HPTN 117

Characterization of Doxycycline Pharmacokinetics and Adherence Post-Single and Repeat Dosing Schemas

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PROTOCOL SIGNATURE PAGE

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07 January 2026

I will conduct the study in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (US) Health and Human Service regulations (45 CFR 46); applicable US Food and Drug Administration regulations; standards of the International Council for Harmonisation Guideline for Good Clinical Practice (E6); Institutional Review Board/Ethics Committee determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (e.g., US National Institutes of Health, Division of AIDS) and institutional policies.

I have read and understand the information in this protocol 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 (print name)

_____ _____
Signature of Investigator of Record Date (DD/MONTH/YYYY)

LIST OF ABBREVIATIONS AND ACRONYMS

AE	Adverse Event
AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine Transaminase
ARV	Antiretroviral
AST	Aspartate Transaminase
BP	Blood Pressure
BV	Bacterial Vaginosis
BUN	Blood Urea Nitrogen
CAB	Community Advisory Board
CAB LA	Cabotegravir (Long-acting Injectable formulation)
CBC	Complete Blood Count
CFAR	Center for AIDS Research
CFR	Code of Federal Regulations
CL	Clearance
CLIA	Clinical Laboratory Improvement Amendments of 1988
CMC	Clinical Management Committee
CPQA	Clinical Pharmacology Quality Assurance
CRM	Clinical Research Manager
CRMS	(DAIDS) Clinical Research Management System
CRPMC	(DAIDS) Clinical Research Products Management Center
CRF	Case Report Form
CSF	Cerebrospinal Fluid
CT	Chlamydia Trachomatis
CTA	Clinical Trials Agreement
DAERS	DAIDS Adverse Experience Reporting System
DAIDS	Division of AIDS
DBS	Dried Blood Spot
DHHS	US Department of Health and Human Services
DOT	Directly Observed Therapy
DR	Delayed Release
DSMB	Data and Safety Monitoring Board
EAE	Expedited Adverse Event
EC	Ethics Committee
EQA	External Quality Assurance
EWG	(HPTN) Ethics Working Group
FDA	(United States) Food and Drug Administration
FTC/TDF	Emtricitabine (FTC) and Tenofovir Disoproxil Fumarate (TDF); Truvada®

GC	Gonorrhea
GCLP	Good Clinical Laboratory Practices
GI	Gastrointestinal
HBcAb	Hepatitis B Core Antibody
HBsAb	Hepatitis B Surface Antibody
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HPTN	HIV Prevention Trials Network
HR	Heart Rate
HSV	Herpes Simplex Virus
ICF	Informed consent form
IDS	Investigational Drug Service
IND	Investigational New Drug
IoR	Investigator of Record
IQA	(DAIDS) Immunology Quality Assurance
IR	Immediate Release
IRB	Institutional Review Board
IR-MALDESI	Infrared Matrix-Assisted Laser Desorption Electrospray Ionization
MSI	Mass Spectrometry Imaging
IUD	Intrauterine Device
LC	(HPTN) Laboratory Center
LDMS	Laboratory Data Management System
LL	Local Laboratory
LOC	(HPTN) Leadership and Operations Center
MIC	Minimal Inhibitory Concentration
MOP	(HPTN) Manual of Operations
NAAT	Nucleic Acid Amplification Test
NIAID	(United States) National Institute of Allergy and Infectious Diseases
NIH	(United States) National Institutes of Health
OHRP	Office for Human Research Protections
PAL	Protocol Analyte List
PBPK	Physiologically Based Pharmacokinetic
PEP	Post Exposure Prophylaxis
PID	Pelvic Inflammatory Disease
PK	Pharmacokinetic
PopPK	Population Pharmacokinetics
PrEP	Pre-exposure Prophylaxis
PRO	(DAIDS) Protocol Registration Office
PSRC	Prevention Science Review Committee
PTID	Participant Identification Number

QA	Quality Assurance
QC	Quality Control
RE	Regulatory Entity
RNA	Ribonucleic Acid
RR	Respiratory Rate
RSC	(DAIDS) Regulatory Support Center
SAE	Serious Adverse Event
SDMC	(HPTN) Statistical and Data Management Center
SMC	Study Monitoring Committee
STI	Sexually Transmitted Infection
SOE	Schedule of Events
SOP	Standard Operating Procedures
SSP	Study-Specific Procedures
UNC	University of North Carolina at Chapel Hill
US	United States
VAMS	Volumetric Absorptive Microsampling

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PROTOCOL TEAM ROSTER

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SCHEMA

Purpose:	The purpose of this study will be to characterize pharmacologic parameters for daily and non-daily doxycycline use and establish adherence cutoffs for daily and non-daily use.
Design:	This is a Phase I, single site, open label, randomized (1:1) pharmacokinetic (PK) modeling study
Study Population:	Healthy individuals who do not have a sexually transmitted infection (STI), including acute (e.g., gonorrhea or chlamydia) or chronic (e.g., HIV or HSV-2) infections
Study Size:	Sixteen healthy individuals, stratified in a 1:1 ratio by sex, will be enrolled in the study.
Study Duration:	Accrual will require approximately 10 months. Each participant will be followed for approximately 1.5 months (37 days). Study duration will be approximately 1 year.
Study Site:	One site in the United States (US) will participate in this study.
Study Regimen:	Sixteen (16) participants (approximately 8 males and 8 females) will undergo two randomization sequences upon enrollment into the study. Participants will be randomized (1:1) to take a dose of 200mg doxycycline hyclate as either 1) 1x 200mg delayed release (DR) tablet or 2) 2x100mg immediate release (IR) tablets. Following administration of this single dose, participants will undergo semi-intensive PK sampling over a 14-day period. Participants will also be randomized (1:1) to receive 200mg doxycycline hyclate (as a DR tablet) either daily (every 24 hours) or intermittently (every 72 hours) for 10 days. Both randomizations (IR vs DR and daily vs intermittent frequency) will be stratified by sex. All doses will be directly observed by study staff. Participants will be followed for 14 days after their last dose. PK sampling will occur at designated times over the course of the study. See Figure 1 for PK sampling timepoints.
Primary Objective(s):	• To estimate the single-dose plasma PK parameters of 200mg doxycycline hyclate immediate release (IR) and delayed release (DR) formulations.

	• To summarize doxycycline plasma concentrations following 10 days of daily or intermittent dosing. • To characterize differences in doxycycline plasma concentrations 24 hours (C_{24}) post-dose between intermittent and daily doxycycline following 10 days of dosing.
Secondary Objectives:	• To assess gastrointestinal (GI)-related adverse events for daily and intermittent doxycycline. • To estimate the single dose PK parameters of 200mg doxycycline hyclate IR and DR in DBS, anorectal fluid and vaginal fluid. • To estimate the single dose pooled PK parameters of 200mg doxycycline hyclate IR and DR in urine. • To summarize doxycycline concentrations in DBS, urine, anorectal fluid and vaginal fluid for intermittent and daily doxycycline.
Exploratory Objectives:	• To evaluate the feasibility of measuring doxycycline via DBS and volumetric absorptive microsampling (VAMS/ Tasso+ device; dried whole blood) to estimate plasma concentrations. • To develop a popPK model describing the compartmental PK of doxycycline and simulate concentrations under various dosing conditions, as well as use physiologically based pharmacokinetic (PBPK) modeling of the data observed herein alongside published CSF data to predict concentrations in the central nervous system given these dosing conditions. • To compare the single dose safety profiles of IR and DR doxycycline. • To characterize doxycycline concentration patterns in hair strands using infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI) mass spectrometry imaging (MSI) and correlate these images with daily and intermittent dosing patterns. • To perform exploratory laboratory assessments that may include evaluation of factors related to STIs and laboratory assays related to the study objectives.

OVERVIEW OF STUDY DESIGN

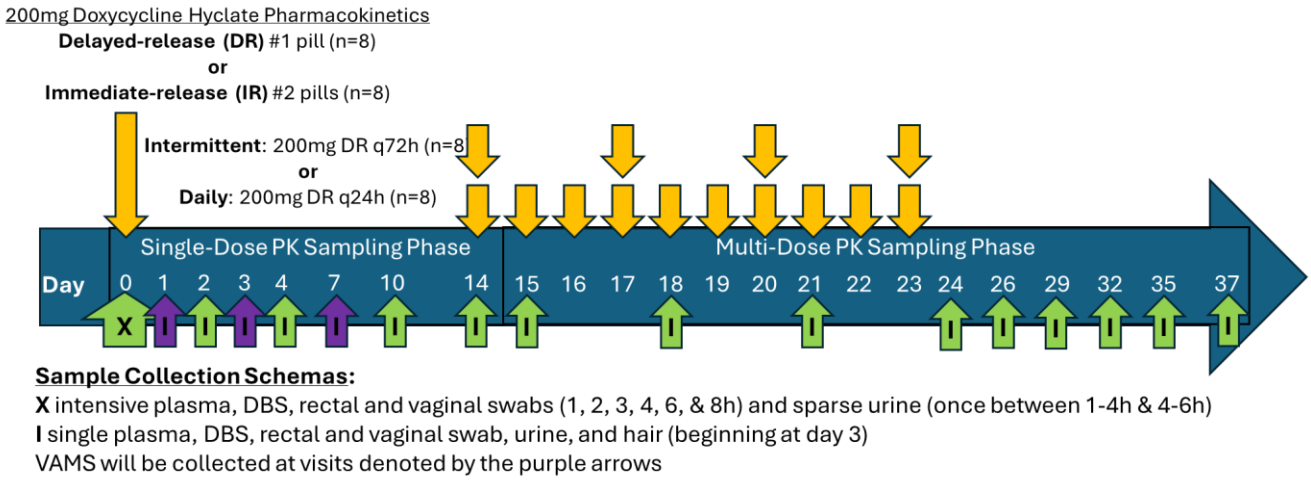


Figure 1. Study Schema.

1.0 INTRODUCTION

1.1. BACKGROUND AND RATIONALE

Rates of gonorrhea (GC), chlamydia (CT), and syphilis have been steadily rising in the United States (U.S.) since 2013, with almost half of incident bacterial sexually transmitted infections (STIs) and 56% of all cases of CT occurring among individuals aged 15-24 with annual incidence rate of 2,998 cases per 100,000 population [2]. These rises in STI rates indicate that current STI prevention paradigms are inadequate and new strategies are required. Recent open-label studies demonstrated that doxycycline 200mg within 72 hours of STI exposure (doxycycline post-exposure prophylaxis [DoxyPEP]) resulted in a 47-66% reduction in STIs among sexual minority men [3, 4]. These observations have led to the endorsement of DoxyPEP as an option for biomedical STI prevention by some public health authorities, including the (U.S.) Centers for Disease Control and Prevention (CDC) [5, 6]. The only trial designed to enroll women (the dPEP Kenya study) demonstrated no effect of DoxyPEP on STI incidence; however, this has been attributed to low adherence based on measurements of doxycycline in hair [7]. A current barrier to evaluating the efficacy and effectiveness of DoxyPEP in clinical trials is the lack of objective biomedical biomarkers of adherence.

1.2. DOXYCYCLINE PHARMACOKINETICS

Doxycycline is a broad-spectrum, second-generation tetracycline antibiotic with bacteriostatic effects that is generally well-tolerated and has been used as primary prophylaxis for bacterial and parasitic infections [8-10]. Doxycycline inhibits the synthesis of bacterial proteins by binding to the bacterial 30S ribosomal subunit, thereby preventing the binding of transfer RNA to messenger RNA at the ribosomal subunit and preventing downstream protein synthesis [11]. Pharmacologically, doxycycline is highly lipophilic and demonstrates a large volume of distribution following efficient gastrointestinal absorption. It is 80-90% protein-bound and does not undergo extensive metabolic conversion; the drug is cleared unchanged via biliary or renal pathways [12, 13].

While the pharmacokinetics (PK) of doxycycline in blood are well-described [6], knowledge gaps exist with intermittent dosing and establishing adherence thresholds for prophylactic use. Further, the multi-compartment distribution of the doxycycline has not been extensively characterized. Recently, Haaland and colleagues conducted a single-dose PK study to evaluate doxycycline PK parameters [1]. Following a single dose of 200mg doxycycline hyclate delayed release (DR) in men and women (n=20), mean C_{max} was 1.04 µg/mL (95% confidence interval [CI]: 0.89-1.22); t_{max} was 2 hours (95% CI, 1-4 hours); $t_{1/2}$ was 20 hours (95% CI, 19-22 hours), and $AUC_{0\text{-}96h}$ was 33.95 µg*h/mL (95% CI: 29.63-39.0 µg*h/mL). Importantly, there were no significant sex-specific differences between male and female plasma doxycycline PK parameters estimated in this study. Blood measurements were measured through 96 hours (Figure 2), and doxycycline was quantifiable in urine for up to one week after the last dose**.** Drug concentrations against GC and CT remained above the 1x minimum inhibitory concentration (MIC) for 45 and

87 hours, respectively. Doxycycline showed equivalent penetration into the anorectal compartment and female genital tract, with concentrations above the 1x MIC in both men and women [1] . The findings that doxycycline exposures in the female genital tract and anorectal compartment are comparable support the notion that the doxycycline PEP dosing strategy proven effective in men should also confer efficacy in women.

While the findings from Haaland and colleagues provide important reference data, several limitations should be noted. First, the sampling period in that study was relatively short, extending up to 4 days for anorectal and genital secretions, and 7 days for plasma and urine. Although this timeframe is sufficient for capturing early PK profiles, it may not fully reflect the pharmacologic behavior of doxycycline in the context of event-driven prevention strategies. In these cases, extended sampling beyond one week is valuable to empirically assess drug persistence and support adherence modeling for intermittent or episodic dosing. Second, the lower limit of quantitation (LLOQ) for plasma doxycycline in the Haaland study was 10 ng/mL, which limits the ability to measure drug concentrations beyond 7 days post-dose, as most concentrations fell below this threshold. In HPTN 117, we will employ more sensitive analytical methods with a plasma LLOQ of 0.5 ng/mL—approximately 1.5 log-fold lower—allowing for quantification of doxycycline at lower concentrations and over a longer post-dosing interval. This enhanced sensitivity will improve our ability to characterize late-phase pharmacokinetics and extend the window for detecting last product use, which is particularly relevant in studies with infrequent follow-up visits or adherence assessments. The improved analytical capacity over previously published studies warrant evaluation of doxycycline PK with single-dose administration.

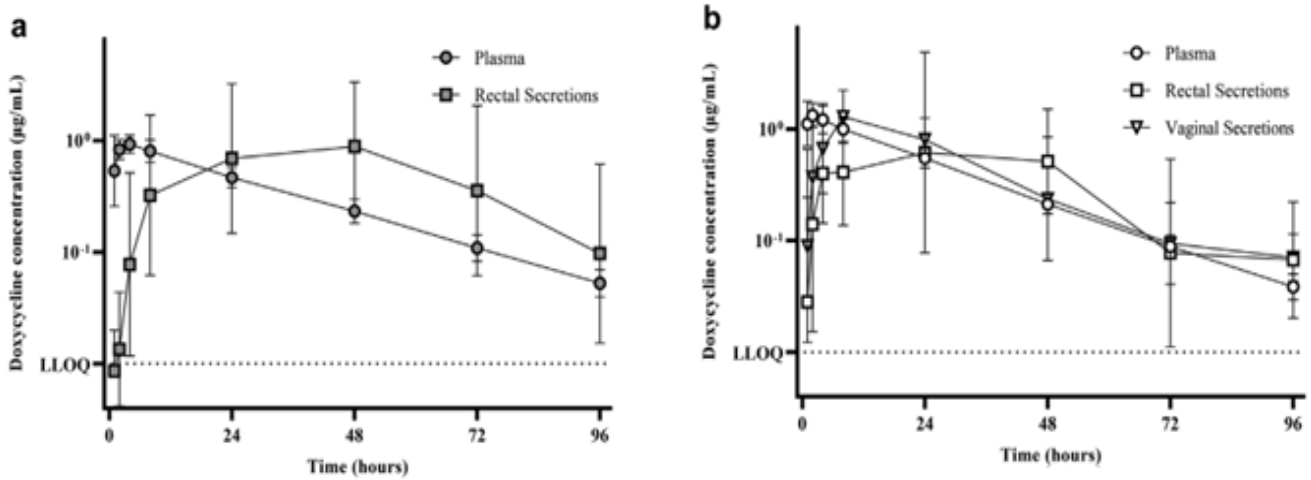


Figure 2. Multi-compartment pharmacology of single dose of 200mg doxycycline hyclate in a) men and b) women. [1]

1.3. DOXYCYCLINE MEASUREMENTS IN HAIR

The lack of efficacy within the dPEP Kenya study was attributed to non-adherence, as less than 29% of a randomly tested subset of participants had quantifiable doxycycline concentrations. Hair has been previously utilized as a non-invasive alternate specimen

type and is predicted to provide insight into more distal drug exposures and product use over time. While analytical methodologies have demonstrated proof-of-concept for doxycycline measurements in hair, the tetracycline molecule degrades over time in this specimen source, thereby confounding interpretation of hair concentrations with infrequent dosing. Based on longitudinal data in individuals on long-term doxycycline therapies, hair measurements reliably provide a look-back of 1-2 months before doxycycline begins to degrade/decline (Figure 3; [14]). Alternative strategies to evaluate doxycycline disposition in hair, beyond bulk measurements, may provide further insight into the utility of this matrix to assess adherence.

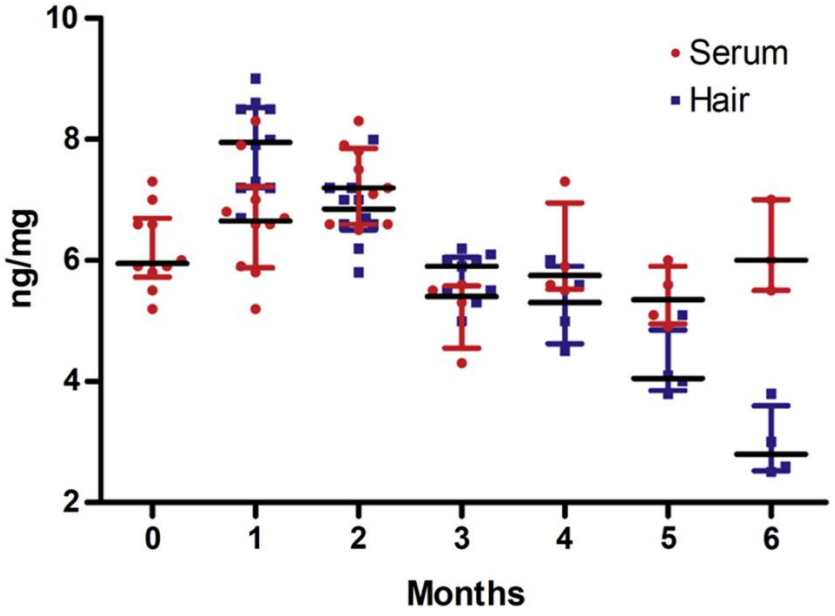


Figure 3. Median ± inter-quartile range levels of doxycycline (ng/mg) in hair and serum samples during time. Doxycycline concentrations significantly decreased over time in hair samples although they remained stable in the serum samples [14]

Although there are confounders to bulk measurement of drugs in hair, a new approach for hair analysis that uses infrared (IR) matrix-assisted laser desorption electrospray ionization (MALDESI) technology for mass spectrometry imaging (MSI) has been developed to evaluate longitudinal daily antiretroviral (ARV) use in hair samples [15]. The technique uses a focused laser to probe 100-micron features along the length of individual hair strands in order to build a picture of drug accumulation. In the resulting image, every pixel represents a separate sampling location representing about 8 hours of hair growth and the position along the hair strand can be associated with a point in time based on the average growth rate of hair [16]. This approach has been applied to the ARV emtricitabine (FTC) [17]. This technique may provide greater granularity on doxycycline use over time, as doxycycline signal may only be detected in the pixels/data points when doxycycline was used. This analytical approach may be amenable to understanding doxycycline adherence, particularly in the background of intermittent or episodic use.

Furthermore, prior studies using high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) have shown that doxycycline remains stable in hair for up to four months post-treatment. Although some degradation has been noted beyond this period, its underlying cause has not been investigated. For the purposes of this study—and in alignment with current standards in the biomarker field—a four-month stability window is considered acceptable for interpretation. Given these considerations, the study team has prioritized imaging-based hair analysis over bulk hair analysis to improve adherence monitoring and better support the study's pharmacologic objectives. To enable future comparative analyses, contingent upon funding availability, bulk hair sample collection will be optional to support comparative testing with hair collected for IR-MALDESI analysis, which will be collected and analyzed at time points described in Appendix I.

1.4. CONSIDERATIONS FOR DOXYCYCLINE IN THE TREATMENT OF NEUROSYPHILIS

While doxycycline is being considered as pre-exposure prophylaxis (PrEP)/ post-exposure prophylaxis (PEP) for treatment of STIs like GC/CT and *Treponema Pallidum* (syphilis), there is interest in evaluating short-term daily doxycycline for the treatment of neurosyphilis. HPTN 116 will evaluate the efficacy of oral doxycycline compared to intravenous penicillin G in the treatment of neurosyphilis and ocular syphilis in approximately 180 participants, aged 18 years or older diagnosed with symptomatic neurosyphilis, or ocular syphilis.

Central nervous system (CNS) penetration of doxycycline is a clinically important consideration, particularly in the context of treating or preventing infections involving the CNS. While this study does not include cerebrospinal fluid (CSF) sampling, existing literature provides valuable data on doxycycline disposition in the CNS. In a study of individuals with latent or neurosyphilis receiving 200mg doxycycline twice daily, Yim et al. ([18]) reported a geometric mean CSF concentration of 1.3 µg /mL, with a mean CSF-to-plasma penetration ratio of 26%. These findings are consistent with those from Dotevall and Hagberg ([19], who observed mean CSF concentrations of 1.1 µg /mL after 5–8 days of 200mg twice-daily dosing. Additionally, Karlsson et al. reported median CSF concentrations of 0.6 µg /mL (range: 0.4–2.5 µg /mL) in patients treated with 200mg once daily for neuroborreliosis, measured four hours post-dose [20]. These studies collectively demonstrate that doxycycline achieves measurable and therapeutically relevant concentrations in the CSF, typically exceeding the MIC_{90} for *Treponema pallidum* (0.06–0.10 µg /mL). Given the adequacy of existing PK data, along with the added participant burden and potential risks associated with lumbar puncture in healthy volunteers, CSF collection will not be included in this Phase I study. Instead, we plan to leverage published CSF data from these prior studies to inform physiologically based PK (PBPK) modeling and estimation of CNS exposures under different doxycycline dosing regimens.

1.5. RATIONALE FOR STUDY DESIGN

While there have been several efforts to characterize doxycycline PK parameters, population pharmacokinetic (popPK) modeling is needed to establish and interpret

adherence benchmarks based on doxycycline use, as well as further interrogation of drug concentrations relative to STI MICs. Easy to implement pharmacologic tools that can assess adherence will be an essential component of future clinical trials for DoxyPEP.

Multiple clinical studies of HIV PrEP demonstrate the need for biometric adherence assessment tools to be incorporated into the clinical trial design and inform study endpoints [21, 22]. For oral medications in the HIV PrEP space, objective biomedical assessments of adherence have become the gold-standard. Using tenofovir disoproxil fumarate/emtricitabine (F/TDF) as an example, intraerythrocytic tenofovir-diphosphate (TFV-DP) and emtricitabine-triphosphate (FTC-TP) concentrations from dried blood spots (DBS) are used to interpret product adherence in the preceding 8 weeks. Adherence thresholds were established via rigorous directly observed therapy (DOT) clinical studies across a broad range of dosing scenarios [23-25]. Additionally, popPK models have been used to characterize intraerythrocytic TFV-DP and FTC-TP concentrations that are associated with dosing patterns not directly observed in clinical studies [26].

The aforementioned F/TDF adherence tools do not currently exist for doxycycline and are needed to evaluate whether the drug exhibits dose proportionality under the real-world clinical dosing scenarios in which doxycycline may be used. To apply the F/TDF approach for doxycycline, we propose a study of directly observed doxycycline dosing to support popPK modeling within several biological matrices that can be collected in a minimally to non-invasive manner. Fitting popPK models within each of these matrices will facilitate testing different hypothetical scenarios of on-demand doxycycline use and provide guidance within the field as to the optimal measures by which to interpret on demand adherence.

Doxycycline hyclate, the formulation to be used in this study, is the most prescribed form of doxycycline worldwide and is available in both immediate- and delayed release tablets and capsules. Tan et al [27] has demonstrated comparable bioavailability between the hyclate and monohydrate formulations. The selection of doxycycline hyclate for this study reflects its broad clinical availability, consistency with prior and ongoing DoxyPEP studies, and relevance to real-world prescribing practices. Doxycycline hyclate is currently marketed by many generic vendors with both immediate release (IR) and delayed release (DR) formulations available [27]. The DR formulation was originally developed by Mayne Pharmaceuticals as a capsule comprising small controlled-release pellets of doxycycline to reduce incidence of nausea. This design prevents dissolution and absorption within the acidic environment of the stomach, which is believed to contribute to esophageal and gastric irritation. In a head-to-head comparison of these formulations, incidence of nausea was ~2-fold and ~4-fold higher for IR when compared to DR and placebo, respectively [28] . While the PK of these formulations have not been directly compared, parameter estimates appear to be similar despite the differences in their dissolution profiles. Time to maximum plasma concentrations (t_{max}), half-life ($T_{1/2}$), and clearance (CL) reported across multiple pharmacokinetic studies of various formulations of IR doxycycline range from 1.5-4 hours, 13-27 hours, and 1.7-3.8 L/hour, respectively [6]. Similarly, the t_{max}, $T_{1/2}$, and CL observed in registration trials for the DR capsules and tablets were ~2.5 hours, ~21 hours, ~5 L/hour respectively [29]. It is not

anticipated that PK parameters and drug exposures will differ significantly between the IR and DR formulations.

The IR formulation was provided in the ARNS IPERGAY and DOXYVAC trials [4, 30] and the DR formulation was provided in the DoxyPEP trial [3]. PopPK models are available in the published literature to describe the plasma PK of the DR formulation [31] and Haaland et al. previously described single-dose compartmental PK (mucosal fluids, tissues, and urine) using the DR formulation. Given the improved side effect profile, lack of product uniformity across published and ongoing clinical trials testing doxycycline PEP/PrEP, and the availability of historic PK data for the DR formulation, this study will focus on the DR formulation. However, given the similarity in PK profiles achieved by IR and DR products, our final recommendations for incorporating biometric adherence measures into clinical trials for doxycycline PEP/PrEP will be fit for purpose to extrapolate across the currently available doxycycline hyclate formulation options. Establishing bioequivalence for doxycycline IR and DR PK parameters would be a costly effort that to the best of our knowledge was not required of Mayne Pharmaceutics in their original licensing of their DR product. Small differences in PK parameter estimates between these formulations will not negate the utility of extrapolating our PK findings to make recommendations for adherence interpretations across formulations. As such, we will confirm the expected similarities of IR and DR doxycycline PK profiles during the single-dose phase of our PK study by randomizing an equal number of participants (sex-balanced) for each formulation.

The purpose of this study is to validate analytical tools that may be used to estimate adherence to doxycycline PEP/PrEP, which is largely taken on an on-demand, or event-driven, basis. During the validation of TFV-DP as a biometric measure of PrEP adherence, high inter-individual variability in TFV-DP DBS concentrations was associated with overlapping concentration profiles observed between individuals assigned to take 33%, 67% and 100% of directly observed daily PrEP doses [23]. Despite this inter-individual variability, the TFV-DP biometric adherence assessment tool has proven to outperform self-reported adherence measures across a wide array of study designs and populations and is more strongly correlated with outcomes of virologic failure in people taking HIV treatment. Further, a desirable attribute of our modeling approach will be the ability to inform fit to our population PK model with single dose data derived from both formulations to affirm this postulate or inform a different set of recommendations.

Given the heterogeneity in doxycycline formulations and how doxycycline may be used in different populations (DoxyPEP, treatment for neurosyphilis), capturing the frequency of gastrointestinal (GI)-related adverse events throughout the protocol will be beneficial and provide a safety readout throughout different product exposures. While this study will focus on comparing the frequency of GI-related AEs with daily vs intermittent DR doxycycline use, we will also document the number of observed and/or reported GI-related AEs single-dose IR and DR doxycycline administration. While doxycycline is commonly used and well-tolerated, these results will yield clinically important information to guide prescribers and implementation scientists in designing doxycycline prevention programs with maximized public health benefits.

Doxycycline PK parameters in plasma and urine have been previously established; however, less is known about its distribution in other biological matrices and its potential detectability using minimally invasive sampling techniques. Expanding our understanding of doxycycline distribution across multiple compartments is essential for optimizing dosing strategies, particularly in the context of adherence monitoring and biomedical prevention efforts. In this study, we will collect plasma, hair, DBS, anorectal and vaginal secretions, and urine from healthy volunteers. Participants will initially receive a single dose of doxycycline, administered as either the IR (2 x100mg) or DR (200mg) formulation and will be followed for 14 days. On Day 14, participants will begin the multi-dose phase of the study based on the second randomization that occurred at enrollment. During this time, they will take 200mg doxycycline daily or intermittently (every 72 hours) for 10 days, using the DR formulation. Biological samples (plasma, hair, DBS, anorectal and vaginal swabs and urine) will be collected throughout this 10-day dosing period and for 14 days following their last dose. While plasma and urine PK of doxycycline have been previously described, this study aims to expand on existing data by incorporating DBS, volumetric absorptive microsampling (VAMS) using the Tasso+ device, and hair samples. These alternative sampling methods will support a more comprehensive assessment of drug distribution and explore less-invasive strategies for monitoring doxycycline exposure and adherence and are particularly relevant for future implementation in real-world settings where conventional PK sampling may not be feasible. Of note, the evaluation of doxycycline in hair samples using IR-MALDESI MSI will facilitate the evaluation of granular accumulation of doxycycline in hair, which can provide greater resolution on intermittent/event-driven product use. Additionally, we will utilize bioanalytical assays that are 1.5-log fold more sensitive than those employed by Haaland (plasma doxycycline LLOQ from Haaland study: 10 ng/mL; plasma LLOQ to be employed in this study: 0.5 ng/mL). The increased analytical sensitivity of the methods to be employed in this study will allow us to evaluate terminal clearance kinetics of doxycycline and determine the duration in which doxycycline is quantifiable after dosing. Lastly, the data generated in this study may be leveraged to build multi-compartment PK models that can be used to conduct simulations to determine doxycycline exposure and adherence under different dosing schedules.

2.0 STUDY OBJECTIVES

The primary, secondary and exploratory objectives of the study are listed below. Endpoints related to each objective are described in Section 8.

2.1. PRIMARY OBJECTIVE(S)

The primary objectives are:

2.1.1 To estimate the single-dose plasma PK parameters of 200mg doxycycline hyclate immediate release (IR) and delayed release (DR) formulations.

2.1.2 To summarize doxycycline plasma concentrations following 10 days of daily or intermittent dosing.

2.1.3 To characterize differences in doxycycline plasma concentrations 24 hours (C_{24}) post-dose between intermittent and daily doxycycline following 10 days of dosing.

2.2. SECONDARY OBJECTIVES

The secondary objectives are:

2.2.1 To assess gastrointestinal (GI)-related adverse events for daily and intermittent doxycycline.

2.2.2 To estimate the single dose PK parameters of 200mg doxycycline hyclate IR and DR in DBS, anorectal fluid and vaginal fluid.

2.2.3 To estimate the single dose pooled PK parameters of 200mg doxycycline hyclate IR and DR in urine.

2.2.4 To summarize doxycycline concentrations in DBS, urine, anorectal fluid and vaginal fluid for intermittent and daily doxycycline.

2.3. EXPLORATORY OBJECTIVES

The exploratory objectives are:

2.3.1 To evaluate the feasibility of measuring doxycycline via DBS and volumetric absorptive microsampling (VAMS/Tasso+ device; dried whole blood) to estimate plasma concentrations.

2.3.2 To develop a popPK model describing the compartmental PK of doxycycline and simulate concentrations under various dosing conditions, as well as use physiologically based pharmacokinetic (PBPK) modeling of the data observed herein alongside published CSF data to predict concentrations in the central nervous system given these dosing conditions.

2.3.3 To compare the single dose safety profiles of IR and DR doxycycline.

2.3.4 To characterize doxycycline concentration patterns in hair strands using infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI) mass spectrometry imaging (MSI) and correlate these images with daily and intermittent dosing patterns.

2.3.5 To perform exploratory laboratory assessments that may include evaluation of factors related to STIs and laboratory assays related to the study objectives.

3.0 STUDY DESIGN

This is a Phase I, single-site, open-label randomized study, during which participants will undergo two randomizations.

Sixteen participants will be randomized (1:1, by sex) to receive a single dose of 200mg doxycycline hyclate as either 1) one 200mg DR tablet or 2) two 100mg IR tablets. Following the administration of this single dose, specimens will be collected over a 14-day period.

Based on the second randomization (which occurred at enrollment), participants (1:1, by sex) will receive 200mg doxycycline hyclate DR either daily (every 24 hours; n=8) or intermittent (every 72 hours; n=8) for 10 days. Following the administration of these doses, participants will be followed for 14 days.

All doses will be directly observed (in-person or virtual) by study staff.

3.1. PARTICIPATING SITES

The participating clinical site is listed in the Study Specific Procedures (SSP) Manual. The single participating site will be in the United States.

3.2. STUDY DURATION

Study duration is approximately one year. Accrual will require approximately 10 months, with individual participants being followed on study for approximately 37 days.

4.0 STUDY POPULATION

Sixteen healthy individuals who are not living with HIV and have not been diagnosed with a current bacterial STI (chlamydia, gonorrhea, syphilis) will be included in this study. Healthy is defined as having no clinically relevant abnormalities identified by a detailed medical history, comprehensive physical examination and clinical laboratory tests.

Participants will be selected for the study according to the criteria in Section 4.1 and 4.2 and the co-enrollment guidelines in Section 4.4. Participants will be recruited, screened, and enrolled as described in Section 4.3. Requirements related to participant retention and withdrawal from the study are described in Sections 4.5 and 4.6, respectively.

4.1. INCLUSION CRITERIA

Individuals who meet the following criteria will be eligible for study inclusion:

1. Aged 18 to 65 years of age at the time of screening
2. Able and willing to follow study participation requirements and provide informed consent to take part in the study

3. Has a non-reactive/negative HIV test results at screening per applicable algorithm in Appendix II
4. Has and is able to maintain a caput (head) of hair, that has not been chemically treated (defined as hair that has been bleached, permed, relaxed or dyed/colored) and is greater than one centimeter in length for the duration of the study
5. *For females of reproductive potential*: Has a negative urine pregnancy test at screening
6. *For females of reproductive potential*: Using at least two effective methods of contraception for at least 30 days (inclusive) prior to enrollment and intending to use an effective method of contraception for the duration of study participation. It is strongly recommended that at least one barrier method (e.g. condoms) in addition to a hormonal contraception method be used. Examples of acceptable and effective methods include:
    a. Hormonal methods (oral pills, vaginal ring, depo, transdermal or implant)
    b. Intrauterine device (IUD) inserted at least 30 days prior to enrollment
    c. Surgical sterilization (of participant or partner(s)) including bilateral tubal ligation or vasectomized male partners
    d. Barrier methods (condom with/without spermicide, sponge, cervical cap, diaphragm)
    e. Self-identifies as having same sex partners
    f. Self-reported sexually abstinent as defined by abstaining from penile-vaginal intercourse for 90 days prior to enrollment and intending to remain sexually abstinent for the duration of study participation
7. Has access to a smartphone and/or laptop and is able and willing to participate in video-based communications with study staff for directly observed dosing requirements
8. In good general health, in the opinion of the investigator of record (IoR) or designee and has no medical condition that would adversely impact the conduct of the study (inclusive of self-reported conditions and/or those found upon medical history and examination or in available medical records). This includes, but is not limited to, having an intact, healthy gastrointestinal tract (without damage or functional disruption) and the ability to swallow pills

4.2. EXCLUSION CRITERIA

Individuals who meet any of the following criteria will be excluded from this study:

1. Per participant report, planned or active use of any anticonvulsants at screening and an unwillingness to restrict use of certain medications (iron, antacids, etc.) for the duration of the study
2. *For females of reproductive potential*: Pregnant or currently breastfeeding, or intends to become pregnant and/or breastfeed during the study
3. Has any of the following laboratory abnormalities:
    a. An estimated calculated creatinine clearance (CrCl) less than 60 mL/min by the Cockcroft-Gault formula at screening
    b. Positive for hepatitis B surface antigen (HBsAg) at screening

c. Has a Grade 2 or higher clinically significant laboratory abnormality as defined by The Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1 - July 2017, except for CrCl level of 75 mL/min or higher at enrollment

4. Per participant reported symptoms or clinical and/or laboratory diagnosis of an active pharyngeal, anorectal, or reproductive tract infection (RTI) requiring treatment at screening and enrollment per current CDC guidelines (https://www.cdc.gov/std/treatment-guidelines/default.htm). Infections requiring treatment include *Neisseria gonorrhoeae* (GC), *Chlamydia trachomatis* (CT), syphilis, active herpes simplex virus (HSV) lesions, or symptomatic genital warts, chancroid, pelvic inflammatory disease (PID), bacterial vaginosis (BV), symptomatic vaginal candidiasis, and trichomoniasis
5. Participation in research studies involving drugs, products, or vaccines within 30 days of the enrollment and for the duration of the study
6. Has donated blood within 8 weeks of enrollment of approximately 1 pint (550 mL)
7. Has a known allergy (adverse reaction) to any of the components of the study product, including known hypersensitivity to tetracycline-class antibiotics
8. Prior use of doxycycline or any other tetracycline-class antibiotic within 30 days prior to enrollment
9. Has an active infection that may be responsive to treatment with doxycycline or another tetracycline antibiotic
10. Has evidence or history of any other condition (e.g., gastrectomy, seizure disorder), that, in the opinion of the IoR or designee, would make study participation unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving study objectives

4.3. RECRUITMENT PROCESS

The site will be responsible for developing appropriate recruitment processes and informational materials that are geared towards their local community. Recruitment processes and strategies will be defined in site standard operating procedures (SOPs). Strategies may include a variety of methods including, but not limited to, disseminating advertising materials throughout public areas community (outpatient clinics, universities, community-based locations) and the site clinic and/or online engagement via networking applications or social media. All recruitment materials will undergo approval by the local Institutional Review Board (IRB) prior to use. Advice regarding these materials may be sought from local and site community representatives before they are submitted to the site IRB for review and approval.

4.4. CO-ENROLLMENT GUIDELINES

Participants must not take part in any other concurrent research studies involving drugs or medical devices during their participation in the study. Co-enrollment in observational or other studies may be allowable with approval of the site IoR or designee and Protocol Chair(s).

Should any participant report concurrent participation in contraindicated studies after enrolling in the study, the IoR or designee will consult the Clinical Management Committee (CMC) regarding ongoing product use and other potential safety considerations associated with co-enrollment.

4.5. PARTICIPANT RETENTION

Once a participant enrolls in this study, the study site will make every effort to retain them in the study for the entire follow up period, to minimize bias associated with loss-to-follow up. Study site staff are responsible for developing and implementing local strategies, outlined in an SOP, to target this goal and ensure high rates of retention. Components of such procedures may include, but not limited to:

- Thorough explanation of the study visits schedule and procedural requirements during the informed consent process and re-emphasis at each study visit
- Thorough explanation of the importance of both treatment groups to the overall success of the study
- Collection of detailed locator information at the study Screening Visit, and active review and updating of this information at each subsequent visit
- Use of appropriate and timely visit reminder mechanisms and provision of visit calendars
- Immediate and multifaceted follow-up on missed visits

4.6. PARTICIPANT WITHDRAWAL

Regardless of the participant retention methods described in Section 4.5, participants may voluntarily withdraw from the study for any reason at any time. The IoR or designee may also withdraw participants from the study to protect their safety and/or if they are unwilling or unable to comply with the required study procedures after consultation with the CMC.

Once a participant is enrolled in the study, the study site will make every reasonable effort to retain the participant for the entire study duration so that the participant is evaluable. Participants may be replaced at the discretion of the Protocol Chair(s), in consultation with the CMC.

Participants also may be withdrawn if the study sponsor, government or regulatory authorities, or site IRB terminate the study prior to its planned end date. For participants who terminate from the study prior to the scheduled study end, every reasonable effort will be made to complete a final evaluation (as described in Section 6.5). Study staff will record the reason(s) for all withdrawals from the study in participants' study records.

5.0 STUDY PRODUCT CONSIDERATIONS

5.1. STUDY PRODUCT REGIMEN(S)

Participants will be randomized at enrollment to receive a single dose of doxycycline hyclate as 1) one 200mg DR tablet or 2) two 100mg IR tablets. Post dose samples will be collected according to the study schema (Figure 1).

On Day 14, participants will begin their assigned dosing group (which was also randomized at enrollment) to receive 200mg doxycycline hyclate DR either daily (every 24 hours) or intermittently (every 72 hours) for 10 days. Study samples will be collected post dose in accordance with the study schema (Figure 1).

Table 1: Study Product Regimen

Study Phase	N	Group	Product
Single Dose PK Phase*	8	DR	One 200mg doxycycline hyclate DR tablet, once
	8	IR	Two 100mg doxycycline hyclate IR tablets, once

**Observed dose in person*

Study Phase	N	Group	Product
Multi-Dose PK Phase**	8	Daily	200mg doxycycline hyclate DR tablet every 24 hours
	8	Intermittent	200mg doxycycline hyclate DR tablet every 72 hours

***Observed dose in person or via video call*

5.2. STUDY PRODUCT ADMINISTRATION

Study participants will be given detailed instructions on the administration regimen they are assigned to. All doses of doxycycline hyclate will be administered orally and directly observed by study staff either in person or via secure video calls.

5.3. STUDY PRODUCT FORMULATION, SUPPLY, DISPENSING AND STORAGE

Doxycycline tablets will be supplied as doxycycline hyclate 200mg DR and 100mg IR tablets. Both the 100mg IR tablets and the 200mg DR tablet will be administered once, during the single dose phase. After the single dose PK phase, the 200mg DR tablets will be dispensed for daily or intermittently (every three days) administration for 10 days.

The doxycycline hyclate 100mg IR and doxycycline 200mg DR tablets will both be purchased by FHI 360 from McKesson.

Study products will be dispensed by the pharmacist to enrolled study participants or to study staff on behalf of the participant, upon receipt of a written prescription from an authorized prescriber. An authorized prescriber includes the IoR, or a licensed clinician designated as a sub-investigator on the FDA Form 1572. Participants will receive a quantity of study product sufficient to ensure an adequate supply for the study duration.

Further details on dispensation will be outlined in the SSP for study product considerations.

Until dispensed to the participants, all study products should be stored in a securely locked, temperature-controlled area, accessible only to authorized site personnel. Doxycycline tablets should be stored at a controlled room temperature of 25°C (77°F); excursions are permitted between 15°C and 30°C (59°F and 86°F).

5.4. STUDY PRODUCT ACQUISITION AND ACCOUNTABILITY

5.4.1 Acquisition

The National Institute of Allergy and Infectious Diseases (NIAID) Clinical Research Products Management Center (CRPMC) will supply the study product received from McKesson. The study site pharmacist can obtain the study product through the CRPMC by following the instructions in the Pharmacy Guidelines and Instructions for Division of AIDS (DAIDS) Clinical Trial Networks, and instructions in the SSP Manual.

Doxycycline 100 mg tablets (IR) will be manufactured at Zydus Pharmaceuticals. Doxycycline 200 mg tablets (DR) will be manufactured at Solco Healthcare US LLC. At the time product is purchased, supply chain issues may necessitate a different manufacturer. In either case, the CRPMC will receive the required documentation from the respective manufacturer.

5.4.2 Study Product Accountability and Retrieval

The study site pharmacist or designee is required to maintain complete accountability records of study product received and subsequently dispensed to study participants. This includes acknowledgment of receipt of each shipment of study product (quantity and condition). All unused study product dispensed to participants must be returned to the site pharmacy, documented and placed in quarantine. Once the study is complete or if it is terminated early, the unused return product will be destroyed by the site.

5.5. CONCOMITANT, PROHIBITED, AND PRECAUTIONARY MEDICATIONS

Except for those listed as prohibited or restricted listed below, participants may use concomitant medications during the study. All concomitant medications, including prescription medications, hair growth medications such as Minoxidil, over-the-counter products, vitamins, nutritional supplements, and herbal preparations—taken within 30 days prior to enrollment and throughout the study, including those used to treat adverse events (AEs), will be recorded on forms designed for that purpose. Prescription and non-prescription medications will be recorded on the case report forms (CRFs) and all others (such as vitamins, nutritional supplements and herbal preparations) will be retained in the participant's file.

5.5.1 PROHIBITED MEDICATIONS

Participants will be advised not to use the following products within 30 days prior to enrollment or during study conduct: iron and anticonvulsants, such as barbiturates, phenytoin and carbamazepine, which may reduce the half-life of doxycycline.

Throughout study participation, participants will be counseled not to use iron and antacids or Pepto-Bismal (including bismuth, magnesium, calcium and aluminum) within 4 hours of dosing as these agents interfere with doxycycline absorption. Should a participant report the use of such medications, sample collection will be performed at the discretion of the IoR or designee.

6.0 STUDY PROCEDURES

An overview of the study visits and procedures is presented in Appendix I. Presented below is additional information on specific study procedures. Additional detailed instructions to guide and standardize all study procedures across sites will be provided in the SSP Manual. Visit windows and visit coding are described in the Data Management SSP Manual.

As part of recruitment efforts, study staff may pre-screen potential participants at on-site or off-site locations. During these interactions, staff may explain the study and assess basic eligibility factors (e.g., willingness to use the study product or follow study procedures). These assessments will be confirmed during an on-site screening visit. Information such as the number of individuals approached and those who appear eligible may be recorded and stored at the study site without written informed consent, as long as no identifying information is collected—unless a waiver has been granted by the local IRB/IEC. All procedures and documentation will comply with local IRB/IEC guidelines.

6.1. SCREENING VISIT

The purpose of the Screening Visit is to assess the prospective participant's eligibility. It is the responsibility of the site to determine the best approach to screening. For each participant, independent written informed consent will be obtained before any study procedures are initiated. Screening procedures may occur over one or more visits [up to and including the day of enrollment], if necessary to complete all required procedures. The Enrollment Visit must occur within 30 days of blood collection for HIV testing at the Screening Visit.

Screening procedures will include:

Administrative

- Review study overview and obtain written informed consent
- Assign Participant Identification (PTID) number
- Assess eligibility
- Collect demographic/locator information

- Provide reimbursement
- Schedule next visit/contact

Counseling

- Provide HIV pre- and post-test counseling
- Provide HIV/STI risk reduction counseling (including provision of condoms)
- Provide protocol adherence counseling

Clinical

- Collect medical history, including concomitant medications
- Perform physical examination (including obtaining vital signs (including vitals, blood pressure [BP], heart rate [HR], respiratory rate [RR], weight, orthostatic BPs and HR)
- Offer Hepatitis B vaccination or referral, if indicated

Laboratory

- Collect blood
    - Complete blood count (CBC) with platelets
    - Chemistry testing (creatinine, BUN, AST, ALT, total bilirubin)
        - Calculated creatinine clearance (CrCl) of at least 60 mL/min by the Cockcroft-Gault formula at screening
    - HIV testing (See Appendix II)
    - Hepatitis B surface antigen (HBsAg)
    - Hepatitis B core antibody (HBcAb; total) or Hepatitis B surface antibody (HBsAb) [*may be performed at screening or enrollment*]
    - Syphilis serology (RPR titer with reflex to TPHA if required)

- Collect urine
    - Urine dipstick (protein and glucose)
    - NAAT for gonorrhea (GC)/chlamydia (CT)
    - Urine pregnancy test (*For females of reproductive potential*)

- Collect pharyngeal (throat) swab
    - NAAT for GC/CT

- Collect mucosal fluids
    - Anorectal
        - NAAT for GC/CT
    - Penile
        - NAAT for GC/CT
    - Vaginal
        - NAAT for GC/CT

If collection of vaginal and/or penile swabs is declined or not feasible, NAAT testing using urine is acceptable.

STI testing will be performed at local laboratories. Participants will be treated or referred for treatment of STIs as per local guidelines. Symptomatic screening for STIs beyond what is required by the protocol will be at a site's discretion and cost. The participant will also be referred to or treated for any pharyngeal, anorectal, or reproductive tract infections, as needed.

Participants will be tested for HBsAg at screening. Participants who have a positive result for HBV will not be enrolled in the study and will be referred to their primary care provider for management. Participants who do not have evidence of immunity to HBV (e.g., negative HBsAb) will be offered HBV vaccination.

Sites will follow the HIV testing algorithm for screening as described in Appendix II. For enrollment of participants without HIV: If any result or interpretation other than negative or non-reactive is obtained for any HIV test, the person is not eligible for the study. Additional testing to confirm suspected HIV infection will be performed during Screening in accordance with local guidelines. If HIV infection is confirmed, participants will receive counseling and be referred for appropriate care, as necessary.

Individuals deemed not eligible will be informed that they do not meet the eligibility criteria for the study and will be referred to appropriate medical care, if necessary.

Potential participants may be rescreened once at the discretion of the IoR or designee. Further re-screening for administrative reasons may be permitted with the approval of the CMC.

6.2. ENROLLMENT AND SINGLE-DOSE DOSING VISIT (DAY 0)

Enrollment must occur no more than 30 days after screening. Participants will be considered enrolled once they have been randomized to treatment/use group. Enrollment procedures will include:

Administrative

- Assess and confirm eligibility
- Review/update locator/contact information
- Provide reimbursement
- Schedule next visit/contact
- Assess adverse events and social harms, as appropriate
- Perform randomizations:
    - Treatment Group (1x 200mg doxycycline hyclate DR tablet or 2x 100mg doxycycline hyclate IR tablets)
    - Use Group (*Daily/Every 24 hours* or *Intermittent [Every72 hours] Use*)

Counseling

- Provide HIV pre- and post-test counseling
- Provide HIV/STI risk reduction counseling (including provision of condoms)
- Provide protocol adherence counseling

Clinical

- Collect medical history, including concomitant medications
- Perform targeted physical examination, if indicated (including vitals, BP, HR, RR, weight, orthostatic BPs and HR)
- Treat or refer for treatment for pharyngeal, anorectal, or reproductive tract infections

Product Administration (directly observed dose)

- DR: Single dose of one 200mg doxycycline hyclate DR tablet
- IR: Single dose of two 100mg doxycycline hyclate IR tablets

Laboratory

- Collect blood
    - HIV testing (See Appendix II)
    - PK samples collected at 1, 2, 3, 4, 6, and 8 hours
        - Plasma
        - DBS
    - Plasma for storage
    - DBS for storage

- Collect mucosal fluids
    - Anorectal swab samples collected at 1, 2, 3, 4, 6, and 8 hours
    - Vaginal swab samples collected at 1, 2, 3, 4, 6, and 8 hours

- Collect urine
    - Urine Pregnancy Test (*For females of reproductive potential*)
    - PK sample collected at two timepoints (timing based on participants' ability to urine)
        - 1-4 hours post dose
        - 4-8 hours post dose

The HIV testing algorithm at enrollment is described in Appendix II. Prior HIV test results must be negative/non-reactive for enrollment; results from samples collected for HIV testing at the Enrollment Visit are not required to proceed with enrollment.

6.3. MULTI-DOSE DOSING VISITS (DAYS 14-23)

Participants, based on their randomization assignment, will begin taking 200mg doxycycline hyclate DR as follows:

- Daily (Group 1): Every 24 hours on days 14, 15, 16, 17, 18, 19, 20, 21, 22, 23.
- Intermittent (Group 2): Every 72 hours on days 14, 17, 20, and 23.

See Schedule of Study Visits and Procedures (Appendix I) for additional administrative, clinical and laboratory procedures to be conducted at this visit.

All doses will be directly observed by study staff, either in person or via video calls.

If sampling is scheduled to occur on the same day as an assigned dose, all samples will be collected before the subsequent dose is given. Collection of blood samples will be prioritized first, if possible.

6.4. SAMPLING VISITS (DAYS 1-35)

PK sample collection will occur on Days 1, 2, 3, 4, 7, 10, 14, 15, 18, 21, 24, 26, 29, 32, and 35 across the single-dose and multi-dose PK phases.

6.4.1 Single Dose PK On Days 1 – 14

Laboratory

- Collect blood (6 mL Potassium-EDTA tube for plasma and DBS PK)
    - Day 1: Single PK sample at 24 hours post last dose
    - Days 2, 3, 4, 7, 10, and 14: Single PK sample

- Collect VAMS (Tasso+ collection device or equivalent) for dried whole blood PK
    - Day 1, Single PK sample at 24 hours post last dose
    - Day 3, Day 7

- Collect mucosal fluids
    - Anorectal swabs
        - Day 1: Single PK sample at 24 hours post last dose
        - Days 2, 3, 4, 7, 10, and 14: Single PK sample
    - Vaginal swabs
        - Day 1: Single PK sample at 24 hours post last dose
        - Days 2, 3, 4, 7, 10, and 14: Single PK sample

- Collect urine
    - Day 1: Single PK sample at 24 hours post last dose
    - Days 2, 3, 4, 7, 10, and 14: Single PK sample
    - Day 14: Urine pregnancy test (For females of reproductive potential)

- Collect hair
    - Plucked strands (about 5 strands)
        - Days 3, 7, 10, and 14: Single PK sample
    - *Cut hair:* (Optional; ~100 strands, cut from the base of the caput)*
        - Day 14: Single PK sample 24 hours after the last dose

See Schedule of Study Visits and Procedures (Appendix I) for additional administrative, clinical and laboratory procedures to be conducted at this visit.

6.4.2 Multi-Dose PK On Days 15 – 35

Laboratory

- Collect blood (6 mL Potassium-EDTA tube for plasma and DBS PK)
    - Days 15, 18, 21, and 24: Single PK sample 24 hours after the last dose
    - Days 26, 29, 32, and 35: Single PK sample

- Collect mucosal fluids (anorectal and vaginal swabs)
    - Days 15, 18, 21, and 24: Single PK sample 24 hours after the last dose
    - Days 26, 29, 32, and 35: Single PK sample

- Collect urine
    - Days 15, 18, 21, and 24: Single PK sample 24 hours after the last dose
    - Days 26, 29, 32, and 35: Single PK sample

- Collect hair
    - Plucked strands (about 5 strands)
        - Days 15, 18, 21, and 24: Single PK sample 24 hours after the last dose
        - Days 26, 29, 32, and 35: Single PK sample
    - *Cut hair* (Optional; ~100 strands, cut from the base of the caput)*
        - Day 24: Single PK sample 24 hours after the last dose

**For potential future testing; pending funding availability.*

See Schedule of Study Visits and Procedures (Appendix I) for additional administrative, clinical and laboratory procedures to be conducted at this visit.

If sampling is scheduled to occur on the same day as an assigned dose, all samples will be collected before the subsequent dose is given. Collection of blood samples will be prioritized first, but participants may yield urine based on urgency at any time during the visit and prior to the subsequent dose.

6.5. FINAL STUDY VISIT (DAY 37)

Procedures to be conducted at the final study visit include:

Administrative

- Review/update locator/contact information
- Provide reimbursement
- Assess adverse events and social harms, as appropriate

Clinical

- Perform targeted physical examination, if indicated (vitals, BP, HR, RR, weight, orthostatic BP and HR)
- Medical history, including concomitant medication
- Treatment and referrals for STIs

Laboratory

- Collect blood
  - Single PK sample (6 mL Potassium-EDTA tube for plasma and DBS PK)
  - HIV testing (See Appendix II)
  - CBC and platelets
  - Chemistry testing (creatinine with a calculated CrCl, BUN, AST, ALT, total bilirubin)
  - Syphilis serology

- Collect urine
  - NAAT for GC/CT
  - Urine pregnancy test (*For females of reproductive potential*)
  - Single PK sample

- Collect hair
  - Plucked strands (about 5 strands)
  - *Cut hair* (Optional; ~100 strands, cut from the base of the caput)*

- Collect pharyngeal (throat) swab
  - NAAT for GC/CT

- Collect mucosal fluid
  - Anorectal
    - Single PK
    - NAAT for GC/CT
  - Penile
    - NAAT for GC/CT
  - Vaginal
    - Single PK
    - NAAT for GC/CT

**For potential future testing; pending funding availability.*

If collection of vaginal and/or penile swabs is declined or not feasible, NAAT testing using urine is acceptable. See Schedule of Study Visits and Procedures in Appendix I for further procedures to be conducted at this visit.

6.6. Procedures for Participants with Suspected or Confirmed HIV Infection

Individuals who have one or more reactive/positive HIV tests at screening are not eligible to participate in this study. The CMC must be notified of any reactive or positive HIV test result obtained. Participants who have any reactive or positive HIV test result at Enrollment and at study end will be referred for care and confirmatory testing according to local standards of care. The participant may not receive additional doses of study product if they have a reactive or positive HIV test, even if further testing indicates that they do not have HIV infection. In accordance with the policies of the US NIH, participants must receive their HIV test results to take part in this study. Participants will be provided with their HIV test results in the context of post-test counseling.

6.7. Procedures for Participants with Suspected or Confirmed pharyngeal, anorectal, or reproductive tract infections

Individuals who are diagnosed with an active pharyngeal, anorectal, or reproductive tract infection at screening are not eligible to participate in this study. Participants who are diagnosed at screening and at the end of study will be treated or referred for care according to local standards of care. The IoR or designee must manage these conditions per current CDC guidelines, available at https://www.cdc.gov/std/treatment-guidelines/toc.htm. If participants are diagnosed with an pharyngeal, anorectal, or reproductive tract infection at end of study the CMC should be notified.

6.8. Procedures for Participants Who Discontinue Study Product Early

Enrolled participants are free to withdraw from participation in the study at any time and for any reason and/or the site IoR or designee may choose to discontinue a participant. Product use may also be temporarily held in response to a clinical event or at the participants' request. If they receive study product, they will be asked to complete a follow-up safety visit. Reasons for participant discontinuation/withdrawal from the study will be documented in the study database.

6.9. Contraceptive Use and Pregnancy

To avoid pregnancy-related confounding factors, female participants who can become pregnant are required to use of an effective method of contraception, including an IUD, hormonal contraception, sterilization, or condoms as outlined in Section 4.1. All participants should also use condoms for prevention of HIV and other STIs. As needed, study staff will provide contraceptive counseling to enrolled participants throughout the duration of study participation and will facilitate access to contraceptive services through direct service delivery and/or active referrals to local service providers.

Female participants of reproductive potential will have pregnancy testing performed as outlined in the Schedule of Study Visits and Procedures in Appendix I. Participants will be encouraged to report any signs or symptoms of pregnancy to study staff.

If a participant becomes pregnant, study product will be discontinued, and the participant will be followed until pregnancy outcome is reached, if the participant agreeable or it is determined that the pregnancy outcome cannot be ascertained. See the Laboratory Manual for details regarding visit procedures and specimens to be collected at follow-up visits in the event of pregnancy. The site IoR or designee will counsel any participants who become pregnant regarding possible risks to the fetus according to site-specific SOPs. The study team will proactively provide any participants who become pregnant with contact information for a local obstetrics and gynecology provider who can assist with prenatal care coordination and provide access to related medical and support resources, as needed. However, the site will not be responsible for paying for pregnancy-related care. All pregnancy outcomes will be reported on relevant CRFs. Once pregnancy outcome is reached, the participant will be terminated from the study.

Participants may not enroll if they are currently breastfeeding, and study product should be discontinued if any participant identifies that they are breastfeeding after enrollment.

6.10. INTERIM CONTACTS AND VISITS

Interim contacts and visits (those between regularly scheduled follow up visits) may be performed at participant request or as deemed necessary by the IoR or designee at any time during the study. All interim contacts and visits will be documented in participants' study records and on applicable CRFs.

Some interim visits may occur for administrative reasons. For example, the participant may have questions for study staff. Interim visits for which no data is collected are not documented on CRFs. Other interim contacts and visits may occur in response to AEs experienced by study participants. Study staff will inquire about AEs participants might have experienced as a result of the study product or procedures performed during the preceding visit. When interim contacts or visits are completed in response to participant reports of AEs and/social harms, study staff will assess the reported event, record the event on the appropriate CRF, and provide or refer the participant to appropriate medical care. Please refer to the Study Procedures SSP for further details regarding interim visits.
Safety Monitoring and Adverse Event Reporting

7.0 SAFETY MONITORING AND ADVERSE EVENT REPORTING

7.1 ADVERSE EVENT DEFINITION AND REPORTING

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 study product.

With appropriate permission of the participant, whenever possible, records from all non-study medical providers related to AEs will be obtained and required data elements will be recorded on study CRFs. All participants reporting an AE will be followed clinically until the AE resolves (returns to baseline) or stabilizes.

Staff will document in source documents and the appropriate CRF all AEs (Grade 1 and higher) reported by or observed in enrolled study participants (from the enrollment visit through the termination visit) regardless of severity and presumed relationship to study product. See Section 7.5 for information on grading of severity of events.

7.2 Safety Monitoring and Clinical Data Review

A multi-tiered safety review process will be followed for the duration of this study. The study site IoR or designee is responsible for the initial evaluation and reporting of safety information at the participant level, and for alerting the CMC if unexpected concerns arise. Detailed SOPs describing methods for AE reporting and toxicity management to ensure that AEs are reported and managed in accordance with the protocol and for alerting the CMC (outlined below) if unexpected concerns arise are required to be developed by the site. For life-threatening events, they will also be instructed to seek immediate emergency care. Where feasible and medically appropriate, participants will be encouraged to seek evaluation where the study clinician is based, and to request that the clinician be contacted upon their arrival.

Close cooperation between the Protocol Chair(s), site IoR or designee(s), DAIDS Medical Officer, LOC CRM, Clinical Research Physician, HPTN Laboratory Center (LC), and other study team members will be necessary to monitor participant safety and to respond to occurrences of toxicity in a timely manner. The study team will have regularly scheduled conference calls during the period of study implementation, and additional ad hoc calls will be convened if necessary.

A sub-group of the Protocol Team, including the site IoR or designee, the Protocol Chairs, DAIDS Medical Officer, DAIDS Protocol Pharmacist, and HPTN Pharmacist, will serve as members of the CMC. Representatives from the HPTN LOC and HPTN LC will be included as observers. The CMC provides support to site clinicians regarding individual participant clinical management (e.g., questions related to eligibility, toxicity management, clinical holds of study product, permanent discontinuations, etc.).

The data management team will review incoming safety data for completeness and consistency on an ongoing basis. Events identified as questionable, inconsistent, or unexplained will be queried for verification. AE reports submitted in an expedited manner to the DAIDS Safety Office will be forwarded to the DAIDS Medical Officer for review.

7.3 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 product.

The relationship of all AEs to study product will be assessed as specified in Version 3.0, dated June 2025, of the Manual for Expedited Reporting of Adverse Events to DAIDS, which is available on the DAIDS Regulatory Support Center (RSC) website at https://rsc.niaid.nih.gov/clinical-research-sites/manual-expedited-reporting-adverse-events-daids.

7.4 EXPEDITED ADVERSE EVENT (EAE) REPORTING

Requirements, definitions, and methods for expedited reporting of adverse events are outlined in Version 3.0, dated June 2025, of the Manual for Expedited Reporting of Adverse Events to DAIDS, which is available on the DAIDS RSC website at https://rsc.niaid.nih.gov/clinical-research-sites/manual-expedited-reporting-adverse-events-daids.

7.5. REPORTING TO DAIDS

The DAIDS Adverse Experience Reporting System (DAERS), an internet-based reporting system, must be used for EAE reporting to DAIDS. In the event of system outages or technical difficulties, EAEs may be submitted via the DAIDS EAE form. This form is available on the DAIDS RSC website at https://rsc.niaid.nih.gov/clinical-research-sites/paper-eae-reporting.

For questions about DAERS, please contact NIAID CRMS Support at CRMSSupport@niaid.nih.gov. Please note that site queries may also be sent from within the DAERS application itself.

For questions about expedited reporting, please contact the DAIDS RSC Safety Office at DAIDSRSCSafetyOffice@tech-res.com.

7.6. REPORTING CATEGORY FOR THIS STUDY

The Serious Adverse Event (SAE) Reporting Category, as defined in Version 3.0, dated June 2025,of the Manual for Expedited Reporting of Adverse Events to DAIDS, will be used for this study.

These reporting requirements are for each study participant from enrollment until follow-up in the study ends. After this time, the site must report serious, unexpected, clinical suspected adverse drug reactions if the study site becomes aware of the event on a passive basis, i.e., from publicly available information.

The study product for which expedited reporting is required is doxycycline hyclate, 100mg IR or 200mg DR tablet.

In addition to submitting EAE information to the DAIDS RSC Safety Office via DAERS, the site IoR or designee may be required to submit AE information to local regulatory agencies or other local authorities. Site staff will report information regarding AEs to

their responsible IRB in accordance with all applicable regulations and responsible IRB requirements.

7.7. GRADING SEVERITY OF EVENTS

The *Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1, July 2017*, will be used for determining and reporting the severity of AEs. The DAIDS grading table is available on the DAIDS RSC website at https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables.

7.8. TOXICITY MANAGEMENT

In general, the IoR or designee has the discretion to hold the study product at any time if they feel that continued product use would harm the participant or interfere with treatment deemed clinically necessary according to their judgment. In addition, as described in section 7.2, the CMC's responsibilities will also include consultation and decision-making regarding eligibility for study participation, management of toxicities, and study product administration, including product resumption after certain types of toxicities and permanent discontinuation.

7.8.1 General Criteria for Adverse Event Management

The following general guidance refers to all AEs; some require consultation with the CMC. The CMC should be notified within 48 hours of site awareness of the AE. Investigators or designee should also consult the CMC for further guidance in restarting the study product following a temporary discontinuation or progressing to permanent discontinuation. Of note, severe skin AEs associated with doxycycline, though rare, include Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and erythema multiforme, typically occurring within the first 10 days of treatment. Early recognition and immediate discontinuation of the study product are essential if suspected or observed.

Grade 1
Participants who develop a Grade 1 AE (regardless of relatedness or systemic involvement), study product use may continue in consultation with the IoR or designee.

Grade 2
Participants who develop an unrelated Grade 2 AE (without evidence of systemic involvement), study product use may continue in consultation with the IoR or designee.

Participants who develop a related Grade 2 AE (with evidence of systemic involvement), study product use will be permanently discontinued. The IoR or designee must consult the CMC within 48 hours. The participant should be monitored and re-evaluated daily for at least 5 days for systemic symptoms, laboratory abnormalities, mucosal involvement, and for any progression of the rash or increase in severity. The participant may be referred to a dermatologist, at the discretion of the IoR or designee.

Grade 3 and Grade 4 AEs

For participants who develop a Grade 3 or Grade 4 AE, study product use must be permanently discontinued. The IoR or designee must consult with the CMC within 48 hours of notification of a Grade 3 or Grade 4 AE.

The participant should be monitored and re-evaluated daily for at least 5 days for systemic symptoms, laboratory abnormalities, mucosal involvement, and for any progression of the rash or increase in severity. The participant may be referred to a dermatologist, at the discretion of the IoR or designee.

7.8.2 General Criteria for Temporary Holds and Permanent Discontinuations

Participants may voluntarily discontinue the study products for any reason at any time. The site IoR or designee may also determine that product use must be temporarily held or permanently discontinued from the study product per protocol for any of the specific criteria below, which may be further clarified in the Adverse Events SSP. The site IoR or designee also may temporarily hold or permanently discontinue participants for reasons not shown here or in the Adverse Events SSP (e.g., to protect participants' safety and if participants are unable or unwilling to comply with study requirements). In such cases, the IoR or designee must first query the CMC for review. The CMC will provide a written response to the site indicating whether the CMC has recommended to hold or permanently discontinue study product based on a careful review of all relevant data. It should be noted that the safety of the participant should always take precedence, and therefore, an IoR or designee may determine whether the study product be held or permanently discontinued before the CMC has time to respond. This is acceptable, and in such cases, the CMC should be notified as soon as possible regarding the nature of the case and the course of action taken.

The criteria for temporary holds of study product are:

- Reported use of prohibited concomitant medications as described in the Clinical Considerations SSP. Study product use may resume upon consultation with the CMC, and when the participant reports they no longer take the prohibited drug, provided other reasons for temporary study product hold/permanent discontinuation do not apply. The CMC should be consulted in all cases where a participant reports taking a prohibited product during the study.
- Inability or unwillingness to comply with required study procedures such as HIV testing and routine laboratory assessments or otherwise might be put at undue risk to their safety and well-being by continuing study product use, according to the judgment of the IoR. The IoR must consult the CMC on all temporary study product holds instituted for this reason for further guidance on continued study product use.

The criteria for permanent discontinuation of study product are:

- Study product AEs that require permanent discontinuation per the guidelines above
- Has one or more reactive HIV test results or expresses concern about having acute HIV infection

- STI acquisition
- Pregnancy
- Breastfeeding

7.9. SOCIAL IMPACT REPORTING

It is possible that participants' involvement in the study could become known to others, and that a social impact may result (i.e., because participants could be perceived as having an STI or at increased likelihood for STI acquisition). For example, participants could be treated unfairly or could have problems being accepted by their families and/or communities.

A social impact that is reported by the participant and judged by the IoR or designee to be serious or unexpected will be reported to the responsible IRB at least annually, or according to their individual requirements.

Social impacts will be collected and reported on CRFs during regular visits. If a participant reports a social impact, every effort will be made by study staff to provide appropriate care and counseling to the participant as necessary, and/or referral to appropriate resources for the safety of the participant. The site will provide such care and counseling in accordance with standardized guidance in the Clinical, Safety, and AE Management SSP Manual. The site is encouraged to engage their Community Advisory Board (CAB) to explore the social context surrounding instances of social impacts, to minimize the potential occurrence of such an impact, while maintaining participant confidentiality in these consultations.

8.0 STATISTICAL CONSIDERATIONS

8.1. OBJECTIVES AND ENDPOINTS

8.1.1 Primary Objective(s) and Endpoints

8.1.1.1 To estimate the single-dose plasma PK parameters of 200mg doxycycline hyclate immediate release (IR) and delayed release (DR) formulations.

Consistent with this primary study objective, the following endpoint(s) will be assessed for both the IR and DR dosing groups (n=8 each):

- Median (IQR) plasma doxycycline hyclate C_{max} (ng/mL units)
- Median (IQR) plasma doxycycline hyclate C_{min} (ng/mL units)
- Median (IQR) plasma doxycycline hyclate T_{max} (hours units)
- Median (IQR) plasma doxycycline hyclate half-life ($T_{1/2,}$ hours units)
- Median (IQR) plasma doxycycline hyclate area under the concentration time curve $(AUC)_{0\text{-}inf}$ (ng*hr/mL units)

8.1.1.2 To summarize doxycycline plasma concentrations following 10 days of daily or intermittent dosing.

Consistent with this primary study objective, the following endpoint(s) will be assessed for both the intermittent and daily dosing schedules:

- Median (IQR) plasma concentration at 24 hours post last dose (ng/mL units)
- Median (IQR) plasma concentration at 48 hours post last dose (ng/mL units)
- Median (IQR) plasma concentration at 72 hours post last dose (ng/mL units)

8.1.1.3 To characterize differences in doxycycline plasma concentrations 24 hours (C_{24}) post-dose between intermittent and daily doxycycline following 10 days of dosing.

Consistent with this primary study objective, the following endpoint(s) will be assessed:

- Median (IQR) plasma doxycycline concentrations 24 hours post last dose (C_{24h}, ng/ml units) for intermittent vs daily dosing schedules (n=8 each)

8.1.2 Secondary Objective(s) and Endpoints

8.1.2.1 To assess gastrointestinal (GI)-related adverse events for daily and intermittent doxycycline.

Consistent with this secondary study objective, the following endpoint(s) will be assessed:

- Frequency of GI-related AEs during 10 days of doxycycline dosing (daily or intermittent) and for 14 days following final dose (through the Final/Day 37 visit).

8.1.2.2 To estimate the single dose PK parameters of 200mg doxycycline hyclate IR and DR in DBS, anorectal fluid and vaginal fluid.

Consistent with this secondary study objective, the following endpoint(s) will be assessed for both the IR and DR dosing groups (n=8 each):

- Median (IQR) DBS doxycycline hyclate C_{max} (ng/mL units)
- Median (IQR) DBS doxycycline hyclate C_{min} (ng/mL units)
- Median (IQR) DBS doxycycline hyclate T_{max} (hours units)
- Median (IQR) DBS doxycycline hyclate half-life ($T_{1/2,}$ hours units)
- Median (IQR) DBS doxycycline hyclate area under the concentration time curve $(AUC)_{0\text{-}inf}$ (ng*hr/mL units)

- Median (IQR) rectal doxycycline hyclate C_{max} (ng/swab units)
- Median (IQR) rectal doxycycline hyclate C_{min} (ng/swab units)
- Median (IQR) rectal doxycycline hyclate T_{max} (hours units)
- Median (IQR) rectal doxycycline hyclate half-life ($T_{1/2,}$ hours units)

- Median (IQR) rectal doxycycline hyclate area under the concentration time curve $(AUC)_{0\text{-}inf}$ (ng*hr/swab units)

- Median (IQR) vaginal doxycycline hyclate C_{max} (ng/swab units)
- Median (IQR) vaginal doxycycline hyclate C_{min} (ng/swab units)
- Median (IQR) vaginal doxycycline hyclate T_{max} (hours units)
- Median (IQR) vaginal doxycycline hyclate half-life ($T_{1/2,}$ hours units)
- Median (IQR) vaginal doxycycline hyclate area under the concentration time curve $(AUC)_{0\text{-}inf}$ (ng*hr/swab units)

8.1.2.3 To estimate the single dose pooled PK parameters of 200mg doxycycline hyclate IR and DR in urine.

Consistent with this secondary study objective, the following endpoint(s) will be assessed for both the IR and DR dosing groups (n=8 each):

- Mean (95% CI) urine doxycycline hyclate C_{max} (ng/mL units)
- Mean (95% CI) urine doxycycline hyclate C_{min} (ng/mL units)
- Mean (95% CI) urine doxycycline hyclate T_{max} (hours units)
- Mean (95% CI) urine doxycycline hyclate half-life ($T_{1/2,}$ hours units)
- Mean (95% CI) urine doxycycline hyclate area under the concentration time curve $(AUC)_{0\text{-}inf}$ (ng*hr/mL units)

Note: As urine concentrations will be determined from pooled samples over a period of time, as opposed to discrete timepoint, it is more appropriate to describe pooled urine doxycycline concentrations and PK parameters as mean values, as opposed to median values.

8.1.2.4 To summarize doxycycline concentrations in DBS, urine, anorectal fluid and vaginal fluid for intermittent and daily doxycycline.

Consistent with this secondary study objective, the following endpoint(s) will be assessed for both the intermittent and daily dosing schedules:

- Median (IQR) DBS concentration at 24 hours post last dose (ng/mL units)
- Median (IQR) DBS concentration at 48 hours post last dose (ng/mL units)
- Median (IQR) DBS concentration at 72 hours post last dose (ng/mL units)

- Median (IQR) urine concentration at 24 hours post last dose (ng/mL units)
- Median (IQR) urine concentration at 48 hours post last dose (ng/mL units)
- Median (IQR) urine concentration at 72 hours post last dose (ng/mL units)

- Median (IQR) rectal concentration at 24 hours post last dose (ng/swab units)
- Median (IQR) rectal concentration at 48 hours post last dose (ng/swab units)
- Median (IQR) rectal concentration at 72 hours post last dose (ng/swab units)

- Median (IQR) vaginal concentration at 24 hours post last dose (ng/swab units)
- Median (IQR) vaginal concentration at 48 hours post last dose (ng/swab units)
- Median (IQR) vaginal concentration at 72 hours post last dose (ng/swab units)

8.1.3 Exploratory Objectives and Endpoints

Consistent with the exploratory objectives described in Section 2.3, the following exploratory endpoints may be assessed following review by HPTN leadership and securing resources.

8.1.3.1 To evaluate the feasibility of measuring doxycycline via DBS and volumetric absorptive microsampling (VAMS/Tasso+ device; dried whole blood) to estimate plasma concentrations.

- Concordance between estimated plasma doxycycline concentrations from whole blood collected as DBS or VAMS/Tasso+ and plasma doxycycline concentrations

8.1.3.2 To develop a popPK model describing the compartmental PK of doxycycline and simulate concentrations under various dosing conditions, as well as use physiologically based pharmacokinetic (PBPK) modeling of the data observed herein alongside published CSF data* to predict concentrations in the central nervous system given these dosing conditions.

*Note: *[18-20] The PBPK model will be fit based on these published blood and CSF concentration vs time data. Then the simulated plasma concentration vs time profiles from our popPK model will be utilized within the PBPK model to predict CSF concentrations across different dosing scenarios.*

- Simulated concentration vs time profiles in plasma, DBS, mucosal fluids and urine for 1000 simulated patients various dosing conditions including 200mg doxycycline once, 200mg doxycycline every 24 hours and 200mg doxycycline every 72 hours
- Predicted CSF concentrations for 1000 simulated patients given various dosing conditions including 200mg doxycycline once, 200mg doxycycline every 24 hours and 200mg doxycycline every 72 hours

8.1.3.3 To compare the single dose safety profiles of IR and DR doxycycline.

- The number of GI-related AEs reported during the single dose phase for IR and DR doxycycline and overall, throughout the study

8.1.3.4 To characterize doxycycline concentration patterns in hair strands using infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI) mass spectrometry imaging (MSI) and correlate these images with daily and intermittent dosing patterns.

- Images of doxycycline concentrations along intact hair strands collected during both the single and multi-dose phases of the study

8.1.3.5 To perform exploratory laboratory assessments that may include evaluation of factors related to STIs and laboratory assays related to the study objectives.

- Time estimates for when doxycycline concentrations remain above literature established MICs for GC and CT

8.2. SAMPLE SIZE

This is a directly observed dosing pharmacokinetic study intended to generate compartmental PK data needed to support the use of biometric adherence tests for doxycycline. Pharmacokinetic literature and industry guidance by the FDA support a long precedent of using 6-8 participants per dosing condition to yield sufficient concentration vs time data by which to estimate the plasma pharmacokinetic parameter endpoints for primary objective 8.1.1.1.[32-34]

While the PK for q72hour doxycycline dosing has not been described, the mean (%CV) steady-state concentrations at 24 hours post dose (C_{24}) for 200mg daily doxycycline hyclate DR have been previously reported as 515 ng/mL (29%) with an elimination rate constant (k_{el}) of $0.0345hr^{-1}$ [29] . As such the accumulation ratio for doxycycline can be calculated by the following equation, $AR=1/(1-exp^{-kel*tau})$. Given an AR of 1.78, we anticipate the C_{24} in our intermittent (q72hour) group will be 317 ng/mL. Based on this anticipated mean difference and a standard deviation of 149 ng/mL, a sample size of 8 participants per group is sufficient to achieve 80% power assuming a one-sided alpha of 0.05. Because it is not biologically plausible that concentrations would be increased by less frequent dosing, our selection of a one-sided test is appropriate.

8.3. ACCRUAL AND RETENTION

A total of 16 participants will be enrolled over the course of about 10 months and will be followed for 37 days. Participants will be deemed evaluable if 80% of study visits are completed. Participants that are deemed non-evaluable will be replaced.
A less than 10% attrition rate is anticipated.

8.4. RANDOM ASSIGNMENT/STUDY ARM ASSIGNMENT

Participants will be randomized twice at enrollment: once for the single-dose doxycycline portion of the study and a second time for randomization into a daily or intermittent oral doxycycline schedule for 10 days. For the first randomization, participants will be randomly assigned, in a 1:1 ratio, stratified by sex, to take a single dose of 200mg doxycycline hyclate (either as 1x 200mg DR tablet or 2x 100mg IR tablets). Participants will be evaluated over 14 days.

For the second randomization (which occurred at enrollment), participants (1:1 by sex) will receive 200mg doxycycline hyclate DR daily (every 24 hours) or intermittently (every 72 hours) for 10 days.

Randomization will occur to allow for equal numbers of females and males for both phases of the study. Randomization procedures will be detailed in the SSP Manual.

8.5. STATISTICAL ANALYSIS

8.5.1 Baseline and Demographic Characteristics and Participant Disposition

Descriptive statistics will be computed for demographic characteristics such as height, weight, age, and other demographic information as well as renal status and CrCl.

Participant disposition including dropout and reasons for dropout will be summarized descriptively and supported with a CONSORT diagram.

8.5.2 Primary Analyses

Single-dose plasma PK analysis (primary objective 8.1.1.1):

- Non compartmental analysis will be performed using WinNonlin software (Certara Inc, Cary NC) to estimate PK parameters (defined as endpoints above) associated with a single 200mg dose of doxycycline IR and DR. AUC will be calculated using the linear-up log-down trapezoidal rule. Half-life ($T_{1/2}$) will be calculated as $T_{1/2}=Ln(2)/k_{el}$ and k_{el} will be estimated by the best fitting linear regression line of concentrations vs time based on the semi-log plot.

Multi-dose plasma PK analysis (primary objective 8.1.1.2 and 8.1.1.3):

- Concentrations at 24hours following the last dose of the multi-dosing period will be summarized as median (IQR) by group (daily vs intermittent) using WinNonlin software (Certara Inc, Cary NC).

- A one-sided two-sample t-test will be performed using SAS software (Cary, NC) to determine whether concentrations at 24 hours post last dose of the multi-dosing period differ between daily vs intermittent doxycycline dosing frequencies.

8.5.3 Secondary PK Analyses

Single-dose DBS, anorectal and vaginal fluid PK analyses (secondary objective 8.1.2.1):

- Non compartmental analysis will be performed using WinNonlin software (Certara Inc, Cary NC) to estimate PK parameters (defined as endpoints above) associated with a single 200mg dose of doxycycline IR and DR. AUC will be calculated using the linear-up log-down trapezoidal rule. $T_{1/2}$ will be calculated as $T_{1/2}=Ln(2)/kel$ and kel will be estimated by the best fitting linear regression line of concentrations vs time based on the semi-log plot.

Single-dose urine PK analysis (secondary objective 8.1.2.2):

- Non compartmental analysis will be performed using the sparse sampling function in WinNonlin software (Certara Inc, Cary NC) to estimate PK parameters (defined as endpoints above) associated with a single 200mg dose of doxycycline IR and DR. The pooled NCA will be run as a naïve-pool agnostic of paired samples. AUC will be calculated using the linear-up log-down trapezoidal rule. $T_{1/2}$ will be calculated as $T_{1/2}$=Ln(2)/kel and kel will be estimated by the best fitting linear regression line of concentrations vs time based on the semi-log plot.

Multi-dose DBS, anorectal and vaginal fluid, and urine PK analysis (secondary objective 8.1.2.3):

- Concentrations at 24, 48, and 72 hours following the last dose of the multi-dosing period will be summarized as median (IQR) by group (daily and intermittent) using WinNonlin software (Certara Inc, Cary NC).

8.5.4 Secondary Safety Analyses

GI-related adverse events will be summarized by computing the proportion of participants with observation of any event. Additional analyses may summarize the frequency and duration of GI-related events ongoing after completion of dosing. Summaries will be prepared corresponding to maximum severity and duration per participant. Proportions will be accompanied by two-sided exact 95% confidence intervals. The proportion of GI-related will be compared using a two-sided Fisher's exact test.

9.0 LABORATORY SPECIMENS AND BIOHAZARD CONTAINMENT

Laboratory procedures are described below, in Appendices I and in Section 5.0; additional tests to be performed at a subsequent visit for participants who have a reactive or positive HIV test interpretation are described in Appendix II.

9.1. LOCAL LABORATORY SPECIMENS

As described in Section 5, the following types of specimens will be collected for testing at the local laboratory (LL):

- HIV testing, Chemistry, Hematology, STI, and pregnancy testing as indicated in Appendix I.
- The HIV testing algorithms at screening, enrollment, and the final study visit are summarized in Appendix II.

The study site must adhere to the HPTN Manual of Operations (MOP), the Laboratory Manual: Specimen Management and Laboratory Related Procedures, and local SOPs, for proper collection, processing, labeling, transport, and storage of specimens at the local laboratory (LL). The site should send these samples to Clinical Laboratory Improvement Amendments (CLIA) compliant laboratories for testing. In addition, each study site must

adhere to the Requirements for Laboratories in Performing Testing for DAIDS-Supported and/or Sponsored Clinical Trials policy (DAIDS-OD-A-POL-00002) as outlined in the DAIDS SCORE Manual and the DAIDS Good Clinical Laboratory Practice (GCLP) as appropriate. Laboratories must also follow the DAIDS Clinical Research Laboratory and Specimens Management policies at https://www.niaid.nih.gov/research/daids-clinical-research-laboratory-specimens-management. Specimen collection and storage at the local laboratory will be documented using the Laboratory Center Specimens Management System (LDMS) as described in the Laboratory Manual: Specimen Management and Laboratory Related Procedures.

9.1.1 Specimen Storage at Study Sites

All biological samples will be stored at the local site throughout the study. A subset of the stored samples will be shipped to the HPTN LC (located in the US) or a laboratory designated by the HPTN LC. Testing on stored samples will be performed by the HPTN LC or another laboratory selected and approved by the HPTN LC. Samples stored at study sites may not be used for LL testing without written approval by the HPTN LC prior to use. Stored samples will be maintained at the site until at least one year after acceptance of the manuscript that describes the primary findings for the study, and after protocol-related testing has been completed. This includes retrospective testing performed at the HPTN LC and laboratories designated by the HPTN LC that are needed to address study objectives. In some cases, the HPTN LC will approve sample destruction before completion of testing for exploratory objectives. The study site may not destroy samples without written permission from the HPTN LC. The HPTN LC will communicate with sites when samples can be destroyed; sites will follow LL policies for sample destruction. A list of samples to be destroyed will be provided by UNC and then will be reviewed and reconciled by the site as part of the sample destruction process.

Stored samples may be used for QC, virology, or other exploratory objectives as related to the study protocol.

There might be a small amount of urine, blood, or vaginal and rectal fluid remaining after all the study related testing is completed. If the participant consents, remaining samples will be stored and may be used for future testing. If the participant does not consent, these samples will be destroyed after the study is completed (including addressing study primary and secondary objectives).

9.1.2 Pharmacology

Samples for pharmacology testing (plasma, DBS, VAMS/TASSO+, DBS, vaginal and anorectal swabs, urine, and hair) will be collected throughout the study from all participants. Procedures for sample processing and shipping are outlined in the Laboratory SSP Manual. Pharmacology testing will be performed at the HPTN LC or another laboratory selected and approved by the HPTN LC. The primary pharmacologic assessments will be performed using assays that have been appropriately validated and approved. Results will not be returned to the study participants. Interpretation of

pharmacologic results, including PK modeling, will be co-led by the Protocol Chair(s), in conjunction with the HPTN LC Pharmacology Core, as needed.

Stored samples may also be tested for the presence of other ARV drugs or other concomitant medications.

9.2. Quality Control and Quality Assurance Procedures

The site will document their clinical laboratories (including clinic labs or other locations where processing and testing are performed) are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-certified or have a CLIA Certificate of Waiver) and/or participate in DAIDS-sponsored EQA programs. Laboratories must also follow the DAIDS requirements (link to policy on DAIDS website https://www.niaid.nih.gov/research/daids-clinical-research-laboratory-specimensmanagement).

LC staff may conduct periodic visits to the site to review the implementation of on-site laboratory quality control (QC) procedures, including proper maintenance of laboratory equipment and use of appropriate supplies and reagents. LC staff may follow up directly with site staff to resolve any QC or quality assurance (QA) problems identified through proficiency testing or on-site visit reviews. Throughout the course of the study, the HPTN LC may select a random sample of stored specimens to test for QA purposes. LC staff may follow up directly with site staff to resolve any QA problems identified through this process.

HIV diagnostic tests will be listed on a US lab information sheet and will be subject to review and acceptance by DAIDS and the HPTN LC.

Throughout the study, QA/QC procedures will be conducted by study staff as outlined in local SOPs.

9.3. HIV Diagnostic Testing

The LL will perform testing for HIV diagnosis at Screening, Enrollment, and Final Study visits as described in Appendix II. Individuals who have one or more reactive/positive HIV tests at screening are not eligible to participate in this study. If HIV tests are reactive/positive from samples collected at the enrollment visit, study product will be held, and the CMC must be notified. In accordance with the policies of the US NIH, participants must receive their HIV test results to take part in this study. Participants will be provided with their HIV test results in the context of post-test counseling.

Participants who have any reactive or positive HIV test result at the Enrollment Visit will be referred for care and confirmatory testing according to local standards of care. The participant may not receive additional doses of study product if they have a reactive or positive HIV test, even if further testing indicates that they do not have HIV infection. The CMC will provide additional guidance on re-initiating study product. All follow-up visits may be discontinued, and the participant may be terminated from the study.

9.4. LONG-TERM LOCAL SPECIMEN STORAGE AND POSSIBLE FUTURE RESEARCH TESTING

Sites are required to store all specimens collected in this study, as described in Section 9.1.1. In some cases, specimens may be stored for longer periods, based upon the capacity of the study site, local regulations, and interests of the study team. In these cases, samples in long-term storage may be available for future research testing by the HPTN LC or other interested investigators, in accordance with HPTN policies for ancillary studies. If a site does not have the capacity to accommodate long-term storage (beyond that outlined in Section 9.1.1), the HPTN LC will work with the site to transfer selected samples (identified by the HPTN LC) to the HPTN LC. Study participants will be asked to provide written informed consent for specimens to be stored long-term for possible future testing. Specimens from participants who do not consent to long-term storage and future testing will only be used for testing that is needed to address protocol objectives.

9.5. BIOHAZARD CONTAINMENT

As the transmission of HIV and other blood-borne pathogens can occur through contact with used needles, blood, and other bodily secretions containing HIV, appropriate precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the United States Centers for Disease Control and Prevention. All specimens will be shipped using packaging that meets requirements specified by the International Air Transport Association Dangerous Goods Regulations for UN 3373, Biological Substance, Category B, and Packing Instruction 650.

10.0 HUMAN SUBJECTS CONSIDERATIONS

10.1. ETHICAL REVIEW

This protocol and the template informed consent form(s) (ICFs) contained in Appendix III will be reviewed and approved by the HPTN Scientific Review Committee (SRC) and NIAID Prevention Science Review Committee (PSRC) with respect to scientific content and compliance with applicable research and human subjects' regulations.

The protocol, site-specific ICF, participant education and recruitment materials, other requested documents, and any subsequent modifications will also be reviewed and approved by the IRB, that is responsible for oversight of this research study.

After initial review and approval, the responsible IRB will review the protocol at least annually. The IoR or designee will make safety and progress reports to the IRB at least annually, and within three months of study termination or completion. These reports will include the total number of participants enrolled in the study, the number of participants who completed the study, all changes in the research activity, and all unanticipated problems involving risks to human subjects or others. Study sites will submit

documentation of continuing review to the DAIDS Protocol Registration Office (PRO), in accordance with the current DAIDS Protocol Registration Policy and Procedures Manual.

10.2. INFORMED CONSENT

Written informed consent will be obtained from each study participant. The study site is responsible for developing a study ICF(s) for local use, based on the template in Appendix III, which describes the purpose of the study, the procedures to be followed, and the risks and benefits of participation, in accordance with all applicable regulations.

Literate participants will document their provision of informed consent by signing their ICF.

Non-literate participants will be asked to document their informed consent by marking their ICF (e.g., with an X, thumbprint, or other mark) in the presence of a literate third-party witness. Further details regarding DAIDS requirements for documenting the informed consent process with both literate and non-literate participants are provided in the DAIDS SCORE Manual. Any other responsible IRB requirements for obtaining informed consent from non-literate persons also will be followed.

Participants will be provided with a copy of their ICF(s) if they are willing to receive them.

In addition to the ICF(s), the Protocol Team will work with study staff and community representatives to develop appropriate materials about the study and a standardized approach to the informed consent process to be implemented at the study site.

The informed consent process will include all elements required by applicable federal regulations (e.g., 45 CFR 46.116) and institutional policies. The process will place particular emphasis on the following areas that are essential for participant understanding and ethical conduct. These include:

- The process of randomization and the importance of participants completing all assigned study sequences to ensure the scientific validity of the trial.
- The critical role of participant adherence to the visit schedule and study procedures in ensuring data integrity and participant safety.
- Potential risks associated with participation, along with information about how participants will be supported should such risks occur.
- Potential social harms, including stigma or privacy concerns, and the steps participants can take—and the study team will take—to address them.
- The possibility of limited personal benefit from participation, with a clear explanation that such benefits are not guaranteed.
- The voluntary nature of participation, including the right to withdraw from the study at any time without penalty or loss of benefits.

The consent process will be conducted by trained study staff in a private setting and will include ample opportunity for the prospective participant to ask questions. Comprehension will be assessed prior to enrollment to ensure informed, voluntary participation.

10.3. RISKS

It is not expected that this study will expose participants to unreasonable risk.

Risks Associated with Blood Draw(s)
Participants may experience possible discomfort from the insertion of the catheter in their vein or from the use of the TASSO+ device to obtain the blood specimens. Other risks may include pain, bleeding, bruising, and swelling as well as a small risk of infection at the site where the catheter was inserted or a slight pinch when the TASSO device is placed. There is also a risk of lightheadedness, fainting, and blood clots.

Risks Associated with Penile, Anorectal and Vaginal Swab Risks
Risks associated with repeated self-collection of anorectal and vaginal fluid by swabs include pain or discomfort and tissue agitation. There is also a very small risk of infection or bleeding during the procedure.

Risks Associated with Doxycycline
Doxycycline is a broad-spectrum bacteriostatic agent within the tetracycline class of antibiotics initially approved and marketed in the early 1960s under the brand name Vibramycin®. Doxycycline is generally safe and the rate of adverse event reporting among healthy study participants using doxycycline for PEP was low with no serious adverse events related to doxycycline use reported over 12 months of follow-up in 224 people [7]. Nausea (reported at 7.2% of visits among those randomized to the treatment group) was the most common adverse event reported followed by vomiting, diarrhea, rash and acne.

According to the Vibramycin® prescribing information [35], additional adverse events include:

- Photosensitivity (manifested by exaggerated sunburn reaction); exposure to sunlight, even for short periods of time, may cause skin rash, itching, redness or other discoloration of the skin, or a severe sunburn.
- Clostridioides difficile
- Hypersensitivity reactions (manifested as severe skin reactions, anaphylaxis, pericarditis)
- Hemolytic anemia, thrombocytopenia, neutropenia and eosinophilia

Additionally, although rare, doxycycline use has been associated with Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN): also known as Lyell syndrome. These are serious, potentially life-threatening skin reactions that often begin with flu-like symptoms, followed by painful rash and blisters. Liver tests may be elevated; and other symptoms can develop a few days later, including painful red or purple skin, blisters on the skin, mouth, nose, and genitals, and red, painful, and watery eyes.

Risks Associated with Urine Collection
There are no foreseeable risks associated with urine sample collection.

Risks Associated with Hair Collection
The only foreseeable risk associated with collecting plucked hair strands is minor discomfort. Risks associated with cut hair collection include minor discomfort during the hair cutting process. The cutting of hair is not expected to be noticeable but there may be a slight cosmetic effect depending on your hairstyle and hair thickness. Staff will make every effort to collect hair from an inconspicuous area to minimize cosmetic impact. Some people may feel self-conscious about hair collection for personal or cultural reasons.

Confidentiality Risks
Participation in clinical research includes the risks of loss of confidentiality and discomfort with the personal nature of questions. Although the study site makes 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. For example, participants could be treated unfairly or discriminated against or could encounter lack of acceptance by their families and/or communities. Several systems are in place to maintain participant confidentiality. All hard copies of study records, including all documents with personally identifiable information, will be kept in a locked drawer in a locked office to which only the study IoR or designee will have access.

Samples, study data, and study report forms will not contain participant names; rather they will be labeled with a unique study identification (ID) number with a combination of letters and numbers. The file that links participant identification numbers to their names and other identifiable information will be password-protected and kept by one study IoR or designee on a secure internal computer network, separate from the study data.

All electronic data for this study will be stored on a dedicated server which contains extensive protections and securities.

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 implement confidentiality protections that reflect the local study implementation plan and the input of study staff and community representatives to identify potential confidentiality issues and strategies to address them.

Though every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, steps allowable by law to protect the privacy of personal information. Additionally, all recruitment and prescreening phone calls will be conducted in a private room with the door closed to prevent inadvertent disclosure of participant information.

10.4. BENEFITS

There may be no direct benefits to participants in this study, however, use of doxycycline has been shown to be effective in treating and/or significantly reducing the acquisition of bacterial STIs, such as chlamydia (CT) and gonorrhea (GC).

Participants and others may benefit in the future from information learned from this study. Specifically, information learned in this study may lead to the development of a safe and effective interventions that prevent STI acquisition. Participants also may appreciate the benefit of earlier diagnosis of STIs and referral. In addition, participants will receive HIV counseling and testing as part of the study screening process as well as evaluations of participant health.

10.5. INCENTIVES

Pending IRB approval, participants will be reimbursed for their time and effort in this study, and/or be reimbursed for travel to study visits and time away from work. Information regarding reimbursement, including what is reimbursed and the specific amount will be decided by the site, in accordance with the local IRB or local regulatory requirements. Reimbursement amounts will be specified in the site specific ICF.

10.6. CONFIDENTIALITY

All study-related information will be stored securely at the study site. All participant information will be stored in locked file cabinets in areas with access limited to study staff. All laboratory specimens, reports, study data collection, process, and administrative forms will be identified by a coded number only to maintain participant confidentiality. All local databases will be secured with password-protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link participant ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access.

Participant's study information will not be released without the written permission of the participant, except as necessary for monitoring by the NIAID and/or its contractors; representatives of the HPTN LOC, and/or LC, other government and regulatory authorities, and/or site IRB.

10.6.1 Certificate of Confidentiality

Research participants in Network protocols are protected by a Certificate of Confidentiality (CoC) from the US National Institutes of Health (NIH), which can prevent disclosure of study participation even when that information is requested by subpoena. Participants are told of the use and limits of the CoC in the study consent form. CoCs are automatically deemed to be issued for any NIH-funded research that collects or uses identifiable, sensitive information that was on-going on or after December 13, 2016.

In accordance with subsection 301(d) of the Public Health Service Act, the recipient of the CoC shall not:

- Disclose or provide, in any federal, state, or local civil, criminal, administrative, legislative, or other proceeding, the name of such individual or any such information, document, or biospecimen that contains identifiable, sensitive information about the individual and that was created or compiled for purposes of the research, unless such disclosure or use is made with the consent of the individual to whom the information, document, or biospecimen pertains; or
- Disclose or provide to any other person not connected with the research the name of such an individual or any information, document, or biospecimen that contains identifiable, sensitive information about such an individual and that was created or compiled for purposes of the research.

Disclosure is not prohibited when:

- Required by federal, state, or local laws (e.g., as required by the Federal Food, Drug, and Cosmetic Act, or state laws requiring the reporting of communicable diseases to state and local health departments), excluding instances of disclosure in any federal, state, or local civil, criminal, administrative, legislative, or other proceeding.
- Necessary for the medical treatment of the individual to whom the information, document, or biospecimen pertains and made with the consent of such individual.
- Made with the consent of the individual to whom the information, document, or biospecimen pertains; or
- Made for the purposes of other scientific research that is in compliance with applicable federal regulations governing the protection of human subjects in research.

As set forth in 45 CFR Part 75.303(a) and NIHGPS Chapter 8.3, recipients conducting NIH supported research applicable to this Policy are required to establish and maintain effective internal controls (e.g., policies and procedures) that provide reasonable assurance that the award is managed in compliance with Federal statutes, regulations, and the terms and conditions of award.

10.7. SPECIAL POPULATIONS

This study is designed to develop a popPK model of doxycycline following single-dose administration in healthy individuals. To ensure scientific validity and minimize confounding factors, individuals from the following populations will be excluded: people living with HIV, pregnant females, and persons under the age of 18.

Pregnant Individuals

Pregnant individuals will be excluded from participation due to known and potential risks associated with doxycycline use during pregnancy. Pregnancy testing will be conducted at Screening, Enrollment, and as clinically indicated during the study. Anyone who tests

positive for pregnancy at the Screening or Enrollment Visit will not be eligible to participate in this study. Should a participant test positive for pregnancy after Enrollment, a product discontinuation will be implemented. During the informed consent process, individuals who can get pregnant will be informed that they must use an effective method of contraception and the effects of the study product on a developing human fetus are unknown.

Individuals Under the Age of 18

Based on the frequency of sample collections, including semi-intensive vaginal and rectal swab collections, this study will not enroll individuals under 18 years of age. These procedures present ethical and logistical challenges in minors, particularly regarding acceptability and feasibility. For these reasons, the exclusion of minors is considered both ethically appropriate and scientifically justified.

Individuals Living with HIV

Individuals living with HIV are excluded from study participation due to potential alterations in doxycycline pharmacokinetics resulting from common concomitant antiretroviral therapies. These factors may confound PK modeling in a small, early-phase study designed to characterize baseline variability in a healthy adult population.

10.8. COMMUNICABLE DISEASE REPORTING REQUIREMENTS

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

10.9. STUDY DISCONTINUATION

The study also may be discontinued at any time by NIAID, the HPTN and/or other government or regulatory authorities, and/or responsible IRB/EC.

11.0 ADMINISTRATIVE PROCEDURES

11.1. SOURCE DOCUMENTATION AND DIRECT DATA ENTRY

Direct data entry or direct data capture of study data into the study database is only allowed when capturing information directly from the participant (participant self-report). Other study data will be sourced from electronic or paper source documents prior to being entered into the database. Source documentation tables will be finalized prior to implementation.

The IoR or designee will maintain, and store securely, complete, accurate and current study records throughout the study. The IoR or designee will retain all study records for at least three years following the completion of the study, unless directed otherwise by

the National Institutes of Health (NIH). Study records must be maintained on site for the entire period of study implementation.

11.2. PROTOCOL REGISTRATION

Initial Registration of the protocol by the DAIDS PRO is required prior to the implementation of this protocol. As part of this process, each site must have the protocol and protocol ICF approved, as appropriate, by responsible IRB and any other applicable regulatory entity (RE). Upon receiving final approval, sites will submit all required protocol registration documents to the DAIDS PRO at the DAIDS RSC. The DAIDS PRO will review the submitted protocol registration packet to ensure that all the required documents have been received. In the case of Initial Registration, the site-specific ICF WILL be reviewed and approved by the DAIDS PRO. Sites will receive an Initial Registration Notification from the DAIDS PRO that indicates successful completion of the protocol registration process. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

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

For additional information on the protocol registration process and specific documents required for initial and amendment registrations, refer to the current version of the DAIDS Protocol Registration Manual, which can be found at: https://www.niaid.nih.gov/sites/default/files/prmanual.pdf.

11.3. STUDY ACTIVATION

Pending successful protocol registration and submission of all required documents, the HPTN LOC staff will "activate" a site. Study implementation may not be initiated until a study activation notice is provided to the site by the HPTN LOC. In addition, if study activation is determined to be necessary for any subsequent amendments, study implementation may not be initiated until a study activation notice is provided to the site by the HPTN LOC.

11.4. STUDY COORDINATION

Study implementation will be directed by this protocol as well as the SSP manual. The DAIDS SCORE Manual, the DAIDS Manual for Expedited Reporting of Adverse Events to DAIDS, and the DAIDS Toxicity Tables, will outline procedures for conducting study visits; data and forms processing; AE assessment, management and reporting; dispensing study products and documenting product accountability; and other study operations.

Study CRFs and other study instruments will be developed by key members of the protocol team.

Data management (data cleaning, reporting and analysis) and coding queries will be generated and applied to the data by the designated staff at the site on a routine basis for verification and resolution by site staff. At study end, data will be transferred to the Statistical and Data Management Center (SDMC) for archival.

Close coordination between protocol team members will be necessary to track study progress, respond to queries about proper study implementation, and address other issues in a timely manner. Rates of accrual, adherence, follow-up, and visit completion rates will be monitored closely by the team. The Study Coordinator will address issues related to individual study eligibility, toxicity management, and AE reporting as needed to assure participant safety, consistent case management, documentation, and information-sharing across sites.

11.5. STUDY MONITORING

Study monitoring will be performed in accordance with DAIDS policies. Study monitors will:

- Verify compliance with human subjects and other research regulations and guidelines.
- Assess adherence to the study protocol, SSP manuals, and local counseling practices.
- Confirm the quality and accuracy of information collected at the study site.
- Verify compliance with GCLP

Monitoring visits may be conducted on-site or remotely. Remote visits may include remote source document verification using methods specified for this purpose by NIAID. Remote monitoring visits may be performed in place of or in addition to onsite visits to ensure the safety of study participants and data integrity. The site must make available study documents for study monitors to review utilizing a secure platform that is HIPAA and 21 CFR Part 11 compliant. Potential platform options include: Veeva SiteVault, site-controlled SharePoint or cloud-based portal, direct access to Electronic Medical Record (EMR), and Medidata Rave Imaging Solution. Other secure platforms that are 21 CFR Part 11 compliant may be utilized, as allowed by the DAIDS Office of Clinical Site Oversight (OCSO).

For on-site visits, site IoR or designees will also allow study monitors to inspect study facilities and documentation (e.g., ICFs, clinic and laboratory records, other source documents, paper or electronic CRFs), as well as observe the performance of study procedures. Investigators or designee(s) also will allow inspection of all study-related documentation by authorized representatives of the HPTN LOC, HPTN LC, NIAID, responsible IRB, and US regulatory authorities (Office for Human Research Protections (OHRP)or other regulatory agencies). A site visit log will be maintained at each study site to document all visits.

11.6. PROTOCOL COMPLIANCE

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

11.7. INVESTIGATOR'S RECORD

The IoR or designee will maintain, and store in a secure manner, complete, accurate, and current study records throughout the study. Under the US Department of Health and Human Services (DHHS) regulations, the IoR or designee is required to retain all study records relating to research for at least three [3] years after completion of the research, or longer if needed to comply with local regulations.

Completion of a clinical research study occurs when the following activities have been completed:

- All research-related interventions or interactions with human subjects (e.g., when all participants are off study).
- All protocol-required data collection of identifiable private information described in the responsible IRB/EC-approved research plan.
- All analysis of identifiable private information described in the responsible IRB/EC-approved research plan.
- Primary analysis of either identifiable private or de-identified information.

Study records include administrative documentation including protocol registration documents and all reports and correspondence relating to the study; as well as documentation related to each participant screened and/or enrolled in the study, including ICFs, locator forms, CRFs, and notations of all contacts with the participant; and all other source documents.

11.8. USE OF INFORMATION AND PUBLICATIONS

Publication of the results of this study will be governed by the HPTN Manual of Operations. Any presentation, abstract, or manuscript will undergo review by the HPTN Manuscript Review Committee and DAIDS prior to submission.

11.9. CLINICALTRIALS.GOV

This protocol is not an FDAAA "applicable clinical trial." However, this study is subject to the NIH Policy on the Dissemination of NIH-Funded Clinical Trial Information. A description of this clinical trial will be available on http://www.ClinicalTrials.gov.

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APPENDIX I: SCHEDULE OF STUDY VISITS AND PROCEDURES

	Screening (up to Day -30)*	Enrollment/ Single Dose Visit Day 0	Multi-Dosing Visits (Days 14-23)	Sampling Visits (Days 1-35)	Final Visit
Administrative and Behavioral Evaluations/Procedures					
Participant eligibility assessment/confirmation	x	x			
PTID Assignment	x				
Informed Consent	x				
Demographic information	x				
Randomizations		x			
Locator/contact information	x	x	♦	♦	x
Social harms		x	x	x	x
AE assessment		x	x	x	x
HIV pre- and post-test counseling	x	x	♦	♦	x
HIV/STI risk reduction counseling	x	x	x	x	x
Protocol adherence counseling	x	x	x	x	
Study product supply/administration with direct observed dosing		x	x		
Schedule next visit/contact	x	x	x	x	
Visit reimbursement	x	x	x	x	x
Clinical Evaluations/Procedures					
Medical history including concomitant medications	x	x	♦	♦	♦
Full/targeted physical examination	x	x	♦	♦	x
Hepatitis B vaccination or referral, if indicated	x				
Treatment for pharyngeal, anorectal, or reproductive tract infections or referral for treatment	♦	♦	♦	♦	♦
Laboratory Evaluations/Procedures					
Hair:					
Plucked hair (5 strands) for PK testing[1]			x	x	x
Cut hair (~100 strands) for future PK testing (*optional*)[1]			x	x	x
Urine:					
Dipstick for protein and glucose (local laboratory)	x				
Pregnancy/hCG (local laboratory)	x	x		x (Day 14 only)	x
NAAT for GC/CT (local laboratory)	x				x
PK testing[1]		x		x	x

	Screening (up to Day -30)*	Enrollment/ Single Dose Visit Day 0	Multi-Dosing Visits (Days 14-23)	Sampling Visits (Days 1-35)	Final Visit
Pharyngeal:					
Pharyngeal Swab for GC/CT NAAT (local laboratory)	x				x
Vaginal[3]:					
Vaginal Swab for GC/CT NAAT (local laboratory)	x				x
PK testing[1]		x		x	x
Penile[3]:					
Penile Swab for GC/CT NAAT (local laboratory)	x				x
Anorectal[3]:					
Anorectal Swab for GC/CT NAAT (local laboratory)	x				x
PK testing[1]		x		x	x
Blood:					
Hematology (CBC with platelets) (local laboratory)	x				x
Renal function tests (BUN, creatinine) (local laboratory)	x				x
Calculated creatinine clearance	x				
Hepatic function tests (ALT, AST, total bilirubin) (local laboratory)	x				x
Hepatitis B: HBsAg, HBsAb, HBcAb (total) (local laboratory)	x	♦			
Syphilis Serology (RPR titer with reflex to TPHA if indicated) (local laboratory)	x				x
HIV testing per Appendix II (local laboratory)	x	x			x
Plasma, DBS for PK testing[1]		x		x	x
VAMS (Tasso+ device) for PK testing[4]				x	
Plasma for Storage [5]		x			
DBS for Storage[5]		x			

s If indicated

[1] Participants will be assigned to provide samples at designated dates and/or times as outlined in sections 6.2, 6.3, 6.4 and 6.5. Plasma, DBS, rectal and vaginal swabs will be collected on Day 0 at hours 1, 2, 3, 4, 6, and 8; sparse urine will be collected between hours 1-4 and 4-8. Subsequent samples will be done at a single timepoint on Days 1, 2, 3, 4, 7, 10, 14, 15, 18, 21, 24, 26, 29, 32, 35, and 37. Hair collection or IR-MALDI analysis will occur on Days 3, 7, 10, 14, 15, 18, 21, 24, 26, 29, 32, 35, and 37. Bulk hair collections on Days 14, 24, and 37 are optional.

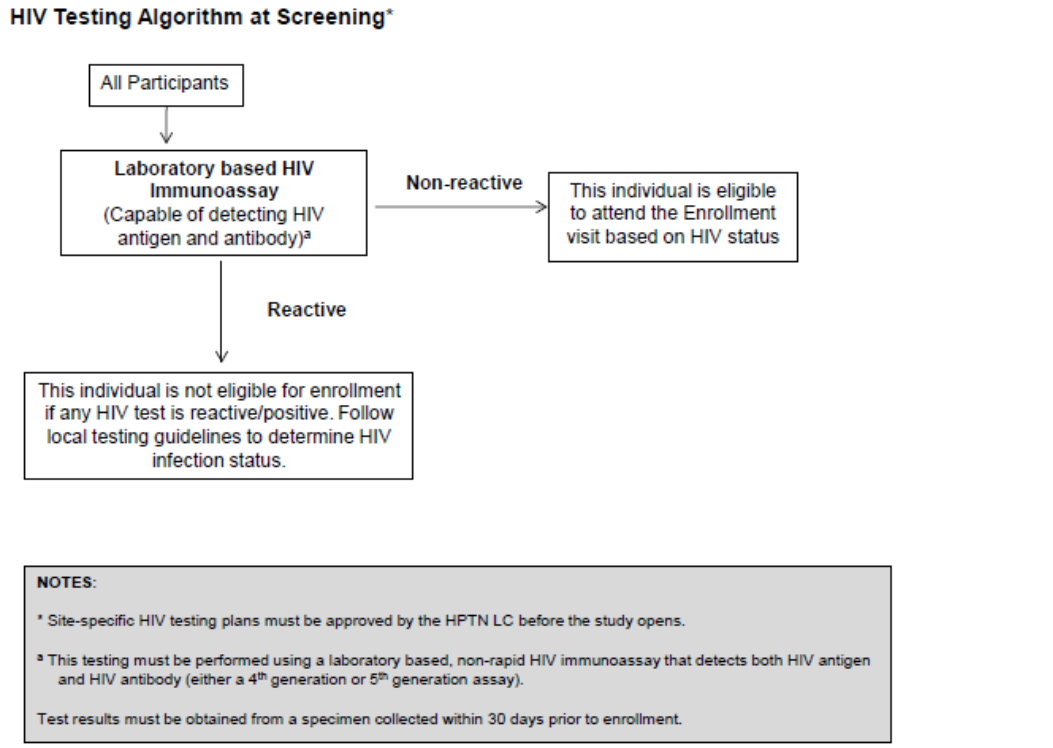
[2] HBcAb and HBsAb testing may be performed at screening or at baseline/enrollment, if indicated.

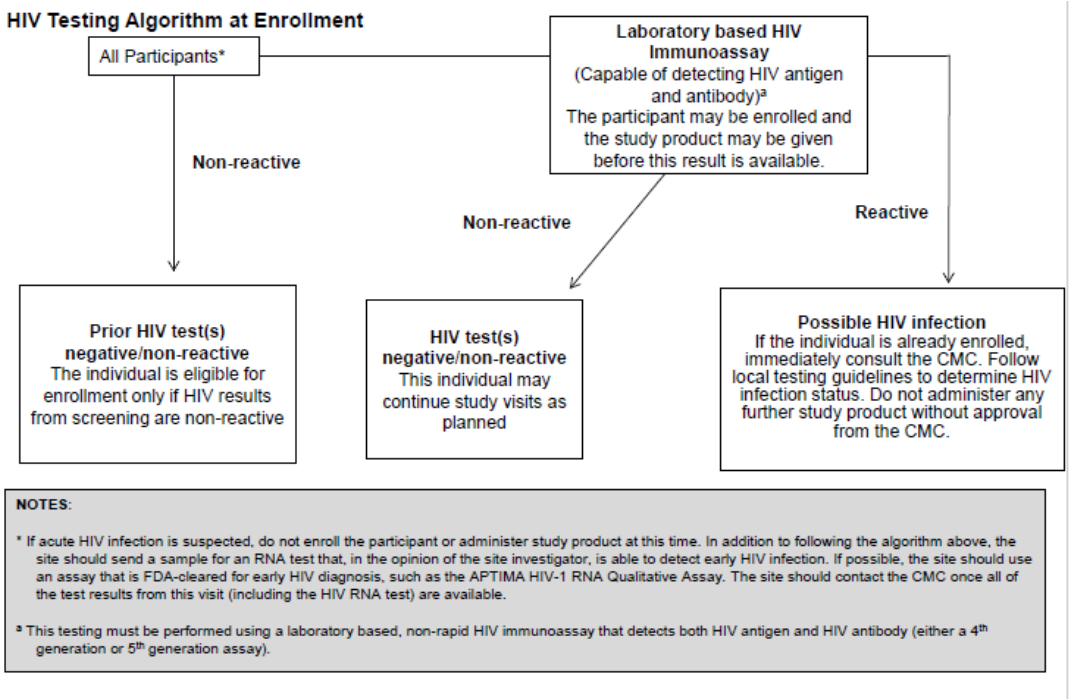
[3] If collection of pelvic swabs (vaginal and/or penile) is declined or not feasible, NAAT testing using urine is acceptable.

[4] Collected on Days 1 (24 hours after single-dose IR or DR doxycycline administrations), and Days 3 and 7.

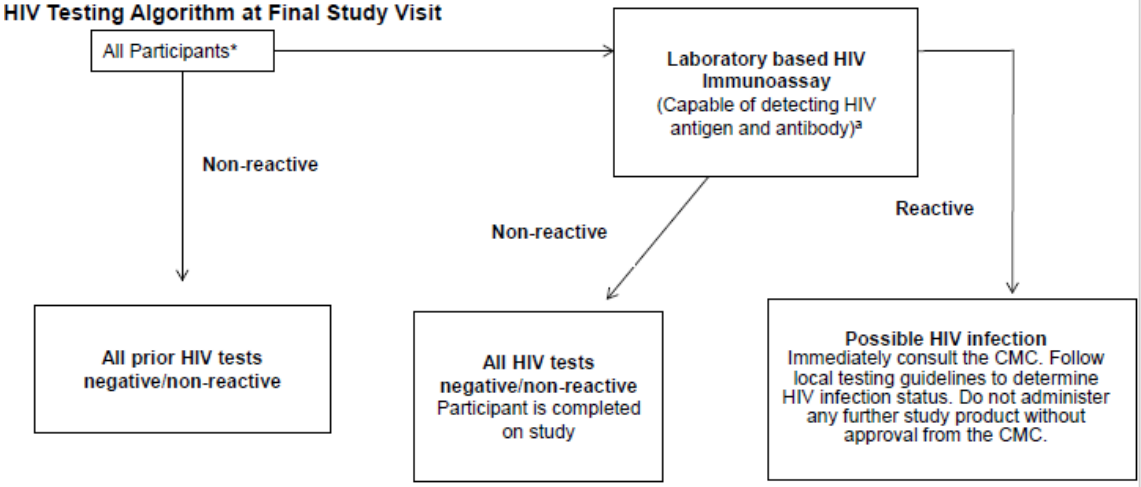
[5] Stored specimens may be used for QC, virology or other exploratory objectives as noted in Section 9.1.1 of the protocol.

APPENDIX II: HIV TESTING ALGORITHM (SCREENING, ENROLLMENT AND FINAL VISIT)





HIV Testing Algorithm at Final Study Visit



NOTES:

* If acute HIV infection is suspected, do not enroll the participant or administer study product at this time. In addition to following the algorithm above, the site should send a sample for an RNA test that, in the opinion of the site investigator, is able to detect early HIV infection. If possible, the site should use an assay that is FDA-cleared for early HIV diagnosis, such as the APTIMA HIV-1 RNA Qualitative Assay. The site should contact the CMC once all of the test results from this visit (including the HIV RNA test) are available.

[a] This testing must be performed using a laboratory based, non-rapid HIV immunoassay that detects both HIV antigen and HIV antibody (either a 4th generation or 5th generation assay).

APPENDIX III: SAMPLE STUDY INFORMED CONSENT FORM (SCREENINGENROLLMENT, AND LONG-TERM STORAGE/FUTURE TESTING)

HPTN 117
Characterization of Doxycycline Pharmacokinetics and Adherence Post-Single and Repeat Dosing Schemas

Version 1.0
07 January 2026
DAIDS Document ID: 39206

Sponsored by: Division of AIDS, US National Institute of Allergy and Infectious Diseases (NIAID), US National Institutes of Health. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study. Study product, doxycycline, purchased from McKesson, by the HIV Prevention Trials Network (HPTN).

PRINCIPAL INVESTIGATOR: [Insert Name]

PHONE: [Insert Number]

1. INTRODUCTION

You are being asked to take part in a research study. It's your choice whether or not to join. You do not have to join, and you can change your mind and leave the study at any time. You won't lose any benefits if you decide not to join or if you leave later.

Before you decide, this form gives you information about the study. Please take time to read this entire form carefully. If you have any questions, ask the study staff. Make sure you understand everything before you decide to join.

If you agree to be in the study, you will be asked to sign this form. The study team will keep the original signed consent form. A copy will also be given to you to keep. A copy of this informed consent will also be placed in your medical record.

Signing the form does not mean you are already in the study. First, you will need to go through some tests to make sure you are eligible to take part.

You should know key information about this study before you join.

Here is a summary of important information you should know about the study:

- This is a research study.
- An objective of this study is to judge the safety of immediate release (IR) and delayed release (DR) doxycycline.
- You do not have to be in this study if you do not want to. If you choose not to join, you can still join another study later if there is one available and you are eligible.

- If you join, you can leave the study at any time. The care you receive at [site name] or other nearby clinics will not be affected.
- The study product is a pill (tablet) called doxycycline. It's an antibiotic often used to treat bacterial infections, including sexually transmitted infections (STIs).
- The purpose of this study is to learn how the body takes in, uses, and gets rid of doxycycline when it is taken by mouth.
- If you join, you will come to the clinic for about 17 visits and be in the study for about 37 days.
- You will be asked to take doxycycline by mouth. You will be randomly assigned to take either 1 or 2 pills at the start. After this first dose, study staff will collect samples of your blood, urine, hair and genital fluids will be collected for research purposes (to see how much of the medicine is in your body). After that, you will then take 1 pill, either daily or every three days. (This will also be randomly assigned). More samples will be collected after each dose.
- Your blood and urine will also be tested for HIV and/or STIs. Samples from your throat and genitals (penis, rectum and/or vagina) will also be collected and tested for STIs.
- You may not get any direct benefit from being in this study. However, you will get information about HIV and STIs and how to protect yourself. You will receive free counseling, HIV and STI testing, physical exams, and routine lab tests. If you have an STI, you may get treatment or be referred for care. We will do our best to protect your privacy, but there is a small chance others may find out you are in the study.
- The most common side effects of doxycycline are nausea, vomiting, diarrhea, and sensitivity to sunlight. These side effects usually stop when the medicine is no longer taken.
- Please tell the study staff if you are in any other research studies or plan to join one—especially if it involves drugs, products, or vaccines. This is important for your safety.
- If you don't want to join this study, there are other ways to help prevent HIV and STIs. These include using condoms and/or taking PrEP (pre-exposure prophylaxis). The study staff can give you more information about these options. You may also be able to join another study in the future if one is available and you qualify.

2. ABOUT THE STUDY

This study is seeking to better understand how doxycycline works within the body.

The HIV Prevention Trials Network (HPTN) and the *[site name]* are doing this study to learn more about doxycycline and to better understand the way the body absorbs, distributes and gets rid of doxycycline, when taken by mouth. This study is also trying to gain a better understanding of the safety of immediate release (IR) and delayed release (DR) doxycycline.

About 16 people will participate in this study from one site in the United States. If you join, you will be in the study for approximately 6 weeks (or 37 days). During this time, you will come to the clinic for about 17 visits.
Doxycycline is a medicine that is approved by the U.S. Food and Drug Administration (FDA). It is used to prevent or treat certain bacterial infections, including STIs like chlamydia. However, Doxycycline does not protect you from acquiring HIV. For this reason it is important that you

use preventive method, while in the study, to protect you from acquiring HIV. There are approved medications to prevent HIV, including:

- daily oral (pre-exposure prophylaxis; PrEP) - a pill you take every day
- long-acting PrEP- an injection you get every few months

Study staff can speak to you about these prevention options and can also refer you to local resources to find the method that works best for you.

You will be placed in 1 of 2 groups.

If you choose to join this study, you will be randomly placed into one of two groups during each of the two study phases. Each group will have the same number of males and females.

In the first part of the study, the group you are in will decide which type of doxycycline pill you take. You will take only one dose (one time). You will get either:

- One 200mg delayed release tablet, or
- Two 100mg immediate release tablets

Both tablets have the same amount of medicine. The only difference is how fast the medicine is absorbed by your body. The delayed release tablet takes a little longer to work, and the immediate release tablets start working sooner.

After taking this first dose, the study team will collect samples from you over a two-week (14 day) period. These samples will be used for research purposes. These samples may include:

- Fluids from the anus and rectum (called anorectal samples)
- Vaginal fluids (if applicable)
- Urine
- Blood
- Hair

Two weeks after the first part, you will take one 200mg doxycycline tablet for 10 days. Based on the second randomization (which occurred at enrollment), you will either take the tablet every 24 hours (daily) or every 3 days (on the third, sixth, and ninth day).

You will be asked to take your pill while a study team member watches. This is called “observed dosing.” You can do this at the clinic in person, or by video call using your phone or computer if you are not at the clinic.

During your time in the study, you should use condoms as a method for preventing HIV and other STIs.

3. JOINING THE STUDY

You must be eligible for the study before you can join.

If you choose to participate in this study, we will begin by performing some tests and collecting some information from you to find out if you qualify. Further information about these tests and the information we will collect are described in the remainder of this form. If you do not qualify, you cannot join the study.

We will examine you and test your blood, urine, as well as penile, rectal and/or vaginal fluid.

To see if you qualify for the study, we will first have a "Screening Visit." This visit will happen after you read, discuss, understand, and sign this form. The Screening visit will take about *[site to insert]* hours.

During the Screening Visit, you will:

- Be asked questions to confirm you are able and willing to join the study.
- Answer questions about where you live, your health, and medical history (including any medications you are currently taking). You will also be asked to give study staff your contact information so they can reach you.
- Talk with a member of the study staff about STIs and HIV. You will also be tested for HIV/STIs. We will give you the results as soon as they are available. A member of the study team will also discuss ways to prevent HIV and STIs.
- Learn about the rules of the study and how to follow them to ensure you are safe. For example, you will be asked not to take certain medication that may negatively interact with the study product you take in the study.
- Have a physical exam, which includes checking your weight, temperature, blood pressure, look into your mouth and throat, listening to your heart and lungs, and examining your abdomen and other areas, if needed.
- Have a swab collected from your throat to test for STIs.
- Have fluids taken from your body to test for STIs. This includes:
    - A swab (like a Q-tip) will be used to collect fluid from your rectum to test for STIs.
    - If you have a penis, fluid will be collected from your penis to test for STIs.
    - If you have a vagina, fluid will be collected from your vagina to test for STIs.
- Have your urine tested for STIs, other infections, and health problems, including kidney and liver issues.
- If you can become pregnant, your urine will also be tested for pregnancy. If you are pregnant, you cannot join this study. A study staff member will talk to you about ways to prevent pregnancy. To join this study, you must use at least two effective methods of contraception. It is strongly recommended that at least one barrier method (e.g. condoms with/without spermicide, sponges, etc.) in addition to a hormonal contraception method be used. You can use methods like birth control pills, other hormonal methods (such as oral pills, vaginal ring, depo), or an intrauterine device (IUD) that was placed at least 30 days before you join. Alternatively, you could choose to abstain from vaginal intercourse

for 90 days before and during the study unless you or your partner have been sterilized or only have sex that cannot lead to pregnancy (for example, same-sex partners).

- Give blood (about 5mL; or about 1 teaspoon) to test for HIV and other infections like Hepatitis B, and syphilis.
- Your blood will also be checked for other health problems, including the health of your liver and kidneys.
- You will be contacted and told the results of HIV and other tests when they are ready. It is expected that all your results will be available *(site to insert)*.
    - If you test positive for gonorrhea, chlamydia, or syphilis, you will be (*site to specify [treated] or [referred for]* treatment).
    - If you do not have Hepatitis B, and you have not been vaccinated and seeking protection against the virus, you will be *referred for* the Hepatitis B vaccine. A study team member will explain how to get the vaccine.
    - To join in the study, you must receive your HIV test results. If the test shows you have HIV, you cannot join the study.
        - Sometimes HIV test results are not always clear. This could mean that you may have HIV, it is too soon for the test to detect it. In this case, you may need another test.
        - If your results show that you have HIV or if the results are unclear, you will be referred for further testing and to health services you may need.
    - If your blood tests show a health problem (like liver or kidney issues), you may not qualify for the study. The study staff will refer you to healthcare services. If these issues are resolved later, you may be able to try again for the study.
    - *(Site to include any other information about local procedures here, as necessary).*
- You will be given reimbursement for this visit and will work with the study team to schedule your next visit, if needed.

We will confirm if you qualify for the study. If you are eligible, you will enter the study.

If you qualify for this study and decide to join, you will be asked to return for an "Enrollment Visit." This visit will happen within 30 days after your Screening Visit.

The Enrollment Visit will take longer than others and will last about *[site to insert]*. During the visit, you will:

- Review and update your contact information, if needed.
- Talk with a study team member about HIV and STIs and how to protect yourself from acquiring these infections. You will also be given condoms.
- Have a physical exam and update the study team on any new medications you are taking and any health issues you may have had since your last visit.
- Be assigned to which tablet you will take during the study. For today, you will either be assigned to take either two 100mg tablets or one 200mg tablet. You will also be told how often you will be asked to take the 200mg tablet, either daily or every 3 days later in the study.

- Be given your study tablet(s), along with instructions on how to take them, as well as any possible side effects. You will take your assigned tablet(s) in front of a study team member to make sure you can tolerate it and take it correctly during the study.
- Have blood drawn at several timepoints to check your safety and for research purposes. You will also provide blood sample *(site to insert amount* to test for HIV.
    - You will also give blood samples at different times [1, 2, 3, 4, 6, and 8 hours after taking the tablet(s)]. Each sample will be about (site to insert). These will help researchers understand how the study product works in your body.
    - Any leftover blood or other samples (such as genital fluid) may be stored for possible future testing. This could be for this study or for HIV research in general. No other samples collected at the time of screening will be used for any other purpose than those listed above.
- You will, for study-related purposes:
    - Provide the rectal fluid after taking the tablet(s). This will be collected at different timepoints (1, 2, 3, 4, 6, and 8 hours) after you take the tablet(s).
    - Provide urine sample at two different times after taking the tablet(s) (between 1-4 hours and 4-8 hours).
    - If you have a vagina, you will also provide vaginal fluid. This will also be collected at different timepoints (1, 2, 3, 4, 6, and 8 hours) after you take the tablet(s).
- If you can become pregnant, you will:
    - Provide a urine sample to test for pregnancy. You will talk with the study team about ways to prevent pregnancy.

You will get your test results as soon as they are available. If needed, the study team will refer you for treatment and other care. Some of the blood and other samples collected will be saved for future study-related testing if there are any questions about your results later.

You will also be given reimbursement for your visit and will work with the study team to schedule your next visit. The team will help you plan your visits and when to take the tablets, depending on which group you have been assigned to. Today, you will learn which visit(s) will require you to stay longer and how long you will need to stay at the clinic.

4. BEING IN THE STUDY

Fourteen days after your Enrollment Visit, you will start taking one 200mg tablet of doxycycline, either every day or every 3 days for 10 days, depending on the group you were assigned to. Each time you take the tablet, you will either come to the clinic or have a video call with a study team member.

You will be asked to come to this clinic approximately *17 times* over the course of the next 6 weeks. Each of these visits lasts about [*site to insert amount of time*]. At each study visit, you will be asked about social harms (negative social, emotional, physical, or economic consequences) and adverse events (undesired effects from the study product). You will also be given HIV/STI risk reduction counseling at each study visit. After each visit, you will be reimbursed and work with the study team to schedule your next visit.

- On Day 1 (24 hours after you take the first dose during your enrollment visit), you will visit the clinic to give a blood sample (about 6 mL or 1.5 teaspoons) and a urine sample for research testing. You will also provide rectal fluid for research testing. If you have a vagina, vaginal fluid will also be collected.
    - Some of your blood will be collected using a TASSO+ device. This is a small, round device that sticks to your upper arm. When placed on your arm, you (or a study staff member) will press the button on the device. When the button is pressed, you may feel a quick pinch (like a rubber band snap). The device then slowly collects a small amount of blood through your skin. After the blood is collected, which will only take a few minutes, we will remove the device.

- On Days 2, 3, 4, 7, 10, and 14, you will visit the clinic to give a blood (about 6 mL or 1.5 teaspoons) and a urine sample for research testing. You will also provide rectal fluid for research testing at this time. If you have a vagina, vaginal fluid will also be collected.
    - On Day 3 and Day 7, some of the blood collected will be done using a TASSO device.
    - On Days 3, 7, 10, and 14, a member of the study team will also collect about 5 strands of hair for research purposes. The hair will be taken from the back of your head (the occipital region). For this, your hair must not be treated with any chemicals such as relaxers, perms, dyes or bleach.
        - On Day 14, if you agree, we will cut a small portion of hair (about 100 strands) with scissors for research purposes. The collection procedure involves cutting the shaft of the hair near the scalp, this way people will not be able to tell that your hair has been removed. Taking a sample of your hair may cause some discomfort. You may also worry that this is painful or affects how you look. If you do not wish to provide this cut hair sample, you can still participate in the study, and it will not affect the care you would normally receive.
    - If you can become pregnant, on Day 14, your urine will also be tested for pregnancy. You will talk to a study team member about ways to prevent pregnancy. If you are pregnant, you will be asked to stop using the study product.

- On Days 15, 18, 21, and 24 (24 hours after you take your last dose), you will visit the clinic to provide blood (about 6 mL or 1.5 teaspoons) and urine for research testing. You will also provide urine and rectal fluid. If you have a vagina, vaginal fluid will be collected.
    - On Day 24, if you agree, we will also cut approximately a small portion of hair (about 100 strands) with scissors for research purposes.

- On Days 26, 29, 32, and 35, you will visit the clinic to provide blood (about 6 mL or 1.5 teaspoons), urine, and hair for research testing. You will also provide rectal fluid; if female, vaginal fluid will also be collected.

Your final visit will be on Day 37 (or earlier if you choose or are asked to leave the study). The procedures done at this visit will be like what you had done during the Screening and Enrollment

Visits. During this final study visit, we will talk with you about the end of the study and when the results of the study will be available. You will:

- Review and update your contact information
- Talk with a study staff member about STIs and HIV. You will also be tested for HIV, and the study team will discuss ways to prevent HIV and STIs.
- Have a physical exam.
- Have a swab collected from your throat to test for STIs.
- Provide bodily fluids taken to test for STIs and for research purposes. This includes:
    - Rectal fluid collected with a cotton swab (like a Q-tip) for STI testing and research.
    - If you have a penis, penile fluid will be collected for STI testing.
- If you have a vagina, vaginal fluid will be collected for STI testing and research. You will have your urine tested for STIs. Some of this urine will be used for research purposes.
    - If you can become pregnant, your urine will also be tested for pregnancy.
- Provide blood (about 10 mL; or about 2 teaspoons) to test for HIV and other infections like syphilis and for research purposes. Your blood will also be checked for other health problems, including the health of your liver and kidneys.
- Provide a hair sample. First, the study team will pluck about 5 strands of hair. Also, if you agree, the study team will cut about 100 stands of hair. The hair will be collected for research purposes.
- Receive reimbursement for your visit.

You will be contacted and told the results of HIV and other tests when they are available. It is expected that all your results will be available in about (site to insert). If you test positive for gonorrhea, chlamydia or syphilis, you will be referred for treatment. If tests indicate you have acquired HIV, you will be referred for further tests to confirm your status and we will connect you with medical care and other services you may need.

You may also be asked to have more visits if you are sick, or we need to check on your health. We may also ask you to provide additional samples if any of the above procedures need to be repeated due to issues with sample processing or testing. Additional testing may also be performed as part of quality control.

If you have to stop taking your study products, we will ask you to stay in the study.

If you stop taking the study tablet(s) for any reason, we may ask you to continue to come for your regular study visits. However, you may not need to do certain procedures. We will fully explain to you what will happen if you stop taking your study product.

If you acquire HIV during the study, we will help you get care and support.

We will test your blood for HIV before you join the study and at your last visit. If you acquire HIV while you are in the study, you will stop taking the study product and we will help you find the care and support you need. However, the study does not cover the cost of this care. You will also be referred for additional testing to confirm your HIV status. If it is confirmed that you have

acquired HIV, your doctor may perform more testing to determine what HIV treatments would work best for you. You may also be referred to other research studies. Since continuing the study would not help you, your participation in the study will be stopped.

We will test you for pregnancy and ask you to take birth control while you are in the study.

During the study, we will require you to use an effective method of birth control. We will talk to you about how to prevent pregnancy. We will test your urine for pregnancy and give you these results each time. If you become pregnant during the study, we will ask you to keep coming to your regular study visits, but you won't have to do certain procedures anymore. We will explain to you what will happen if you stop taking your study products. If you are pregnant at the last study visit, we may ask to contact you later to learn what happened with the pregnancy. This is important because it helps researchers understand if the study product had any effects during pregnancy. You do not have to agree to be contacted. We will only contact you if you say we can, and you can choose not to share any information if you do not want to.

The study cannot provide medical care related to a pregnancy or delivery of a baby. The baby will not be in the study. Therefore, it is important to receive medical care for a pregnancy outside the study. We will tell you where you can go for the care you need.

Use of stored and/or leftover specimens. Some of your specimens may be left over at the end of the study and may be used for future research.

Some of the blood samples we collect may be stored for future research. Other leftover samples from the study may also be kept after the study is over. Once all study-related testing is completed, these samples may be stored and used for future research. No genetic testing will be done on these samples. Samples stored in the study may be used for quality control testing (to confirm results obtained in site laboratories).

If you acquire HIV during the study, some of the stored blood may be used to study HIV/STIs and your body's response. Your stored samples may also be used for future research related to HIV, hepatitis, and other STIs, and to better understand laboratory tests related to this study, including measurement of study products or other drugs relevant to HIV, hepatitis, or STIs.

If you agree to participate in this study, you will be asked to give permission to use your stored samples for future research. If you agree, all personal identifiers such as your name, birthdate and any other information that could identify you personally will be removed. The stored samples will be labeled only with your study number and will be tested at special laboratory facilities that may be in the US. The laboratory will not know who you are. Only approved researchers can access your samples. Results from this testing will not be returned to the study site or you. Your samples will not be sold or directly used to produce commercial products or for commercial gain. All proposed research studies using your samples will be reviewed by the US National Institutes of Health (NIH), the organization funding this study.

Before any research can happen with your stored samples, it will be reviewed by the HPTN Laboratory Center (LC) and Executive Committee. If a researcher wants to use your de-identified stored samples and information for future research studies, their research plan must be

reviewed and approved by the HPTN Executive Committee. [*Site: If review by your site institutional review board (IRB) is also required, insert a sentence stating this*.] IRBs protect the rights and well-being of people in research. If the research plan is approved, the HPTN will send your deidentified stored samples and information to the researcher's location.

At the end of this form, you will be asked to give permission for your leftover and stored samples to be used in future research. If you agree to have your stored samples used for future research, there is no time limit on how long your samples will be stored. If you give permission, you will not be asked to give permission again during the study, and additional informed consent will not be obtained.

If you don't want your samples stored for future research, you can still be part of the study. You can change your mind at any time and withdraw permission for your samples to be used in future research. If you withdraw your consent, we will destroy your samples once all study-related testing is done.

5. RISKS OF THE STUDY

There may be risks to being in this study.

BLOOD DRAWS

Risks with blood draws include pain, bleeding, bruising, infection, or swelling at the site of the needle sticks**,** as well as the possibility of dizziness or fainting during needle sticks. Very rarely, the site can become infected. These risks will be minimized by using an aseptic (clean) technique as well as having experienced staff perform blood draws.

HAIR COLLECTION

Taking a sample of your hair may cause some discomfort. You may worry that this is painful or affects how you look. The collection procedure involves cutting the shaft of the hairs near the scalp; the root of the hair will not be collected. Each hair will be cut carefully and will be sampled around your scalp, this way people will not be able to tell that your hair has been removed.

CONFIDENTIALITY

Taking part in clinical research comes with some risks, like the chance that your personal information could be shared or that you may feel uncomfortable with some of the personal questions. Even though the study team works hard to keep your information private, there is still a small risk that other people might find out you are in the study, which could lead to social harm.

SIDE EFFECTS OF THE DOXYCYCLINE

Doxycycline is generally safe, but it can cause some side effects, some of which are listed below. Please note that these lists do not include all possible side effects. Below are the more serious or common side effects with a known or possible relationship to doxycycline. These side effects may go away as your body adjusts to taking the drug.

The most common side effect reported is nausea. Other side effects that have been reported include vomiting, diarrhea, stomach cramping, rash, acne, and rare serious allergic reactions. Taking doxycycline with food (a small meal or snack) and a full glass of water can help reduce some of these side effects. To help reduce the chance of irritation in your stomach, it may help to remain upright (sitting or standing) for at least 30 minutes after taking.

Another side effect is photosensitivity, which means your skin may become more sensitive to sunlight. People taking doxycycline and other types of antibiotics have reported this. While you are in this study, you should be extra careful to avoid sunburn. For example, you should wear protective clothing, use sunscreen often, and limit your time in the sun.

Stevens-Johnson Syndrome (SJS)/Toxic Epidermal Necrolysis (TEN) – also called Lyell syndrome: These are very serious skin reactions that can be life-threatening. They often start with flu-like symptoms, then a painful rash and blisters appear. Blood tests may show liver problems. Other symptoms can appear after a few days, including:

- Painful red or purple skin that looks burned and may peel off
- Blisters on the skin, inside the mouth, nose, and genital area
- Red, painful, and watery eyes

If you have any questions or experience any of these symptoms, you are asked to let the study staff know immediately.

SOCIAL

There may also be some social risks to participating in this study. You may feel embarrassed or uncomfortable with some of the questions you will be asked and/or some of the procedures that will be done. You may feel anxious while waiting for your test results, and after receiving them. You can talk to a member of the study team to help you deal with any feelings or questions you have. You do not have to answer any questions that you do not want to. You can stop answering the questions at any time.

Some people might treat you unfairly or make assumptions about you because you are part of a study involving a drug that treats and prevents STIs. For example, they might think you have an STI, or your family or friends might feel upset, worried, or angry. You may feel uncomfortable telling your sexual partner about your involvement with this study, or use of the study product.

SWAB COLLECTION

You may feel pain or discomfort or pressure in your rectum, penis or vagina when swabs are taken. There could be a small amount of bleeding after use of the swabs. For the pharyngeal (throat) swab, some people feel a momentary gagging reflex (feeling like you want to vomit). There is also a slight risk of infection.

HIV AND OTHER STIs

We told you earlier that study product does not protect you from getting HIV. Because of these risks, it is very important that you use condoms every time you have sex, no matter what group you are in. There are other known effective ways to reduce your risk of getting HIV. This includes the use of PrEP or post-exposure prophylaxis (PEP) medication. Truvada®, Descovy® and Apretude® are HIV prevention methods in which people who do not have HIV take to reduce their risk of getting the virus. If you're interested in learning more about PrEP or PEP, the study team can give you more information, and you might also want to talk to your doctor.

Finding out your HIV or STI status might cause problems between you and your partner(s).

Telling people about your HIV and STI status may cause worry, sadness, depression, or loneliness.

If you have any problems, the study team is available to talk with you and/or your partner to try to help.

PREGNANCY

To join this study, you must use at least two effective methods of contraception for at least 30 days before you start the study and throughout the study. It is strongly recommended that at least one barrier method (e.g. condoms with/without spermicide, sponges, etc.) in addition to a hormonal contraception method be used. You can use methods like birth control pills, other hormonal methods (such as oral pills, vaginal ring, depo), or an intrauterine device (IUD) that was placed at least 30 days before you join. You could also choose to abstain from vaginal sex for 90 days before and during the study unless you or your partner have been sterilized or only have sex that cannot lead to pregnancy (for example, same-sex partners).

We do not know what effect the study product may have on pregnancy, including the effect on the fetuses of individuals who use the study product when pregnant. or this reason, people who are pregnant cannot join the study. If you become pregnant during the study, you will be stopped from using the study product, and you will be referred to medical care for you and your baby. However, the study does not pay for this care. You will also have one final visit before leaving the study.

OTHER RISKS

We do not know if using herbal treatments or supplements while taking the study product might cause any problems. If you are using any herbal treatments, supplements, or other medications,

you must tell the study staff. Some medications and supplements can interfere with how well doxycycline works in your body. Certain medications, such as phenytoin (Dilantin), carbamazepine (Tegretol), and barbiturates, may cause your body to remove doxycycline faster, making it less effective. These medications are sometimes used to treat conditions other than seizures. Doxycycline may also interact with other medications in ways that could increase the risk of seizures. If you have a history of seizures or are currently taking any medications for a seizure disorder, it is very important that you inform the study team. Antacids that contain aluminum, calcium, or magnesium (such as Tums, Mylanta, Rolaids, or Milk of Magnesia) and products that contain iron or bismuth (like Pepto-Bismol) may block your body from fully absorbing doxycycline. If you are currently taking any of these medications or start using them during the study, please tell the study team right away. You may be asked to stop using certain products while you are in the study.

There could also be rare or unknown risks that might happen during the study, so it's important to report any issues or problems right away to the researchers.

6. BENEFITS OF THE STUDY

There may be no direct benefit to you by participating in the study.

There may not be any direct benefit to you from participating in this study. However, there are still some helpful things you might gain from it. Throughout the study, you will be tested for HIV and other STIs. You'll also get counseling that can help you learn how to avoid HIV and STIs. If you do acquire HIV or an STI, you will be referred to medical care and treatment. The study will also check for hepatitis B infection at the screening visit and provide you with a hepatitis B vaccine or referral if needed.

You will get physical exams during the study. If these exams show that you have any health problems, you will be told about medical care and other services that you can access. This will be available to you even if you do not enroll in this study. This study cannot provide you with general medical care, but study staff can refer you to other available resources, if needed. During the study you will have tests to check the health of your blood, liver, and kidneys.

While there may not be direct benefits to you, the study may help improve health and prevention methods for other people, including those in your community. The information from this study could help prevent infections and improve treatments in the future, which could benefit both you and others in your community.

7. OTHER INFORMATION ABOUT THE STUDY

We will tell you any new information that may affect your decision to be in the study.

If we learn anything new during the study that might affect your decision to stay in, we will let you know. For example, if we find out that the study product is causing serious side effects, we will tell you.

You will also be told when the results of the study may be available, and how to learn about them.

You may be withdrawn from the study without your consent.

We may take you out of the study at any time without your consent. This may happen if:

- You are unable or unwilling to follow all the study procedures or instructions.
- You acquire HIV or an STI
- You become pregnant or begin breastfeeding
- The study is stopped or canceled.
- The study staff feels that staying in the study or continuing to take the study product may be harmful to you.
- Other reasons, as decided by the study principal investigator listed on page 1 or their designee.
- If you leave the study early or are asked to leave, we may ask you to return to the clinic one last time. This visit may include blood tests, an exam, and study procedures described for Day 37, if you are willing.

You have other choices if you choose not to be in this study.

[*Sites to include/amend the following if applicable]*

There may be other studies going on at this clinic or in the community that you may be eligible for. If you wish, study staff can tell you about other studies that we know about. The study staff can also tell you about other studies or places where you can receive HIV counseling and testing. If you choose not to be in this study, your regular medical care will not be affected.

Will it cost you anything to be in this study?

It won't cost you anything to be in this study. All study visits, study products, physical exams, lab tests, and other procedures are free. If you need treatment for infections spread through sex, it will be provided at no cost, or you will be referred to available treatments during the study. If you ever get a bill related to the study, please let the study team know right away.

We will give you [site to insert amount] for each study visit.

[Site to insert/update information about local reimbursement for the study.]

You will receive [$XX] for each study visit. You will be paid for your time and participation. If you do not complete the study, payment will be based on the parts you completed.

We will do our best to protect your private information.

Your personal information will not be shared without your written permission. Study staff will protect your privacy in the following ways:

- To keep your information private, you will be given a study ID number that only has a set of numbers. Your name will not be used on samples or records. Only the study team can link your name to your study ID Your name, where you live, and other personal information will be protected. The file that connects this ID number to you will be securely stored on a protected computer network.
- Records will be kept in locked areas or secure computer systems. that only study staff can access with a password.
- Study staff will not leave messages with personal details on your voicemail. They will only contact you the way you prefer.

Your samples will never be used for profit. Study results will not include information that identifies you. When you take the study product with staff watching, your privacy and safety will always come first. This may be done either in person during your clinic visit or through video if you prefer. If it's done in person, it will be in a private space. If it's done virtually, a secure and private video platform will be used to protect your privacy.

Although the study team will do everything possible to keep your information private, there may be times when law requires to share information, including personal information. This is rare, but if it happens, the study team will take steps to protect your privacy as much as possible. In some cases, your information in this research study could be reviewed by representatives of the study team, research sponsors, or government agencies (for example, the NIH) to make sure everything is done properly.

Certain diseases, such as syphilis, gonorrhea, and chlamydia, must be reported to the state by law. If you test positive for one of these diseases during the study, the researchers are required to report it to the state.

Clinic staff will have access to your study records. Your records may also be reviewed, under guidelines of the US Federal Privacy Act, by:

- The [*insert name(s) of site local and/or national Institutional Review Board (IRB) or Ethics Committee*]
- The sponsor of the study (US National Institutes of Health [NIH]), its contractors, and its study monitors
- The US Office for Human Research Protections (OHRP)
- Other local or US regulatory authorities/entities
- The HPTN that is conducting this study
- And (*insert any other applicable local authorities*]

To help us protect your privacy, the study team has obtained a Certificate of Confidentiality from the National Institutes of Health (NIH). With this certificate, the researchers cannot be forced to disclose any information that can be used to identify you, even by court subpoena, in any federal, state, local or civil, criminal, administrative, legislative or other proceedings. The researchers would use the certificate to resist any demands for information that would identify you, as described.

The Certificate cannot be used to resist a demand for information from personnel of the United States government that is used for auditing or evaluation of federally funded projects or for information that must be disclosed in order to meet the requirements of other regulatory agencies.

You should understand that a Certificate of Confidentiality does not prevent you or a member of your family from voluntarily releasing information about yourself or your involvement in this research. If an employer, insurer, or other person receives your written consent to receive research information, then the researchers may not use the Certificate to withhold that information.

The Certificate does not limit the researchers from disclosing information that may include details about child abuse, homicidal or suicidal intent, or other information deemed appropriate. The researchers will not voluntarily release any information about you under this Certificate.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov. This Web site will not include information that can identify you. At most, the Web site will include a summary of the study results. You can search this Web site at any time.

If you get sick or injured during the study, contact us immediately.

All research carries some risk, meaning there is a chance that something bad might happen to you. If you get hurt, become sick, or have a bad reaction from something that was done as part of this study, the study team will help you get medical care. However, the study team nor the National Institute of Health (NIH) will not pay you for any injuries, illnesses or reactions, or the related medical care need. Any costs for medical expenses will be billed to you or your insurance company. You may have to pay for any co-payments and your insurance may not cover the costs of study related injuries. If you think you have been injured from taking part in this study, call (*site to insert*). You do not give up any legal rights by signing this consent form.

What if you have questions about this study?

It may be that you have questions about your rights as a study participant or that you think you have been injured because you were in this study. In this case, you should contact the researchers listed on the first page of this form.

All research on human participants is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research participant, or if you would like to get information or give input, you may contact the IRB at (*site to insert contact information*).

SCREENING, ENROLLMENT AND SPECIMEN STORAGE/FUTRE TESTING CONSENT
SIGNATURE PAGE

HPTN 117
Characterization of Doxycycline Pharmacokinetics and Adherence Post-Single and Repeat Dosing Schemas

Version 1.0
07 January 2026
DAIDS Document ID: 39206

CONSENT FOR SCREENING AND ENROLLMENT

If you have read this consent form, or had it read and explained to you, and you understand the information, and you voluntarily agree to join the study, please sign your name or make your mark below. Also, please indicate by providing your initials in the spaces below regarding deidentified data, sample collection and long-term storage and future testing that you agree to.

____ I **agree** to take part in this study.

____ I **do NOT agree** to take part in this study.

CONSENT FOR CUT HAIR COLLECTION

As part of this research study, we are asking for your permission to collect a small hair sample (about 100 strands, cut close to the scalp) at select study visits. These samples may be used in the future for additional testing, such as understanding how the study drug moves through the body. This hair collection is optional and will not affect your participation in the rest of the study. Please indicate your choice by initialing next to one of the statements below:

____ I **agree** to have a small amount of my hair cut at select study visits.

____ I **do NOT** agree to have my hair cut at select study visits.

CONSENT FOR LONG-TERM STORAGE AND FUTURE TESTING OF SPECIMENS

____ I **agree** to have samples of my blood and leftover urine, and/or rectal, vaginal and penile fluid stored for study-related testing and/or used for potential future studies. I understand that any samples given will be deidentified. I will not be asked to give permission again for stored samples during the study, and additional informed consent for stored samples will not be obtained. However, I may withdraw my consent at any time before I complete the study.

____ I **do NOT agree** to have samples of my blood and leftover urine, and/or rectal, vaginal and penile fluid stored and used for potential future testing and studies.

Insert signature blocks as required by the local IRB

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