LL			X	▲	▲	X	

*** - Items marked with * indicates those specimens will be batched for shipment**

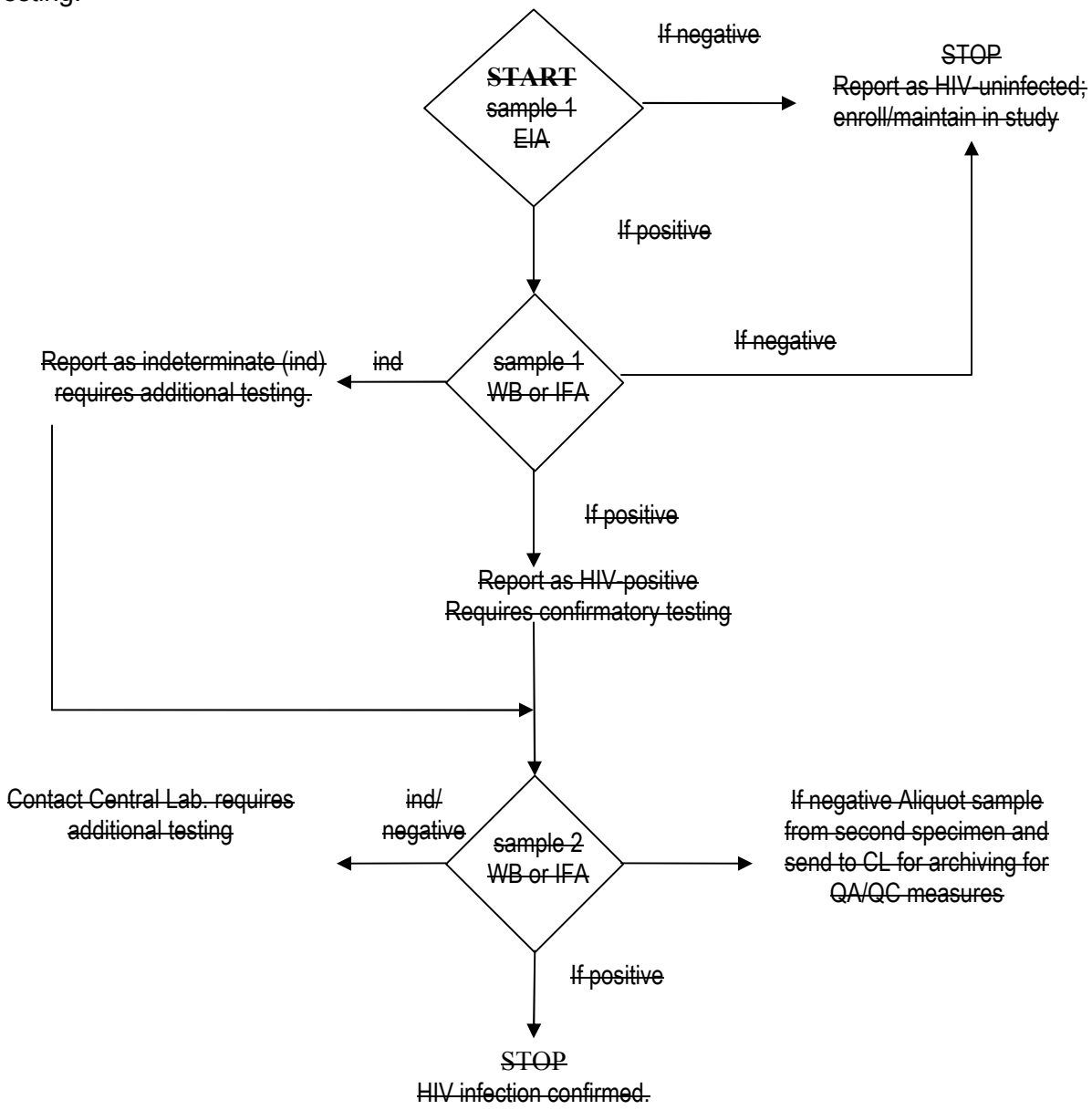
47. In Protocol Appendix I, Schedule of Study Visits and Evaluations, made the following changes to clarify which specimens will go to CL or LL:

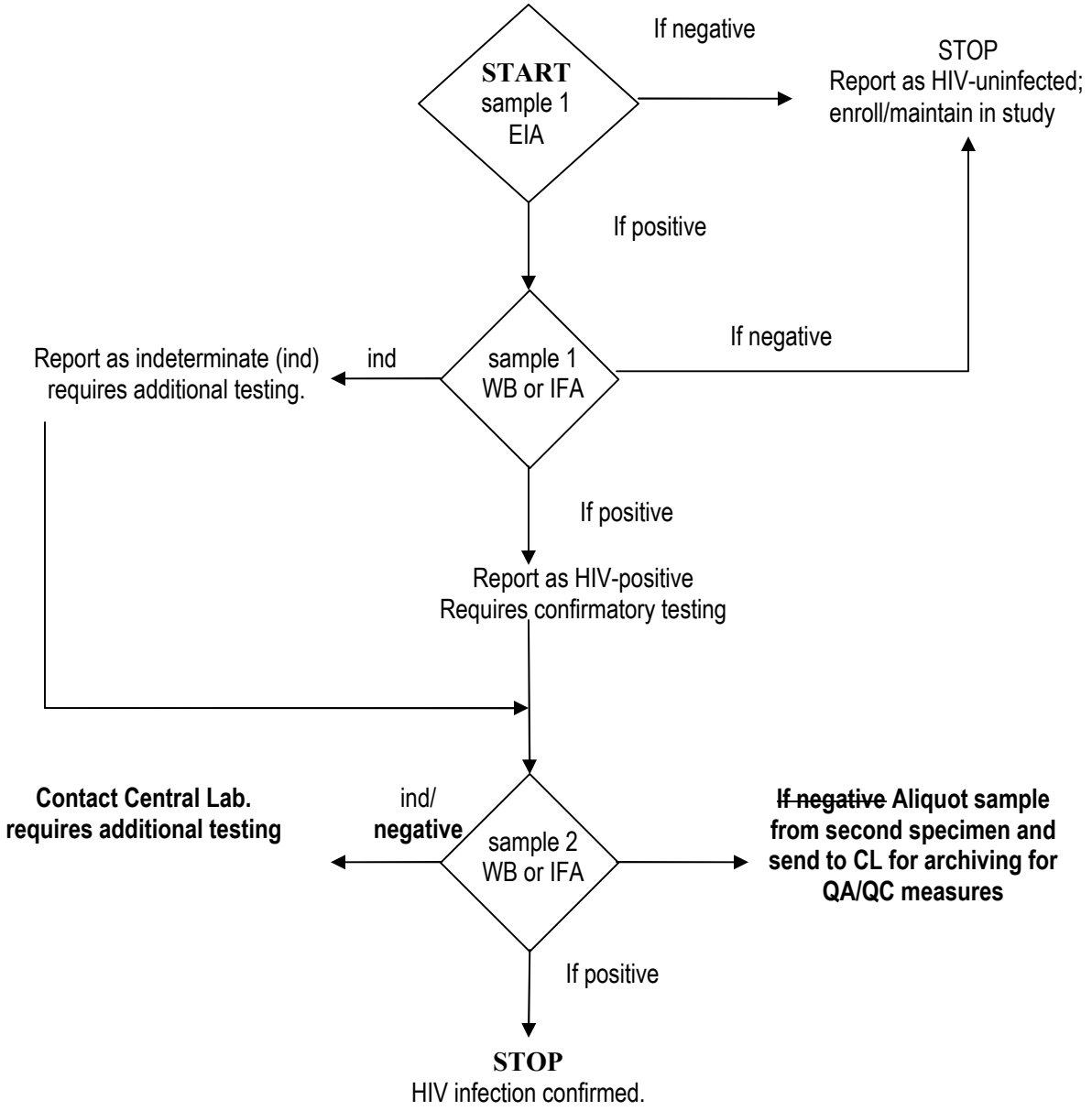
PERFORM LABORATORY EVALUATIONS:							
*PK sampling – ~~LL~~**CL**				X	X[c]		
*HSV-2 serology – LL **or CL**	X		X	▲	▲	X	

48. In Appendix II, Outcomes, Diagnostics, and Follow up Evaluations, Page 2 of 2, fifth row on EAE's (last row), revised to clarify Study Gel Use Procedures:

EAE that is judged by the site investigator or designee to be definitely, probably, possibly, or probably not related to the study gel or applicator	**For Grades 1, 2, and 3** - Hold study Gel (until evaluated) **For Grade 4 – Permanent Discontinuation**	Evaluate as according to current clinical practice at the site **Not applicable**	Provide treatment as clinically indicated, when resolved reinstate **study** gel use at clinician's discretion **Not applicable**

49. In Protocol Appendix III, Revised HPTN HIV Antibody Testing Algorithm, Non Rapid Testing:





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

50. In Appendix IV, Screening Informed Consent, Page 2 of 9, third Paragraph:

> The United States National Institutes of Health is providing funds for this study to take place. ~~About~~ A total of 200 women from Pune, India, **Alabama, USA**, and New York, USA, will join this study (100 ~~at each site~~ **in India and 100 in the US**). About ~~100~~ **[INSERT NUMBER]** women will be in the study here at – [INSERT NAME OF SITE].

51. In Appendix IV, the Screening Informed Consent, Page 2 of 9, the following added to the fourth paragraph:

    Oral tenofovir is known to have an effect on the virus that causes hepatitis B virus. Participants with chronic hepatitis B will have extra tests to find out whether the tenofovir gel had an effect on their levels of hepatitis B. If you have chronic hepatitis B.

52. In Appendix IV, the Enrollment Informed Consent, Page 2 of 17, fourth paragraph:

    The United States National Institutes of Health is providing funds for this study to occur. ~~About~~ A total of 200 women from Pune, India, **Alabama, USA**, and New York, USA, will take part in this study (100 ~~at each site~~ **in India and 100 in the US**). About ~~100~~ **[INSERT NUMBER]** women will be in the study here at – [INSERT NAME OF SITE].

53. In Appendix IV, the Enrollment Informed Consent, Page 3 of 17, sixth paragraph:

    Oral tenofovir is known to have an effect on the virus that causes hepatitis B infection. Participants with chronic hepatitis B will have extra tests to find out whether the tenofovir gel had an effect on their levels of hepatitis B. If you have chronic hepatitis B, you will have the six monthly visits and an additional three **monthly** visits. The additional three **monthly** visits will occur during the three months ~~every month~~ after you have stopped using the study gel, ~~for three months,~~ for a total of ten study visits including today's visit.

54. In Appendix IV, the Enrollment Informed Consent, Page 6 of 17, fourth bullet from the top:

    - Douche or otherwise clean the vagina, or insert other products into your vagina, two hours before and two hours after ~~having sex~~ **using the study gel (menstruating participants are allowed to use tampons as needed)**

55. In Appendix IV, the Enrollment Informed Consent, Page 7 of 17, Sub Section Visits 2, 4 and 5 (Months 2, 4 and 5), add the following text under the first bullet:

    - Insert study gel two to six hours before your visit. All participants will be asked to do this whether you are in the group that uses the gel every day, or only with sex.

56. In Appendix IV, the Enrollment Informed Consent, Page 10 of 17, under section "How Many Women Will Take Part In This Study?":

    About 200 women will take part in this study. About 100 women will be from New York **and Alabama**. About 100 women will be from India.

57. In Appendix IV, the Enrollment Informed Consent, Page 10 of 17, deleted the third bullet under "Why Would the Study Doctor Take Me Off This Study Early?"

    - ~~You are not able to come to the study visits or follow the procedures required by the study.~~

58. In Appendix IV, the Enrollment Informed Consent, Page 11 of 17, second and third bullet from the top of the page:

    - The study doctor decides that ~~continuing in the study~~ **using the study gel** would be harmful to you or your partner.
    - You ~~need~~ **require** a treatment that you may not take while ~~on this study~~ **using the study gel.**

59. In Appendix IV, the Enrollment Informed Consent, Page 12 of 17, third paragraph, last bullet:

    - 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. ~~In children, some decreases in bone thickness (density) has been seen.~~ **Bone thinning has been seen in adults and children taking oral tenofovir.**

60. In Appendix IV, the Enrollment Informed Consent, Page 12 of 17, the following fourth paragraph added after last bullet:

    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, the risk of changes to the bones when using the gel is low.