Protocol Signature Page

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 U.S. Food and Drug Administration regulations; standards of the International Conference on Harmonization 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.

November 11, 2021

Signature of Investigator of Record **Date (Month/Day/Year)**

Monica Gandhi MD, MPH

Investigator of Record (PRINT NAME)

Full Protocol Title: Point-of-care Urine Monitoring of Adherence (PUMA): Testing a Real Time Urine Assay of Tenofovir in PrEP

DAIDS Protocol Number: DAIDS ES-ID 38665
DAIDS Protocol Version: 6.0
Protocol Date: November 11, 2021

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November 11, 2021

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STUDY PROTOCOL

Point-of-care Urine Monitoring of Adherence (PUMA): Testing a Real-Time Urine Assay of Tenofovir in PrEP

Pilot trial to examine the feasibility, acceptability and impact on longer-term adherence of an intervention using a new urine-based tenofovir adherence assay

Funded by:
US National Institute of Allergy and Infectious Diseases

VERSION 6.0

November 11, 2021

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TABLE OF CONTENTS

Abbreviations

AIDS	Acquired Immunodeficiency Virus
AE	Adverse Event
ART	Antiretroviral Therapy
ARVs	Antiretrovirals
ARC	AIDS Related Complex
CDC	Centers for Disease Control and Prevention (US)
DAIDS	Division of AIDS (NIH)
DBS	Dried Blood Spots
EC	Ethics Committee
FDA	Food and Drug Administration (US)
FTC	Emtricitabine
FTC-TP	Emtricitabine-triphosphate
HAL	Hair Analytical Laboratory
HIV	Human Immunodeficiency Virus
HTTP	Hypertext Transfer Protocol (presentation of web data)
IRB	Institutional Review Board
KEMRI	Kenya Medical Research Institute
LC-MS/MS	Liquid chromatography/tandem mass spectrometry
LFA	Lateral flow assay
MSM	Men who have sex with men
NIH	National Institutes of Health (US)
PrEP	Pre-exposure prophylaxis
POC	Point of care
RCT	Randomized controlled trial
REDCap	Research Electronic Data Capture
SAS	st Software
SDC	Serodiscordant couples
SOC	Standard of care
STI	Sexually transmitted infection
TDF	Tenofovir disoproxil fumarate
TFV	Tenofovir
TFV-DP	Tenofovir diphosphate
UCSF	University of California, San Francisco
UNAIDS	Joint United Nations Program on HIV/AIDS
US	United States
UW	University of Washington
VCT	Voluntary counseling and testing
WHO	World Health Organization

1. STUDY SUMMARY AND SCHEMA

1.1. Lay Summary

Pre-exposure prophylaxis (PrEP) with oral tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) prevents HIV infection if taken every day. However, previous PrEP trials demonstrated that many women in Africa at risk for HIV who are not in couples (with an HIV-positive partner) need help remembering to take the pill every day. Also, patients on PrEP may not always report their adherence to the pill accurately and "objective" measures of adherence (where PrEP drug levels were measured) are far superior to self-report. We have reason to believe that telling a patient his/her PrEP drug levels, and subsequent counseling on adherence, can motivate better adherence in the future. The usual ways to measure PrEP drug levels are very complicated and require expensive equipment and trained laboratory staff. However, our group has now developed a new and innovative way to measure adherence to PrEP (specifically tenofovir, TFV) via an antibody-based test ("immunoassay") in urine that can be performed cheaply at the point-of-care (POC) by any healthcare worker (like a urine pregnancy test). A few drops of the patient's urine is dropped into the test and tells the provider "yes" or "no" if the patient has been taking PrEP recently. This protocol is for a pilot trial where we randomize women on PrEP (who are not in serodiscordant couples) at Thika Clinic to having information from the urine assay delivered to them (or not delivered to them in the control group) in order to provide feedback on adherence from this test in a supportive, motivating manner and enhance adherence counseling. The pilot trial will assess if the urine tool is acceptable to participants, feasible for the clinic to perform, and if providing feedback on adherence from the urine tool to the participant increases longer-term adherence, as assessed by measuring PrEP drug levels in small hair samples. Good adherence to PrEP will eventually lead to fewer HIV infections worldwide.

1.2. Study Summary

Worldwide expansion of pre-exposure prophylaxis (PrEP) will be critical to ending the HIV epidemic. The original PrEP clinical trials showed us that 1) adherence is critical to effectiveness; 2) daily PrEP adherence was difficult to maintain, especially among women not in serodiscordant relationships; 3) objective adherence measures (e.g. measuring PrEP drug levels) are more reliable than self-reported adherence; and 4) real-time monitoring of PrEP drug levels with feedback to the patient could improve subsequent adherence. Objective measures of PrEP adherence, especially point-of-care measures that enable real-time assessment, intervention and feedback, will be important for both interpreting and optimizing effectiveness during PrEP implementation. Our group has developed a novel point of care urine-based measure of PrEP adherence which has been validated among HIV-uninfected volunteers who were administered TDF/FTC.[1-5] The test has now been developed into a lateral flow assay (LFA), allowing real-time monitoring.[4,5] This test is cheap, easy to perform and can be done at the point-of-care. However, this tool has not yet been tested among participants on PrEP in a trial to see if real-time monitoring

of adherence using the urine assay is feasible, acceptable and motivates improvements in long-term adherence as assessed by hair levels.

Pharmacologic measures of adherence to TDF/FTC-based PrEP, where tenofovir (TFV) drug levels are measured in a matrix such as plasma,[6] dried blood spots (DBS),[7] or hair (studied extensively as an adherence metric by our group)[8-48] capture drug ingestion and predict outcomes more accurately than self-reported adherence.[13-15,17,19,20] Pharmacologic adherence monitoring is especially important in PrEP, where a surrogate biomarker of response (e.g. HIV viral loads during HIV treatment) is not available. However, current methods to analyze PrEP drug levels in any matrix, including easily accessible urine,[49] require liquid chromatography-tandem mass spectrometry (LC-MS/MS), which cannot be performed in real-time because expensive equipment and trained laboratory staff are needed. Antibody-based tests allow for point-of-care testing.

The UCSF Hair Analytical Laboratory (directed by Dr. Monica Gandhi) has now developed a collaboration with Alere™ (recently bought by Abbott Rapid Diagnostics), a company with vast expertise in point-of-care (POC) diagnostics, to develop one of the first immunoassays that detects TFV in urine. The test is sensitive, specific, precise, and quantitates TFV levels in urine as accurately as LC-MS/MS,[1,2,4] or enzyme-linked immunoassay[5] allowing for low-cost POC adherence monitoring. The first iteration of the lateral flow immunoassay (LFA) has been developed[4] and allows for real-time testing, giving the provider qualitative yes/no information on recent adherence to PrEP. This novel POC adherence assay has the potential to address key knowledge gaps in PrEP implementation to both interpret and improve adherence.

Aim: To perform a pilot randomized controlled trial (RCT) in Kenya to assess the acceptability of the urine POC TFV assay to participants, the feasibility of administering the test for providers, and the impact of real-time adherence monitoring/feedback via the POC test on increasing PrEP adherence over time (as established by a long-term metric of adherence using hair levels).

Hypothesis: Providing real-time monitoring/feedback via the POC assay will be acceptable, feasible, and improve adherence over time among adherence-challenged women on PrEP in Kenya.

Approach: Given known PrEP adherence challenges among women not in serodiscordant couples and the likely need for daily drug-taking among women for efficacy,[50] we will conduct a pilot randomized controlled trial by randomizing women not in serodiscordant couples on PrEP in Kenya to standard of care adherence counseling (n=60) vs real-time adherence feedback and enhanced adherence counseling using information from the POC urine assay (n=60). Using mixed-methods, we will assess the acceptability of the test to participants and the feasibility of administering the test for providers via triangulating data from

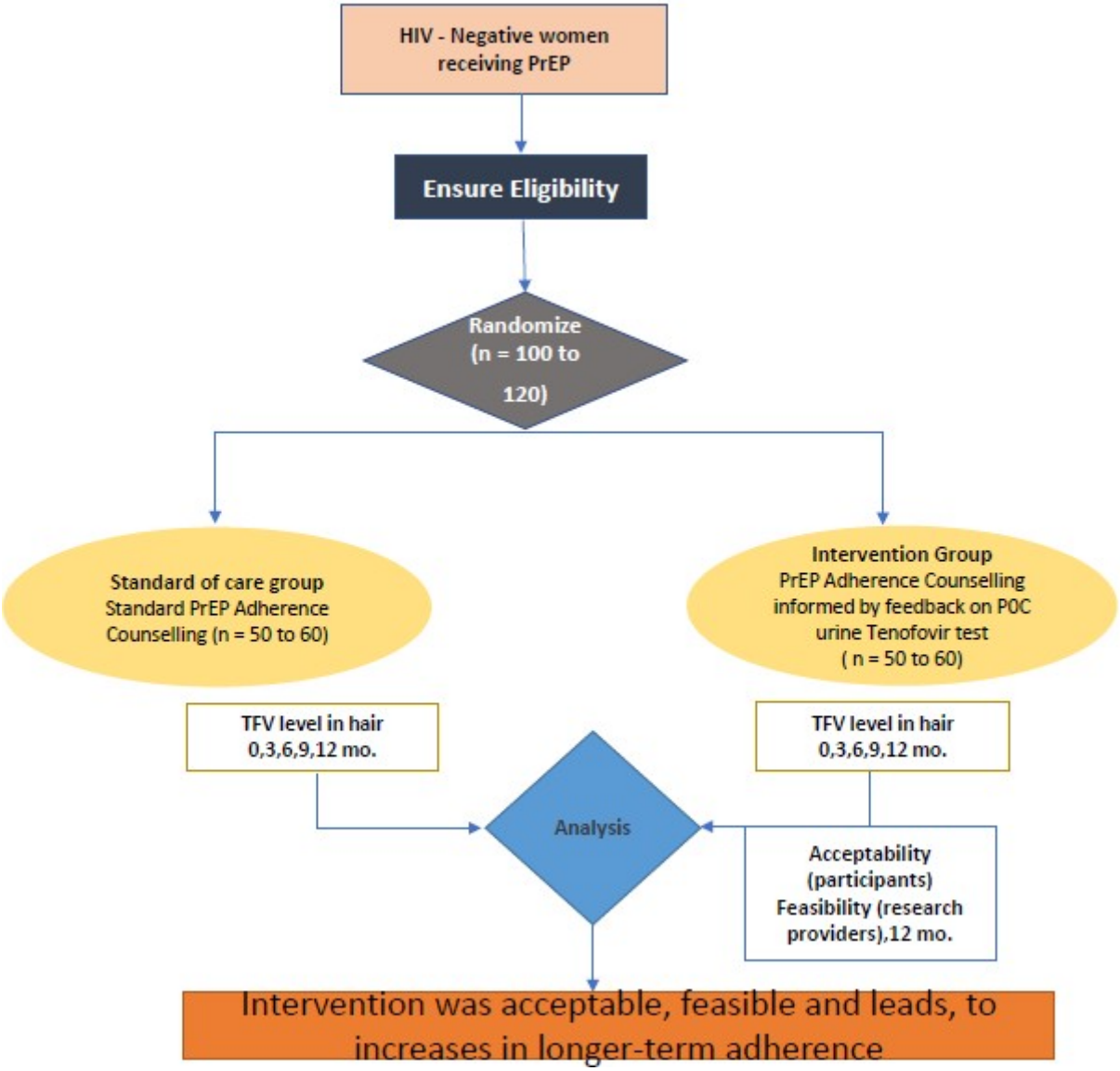
structured surveys and qualitative interviews. The effect of the feedback on long-term adherence over time measured via tenofovir (TFV) levels in hair samples will be assessed.

At study end, we will have conducted a field test of a novel lateral flow assay to detect TFV in urine as the first POC low-cost objective adherence metric available for participants on PrEP. The assay will launch a novel modality for adherence intervention. Moreover, study findings will inform a larger-scale PrEP demonstration trial assessing the impact of real-time adherence monitoring/feedback via the assay on seroconversion rates. The pilot trial proposed here will examine the acceptability, feasibility and impact of this novel tenofovir adherence assay in urine on a longer-term metric of adherence for the first time in any population.

1.3. Schema

Purpose:	To determine if providing real-time monitoring/feedback via a POC tenofovir urine assay will be acceptable to women, feasible for providers, and improve adherence over time using a long-term metric of adherence among women on PrEP in Thika, Kenya.
Design:	A pilot randomized controlled trial of women receiving PrEP will be randomized (1:1) to standard of care adherence counseling (n=60) vs real-time adherence feedback and enhanced adherence counseling using information from the POC urine assay (n=60)
Population:	HIV-uninfected women ≥18 years old receiving PrEP in Kenya
Study Size:	a minimum of 100 and not exceeding 120 participants equally randomized to each arm.
POC Testing:	Urine tenofovir testing by lateral flow assay
Outcome Measurement:	The primary outcomes for this study will be acceptability to participants, feasibility for providers, and impact of the POC adherence test on measures of tenofovir in hair samples 12 months after randomization.
Study Duration:	24 months, including enrollment period, and 12 months of follow-up per participant.
Primary Objectives:	To test the acceptability to participants, feasibility for providers, and impact on a longer-term metric of adherence among participants (assessed via tenofovir levels in hair) of implementing POC urine tenofovir testing and providing real-time feedback among women receiving PrEP in Thika, Kenya.
Study Site:	The Thika Clinic, which is located in an urban center about 40 kilometers outside of Nairobi, has been a center of excellence for PrEP delivery in Kenya and a longstanding clinical research partner with KEMRI and the University of Washington.

2. CONSORT DIAGRAM



3. BACKGROUND

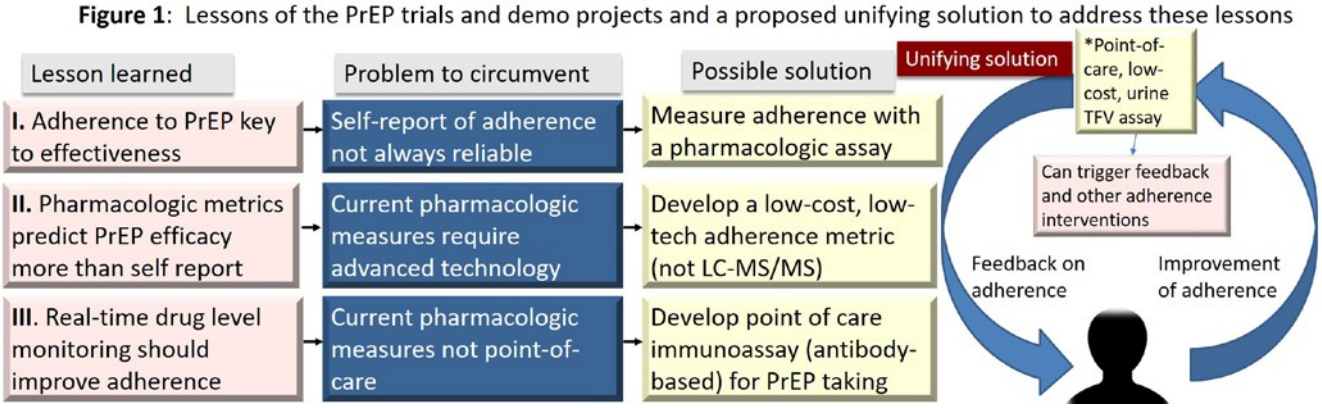
3.1. Significance

Lessons from the PrEP trials and demonstration projects indicate a need for real-time monitoring of PrEP adherence using a pharmacologic measure.

Massive advances in HIV biomedical prevention strategies have allowed the end of AIDS to finally come into sight.[51] Along with treatment as prevention,[52-55] pre-exposure prophylaxis (PrEP) with oral tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) [56-59] is one of the most effective strategies to prevent HIV acquisition among at-risk individuals. PrEP, now broadly recommended by the Centers for Disease Control and Prevention (CDC)[60] and the World Health Organization (WHO),[61] is in an exciting phase of global implementation.

The PrEP trials and demonstration projects to date; however, have highlighted four major lessons that should be addressed in order to increase the effectiveness of this important prevention strategy during implementation **(Figure 1)**. First, there is a profound relationship between adherence and effectiveness.[62] Second, taking a pill every day for prevention (e.g. maintaining adherence) is difficult, especially among certain key groups such as heterosexual women who are not in a relationship with an HIV-positive man. Third, pharmacologic measures – where drug levels of PrEP drugs are assayed in a biomatrix – predict the efficacy of PrEP more accurately than self-reported adherence.[63-69] Fourth, real-time monitoring of PrEP drug levels, as in other disease states,[70-90] may improve subsequent PrEP drug-taking.[91-95] We have now developed one of the first point-of-care tools to assess PrEP adherence objectively[1,3,96,97] with the potential to address all four lessons.

Figure 1: Lessons of the PrEP trials and demo projects and a proposed unifying solution to address these lessons



The first two points – that adherence is necessary for PrEP effectiveness and daily adherence is hard to maintain – are well documented.[98] Although PrEP was effective in placebo-controlled trials among men-who-have-sex-with-men (MSM) and transgender women,[56] among intravenous drug users,[59] and among both men and women in serodiscordant couples,[57,58,99] there was no efficacy of oral PrEP observed in two large trials (FEM-PrEP and VOICE) conducted among young sexually active women in Africa who were not in serodiscordant relationships.[63,65] Low rates of adherence to TDF/FTC were largely responsible for the lack of efficacy in FEM-PrEP[65] and VOICE,[63] where pharmacologic measures of adherence were critical to study interpretation.[66] Women in both trials self-reported >95% adherence to study drug, but random plasma tenofovir (TFV) levels among women on active drug were detectable in fewer than 30% of participants.[63,65]

The third point – that pharmacologic measures predict the efficacy of PrEP better than self-report – was true not only in the placebo-controlled trials, but also in open-label extension studies of PrEP, where adherence has generally been higher than in the original trials. Pharmacologic measures examine drug concentrations in a biomatrix such as plasma,[6] peripheral blood

mononuclear cells (PBMCs),[98] hair,[31] or dried blood spots (DBS).[7,100] Substantial discordance between self-reported measures of adherence and drug detection in trials and demonstration projects[34,35,38,56,58,63,65,66,101-105] have highlighted the crucial importance of incorporating objective pharmacologic measures of adherence when interpreting PrEP effectiveness.[69,98,101,102] For instance, in the iPrEx trial, the efficacy of TDF/FTC rose from 44% to an estimated 92% among those with detectable blood drug levels.[56]

The fourth and final point, gleaned from the VOICE,[91,94] iPrEx open label extension (OLE) study,[95,106] and a demonstration project among MSM in Los Angeles,[107] is that real time monitoring of PrEP drug ingestion is acceptable and motivating to patients and could improve subsequent PrEP adherence.[91-95] However, a knowledge gap exists regarding the impact of this unique adherence intervention because no truly point-of-care (POC) pharmacologic measure of PrEP adherence has existed until now.[97] Therefore, a low-cost, low-technology method to assess objective adherence via drug detection in real-time could address the four major lessons from PrEP studies (**Figure 1**) and optimize outcomes during PrEP roll-out by allowing for immediate feedback, enhanced adherence counseling, and targeted adherence interventions, including counseling to address specific barriers and addressing structural barriers..

Pharmacologic adherence measures in past were expensive; we have now developed a cheap, point-of care test.

Plasma levels of PrEP drugs[6] assess short-term adherence (~1-4 days) and were measured in every placebo-controlled PrEP trial to interpret effectiveness.[56-59,63,65,69,102,108,109] TFV-diphosphate (TFV-DP) and FTC-triphosphate (FTC-TP) concentrations in DBS[7] reflect longer-term adherence and multiple PrEP demonstration projects have used PrEP metabolite levels in DBS to assess adherence.[34,37,38,106,110,111] Our UCSF group has developed methods to analyze PrEP drugs in hair,[31] which assess adherence over weeks to months.[8] Hair TFV levels have the best discriminatory ability as a single measure to predict long-term adherence[39] and have been used to interpret PrEP adherence in multiple settings.[32-35,37-39,41-44,46,48]

The current techniques to measure PrEP or PrEP metabolites, however, all require liquid chromatography/tandem mass spectrometry (LC-MS/MS) or other spectrometry[112] machines, all of which are expensive. Moreover, the techniques to extract drug and assess levels via any sensitive spectrometry-based method, even using small benchtop machines, are laborious and require specialized personnel. Most of the urine assays developed to date also all involve LC-MS/MS[49,1-115] and, therefore, cannot be performed at the clinical point-of-care. Antibody-based tests (lateral flow immunoassays)[116,117] are low-cost, enable drug detection to occur within minutes, and are easy to perform.[118]

We have now developed one of the first such antibody-based (immunoassays) for TFV. Our immunoassay is highly specific (100%), sensitive (96%) and provide TFV levels in urine which correlate strongly with LC-MS/MS-measured levels (r=0.95) in a study of volunteers administered

daily TDF/FTC.[1] In a larger study where TDF/FTC was administered to HIV-noninfected volunteers at 2, 4 and 7 doses a week, the assay showed the same excellent performance characteristics and we were able to establish the adherence cut-off (specifically 1500 nanograms/milliliter) for TFV in urine for the POC assay.[2] This cut-off minimizes misclassification of adherence and the assay has now being translated to a corresponding lateral flow assay[4] in a strip format (like a urine pregnancy test, **Figure 6**) to use for this pilot trial. The lateral flow assay has high accuracy compared to the laboratory based enzyme-linked immunoassay (ELISA). See the preliminary data section (**Section 3.3**) for more details on the lateral flow assay.[4,5]

Real-time feedback of pharmacologic adherence metrics during PrEP will likely increase adherence.

In VOICE, at least 50% of women on active drug had undetectable TFV in *all* plasma tested,[91] but over-reported adherence, even at the last study visit where accurate reporting would not affect study participation.[93] Further qualitative work to understand non-adherence in VOICE was performed through the Microbicides Trials Network (MTN)-003D study[91] which recruited participants with pharmacokinetic (PK) data consistent with low (0% of plasma samples having detectable TFV), inconsistent, or high adherence for retrospective disclosure of plasma TFV results and subsequent capture of reactions. Women with low adherence first expressed surprise at the PK results, then acknowledged they were true and revealed reasons for non-drug-taking during in-depth interviews. *Women in all three categories stated that real-time monitoring and feedback in future trials would improve adherence.*[91] Women also said self-report would be more honest if presented with objective adherence metrics.[94]

In the open-label extension (OLE) study of iPrEx, drug level testing was performed in plasma and DBS.[106] The results of plasma drug levels over prior weeks were shared with individuals at a later visit, which was reported as highly acceptable.[106] Those with detectable drug in plasma appreciated receiving validation of adherence and those without drug detection were not surprised.[106] An in-depth qualitative analysis from iPrEx OLE confirmed the acceptability of drug level feedback (**Figure 2**)[95] and, for those who were not adherent, the motivating effect of receiving such feedback on subsequent adherence.[95] The results of these interviews led authors to conclude that 1) drug level feedback should be provided as quickly as possible, so new methods should be sought to provide rapid feedback; and 2) drug level feedback will encourage frank adherence discussion and should be provided in the context of adherence counseling.[95]

Figure 2: Quotes from VOICE on iPrEx OLE participants on importance and motivating effect of drug level feedback in real time

"If the results of the blood tests come out immediately, then it can also be immediately established whether you were using [PrEP]…Then, they should tell you, your…results. This approach will make you feel more compelled to use the products properly."

"You cannot tell someone that 'you did not use' when the wall is your only witness. You cannot, unless they bring equipment that detects the quantity …[of]..medication"

"You are not present when I am taking the tablets; why don't I say that I am good?...the other thing is that the blood needs to analysed immediately"

"It's just that it [drug level testing] solidifies that all your efforts are..not in vain"

"After hearing that [drug level results], that made me really wanna make sure that I take it every day. 'Cause I feel like why take it at all if it's not effective?"

A demonstration project in Los Angeles MSM[107] demonstrated high acceptability of plasma TFV level adherence feedback, with a trend towards greater adherence in those who received drug level results with enhanced adherence counselling. MSM found drug-level feedback highly acceptable, with participants competing to achieve the highest drug level result.[107,119] Finally, a study which provided urine drug-level results to MSM in Philadelphia (not at the point-of-care) was also highly acceptable.[120,121] In spite of these encouraging results, participants in these two studies requested that drug-level feedback should be provided as close as possible to the clinical visit to help motivate subsequent adherence.

Finally, in the phase III placebo controlled trial of the dapivirine vaginal ring for HIV prevention,[122] plasma was collected quarterly and assayed for dapivirine levels.[92] The data center provided feedback on drug levels to study and site leadership (but not at the individual level) to help motivate staff and counsel participants to use the ring. From 2013 to 2015, the proportion of participants in the active arm with dapivirine blood levels consistent with good adherence increased from 63% to 84% ($p<0.0001$).[92] This study demonstrated the feasibility and possible impact of drug level monitoring with even general (albeit not specific nor real-time) feedback to staff and participants.[92]

Cumulatively, these findings have led to a widespread call for drug-level monitoring and feedback as close to real-time as possible during PrEP implementation. The HIV Prevention Trials Network (HPTN)-082 study, an open-label PrEP study of high-risk sexually active young women (ages 16-25) in Africa,[111] included drug level feedback (using PrEP metabolite concentrations in DBS) at 8 and 13 weeks for those randomized to an enhanced adherence support arm. Since drug level testing in DBS using LC-MS/MS cannot be performed in real time; however, feedback was delayed by at least a month, which could have led to the negative results of this study.[123] The next iteration of the study (PrEP-SMART) will try providing real-time feedback of adherence via the

urine assay we have developed. Only a truly real-time assay for monitoring PrEP drug-taking and providing feedback on the spot will allow for immediate counseling and intervention.

Finally, the hypothesis that real-time monitoring and feedback on adherence may increase adherence among those on PrEP must be seen in the context of other structural and perceived barriers to PrEP use, including erroneous assumptions of one's risk, transportation barriers, economic barriers, lack of peer support, depression, etc. However, the ability of the POC TFV assay to provide an objective metric of adherence in real-time to providers serving clients on PrEP can eventually allow adherence interventions to be tailored to an individual's specific barriers (e.g. transportation vouchers if transportation is an issue; treatment of depression; discussion of risk; adherence clubs; contingency management etc). Moreover, enhanced adherence counseling itself is a tailored intervention in that the counseling takes into account the individual's particular concerns about and barriers to adherence. If a provider relies on an individual's self-reported adherence and that adherence reporting is inaccurate (e.g. inflated), an opportunity for a tailored adherence intervention and/or enhanced adherence counseling will be lost. An objective metric of adherence will allow providers to know if a patient is not taking PrEP and, thereby, tailor the adherence counseling and subsequent interventions appropriately."

Real-time monitoring of adherence and feedback in other disease states improves adherence and outcomes.

Beyond the field of HIV prevention and even HIV treatment, other chronic diseases states are threatened by adherence difficulties. Patients adhere to life-saving cardiovascular and diabetes medications approximately 50% of the time.[124] Hypertension and diabetes treatment have surrogate markers of adherence monitoring (e.g. blood pressure control and glucose or hemoglobin A1c levels, respectively) which can allow for monitoring and feedback. Real-time monitoring, accompanied by feedback to patients on results, have led to increases in adherence (as evidenced by increases in drug levels)[70-72] and better outcomes[73-79] in a variety of disease states.[80-82] For instance, urine monitoring for anti-hypertensives,[83-86,125] with subsequent feedback to patients on drug levels, increases both urine anti-hypertensive drug levels and blood pressure control.[83] Another major field in which real-time monitoring and feedback is performed is in the context of screening for prescribed opioids (as well as for substance use).[87-90] Immunoassays are the recommended first-line screening modality for opioid or substance use, since antibody-based tests allow for POC testing and rapid response at the clinical point-of-care.[88,87-90,118]

Potential utility of PrEP adherence monitoring in urine and addressing "white-coat" adherence.

Urine is a suitable matrix for POC testing for PrEP adherence since urine collection is noninvasive,[49] preferred among youth over blood sampling,[126,127] and TFV levels in urine correlate with adherence to TDF.[49] Urine levels reflect plasma levels,[128] assessing recent adherence,[129] which may be particularly useful when daily PrEP adherence is necessary, as in women.[50] Plasma

and urine PrEP drug levels reflect only short-term exposure, and thus monitoring via these matrices may be susceptible to "white coat adherence". White-coat adherence is a phenomenon where adherence improves transiently before a clinic or a study visit.[130] Despite this theoretical concern, this phenomenon has not yet been observed in PrEP. Indeed, plasma levels of TFV/FTC served as the "gold standard" adherence metric in every one of the placebo-controlled trials of TDF/FTC-based PrEP.[3,4,57,59-63,79] Across these trials, plasma PrEP levels provided robust and critical information for interpreting trial results. For example, in iPrEx[56] and Partners PrEP,[58] plasma levels demonstrated that the use of PrEP was associated with >90% protection against HIV, and in FEM-PrEP[65] and VOICE,[63] plasma TFV/FTC levels showed that very low adherence explained the trials' results, further emphasizing that a white coat effect was not widespread in those trials. Our group found no evidence of white-coat adherence when examining a combination of long and short-term adherence metrics (assessed via PrEP drug levels in hair and plasma samples) in VOICE.[35] In iPrEx OLE, there was no evidence of "white coat adherence" despite participants knowing drug level results were being monitored. Moreover, qualitative data from those who received plasma TFV level feedback found feedback "motivating" – not to impress study coordinators before a visit – but to maintain long-term adherence (last quote, **Figure 2**).[95]

In research studies, a combination of short- and long-term metrics may provide the most complete detail to examine patterns of adherence[35,39,41,48,131] and enables examination for the phenomenon of white-coat adherence. In this study, we plan to combine urine and hair PrEP measures to adjudicate white coat effects and confirm the benefit of the urine assay on adherence over time. Hair levels measure cumulative drug adherence over time and are not influenced by short-term fluctuations in adherence (analogous to a hemoglobin A1c level compared to a single glucose level in diabetes monitoring).[8] Therefore, in this study, we will be able to distinguish between white coat adherence and long-term adherence occurring at each time point by comparing the urine and hair levels of tenofovir, respectively (for instance, good urine levels but low hair levels are suggestive of white-coat adherence). Another way to minimize the concern in future studies or the clinic is to perform unannounced urine assays[125,132-135] and then follow those with adherence challenges more closely (via differentiated service delivery[136]), both with a combination of short-term and longer-term metrics (e.g. drug levels in hair, DBS) and interventions.

Women at risk need PrEP and need adherence support

Adolescent girls and young women in sub-Saharan Africa have unacceptably high HIV incidence rates.[137,138] PrEP has the potential to dramatically reduce HIV incidence in this population.[139,140] However, among women not in serodiscordant couples (FEM-PrEP[65]; VOICE[63]), adherence was too low to result in PrEP effectiveness. Recent demonstration projects show higher adherence among women at risk in Africa than in the initial randomized trials early on, with subsequent

adherence waning.[141] The recently completed HPTN 082 Evaluation of Daily Oral PrEP as a Primary Prevention Strategy for Young African Women Study (n=427) found that 25% of participants had tenofovir diphosphate (TFV-DP, measured in dried blood spots) levels consistent with 4 doses a week (albeit women may need dosing 7 doses a week) at 3-months with only 9% of women with similar levels at 12 months.[142] Pharmacokinetic modeling indicates that, due to differences in TFV exposure in vaginal versus rectal tissues,[50] women likely require higher levels of adherence, specifically 6-7 doses PrEP/week.[143] These data highlight the importance of bringing novel adherence interventions to women on PrEP, which is why we chose to study women in our initial pilot study of this important new assay.

3.2. Innovation

Although pharmacologic adherence measures of PrEP are available, no POC method to assess adherence to PrEP via drug detection has yet been scaled. We have developed the first PrEP immunoassay, which is now available as a point-of-care test,{Gandhi, 2020 #1196} allowing for real-time adherence monitoring. The primary innovation of this study is that we propose to test the acceptability, feasibility and utility of this novel low-cost real-time metric of adherence to PrEP for the first time.

Further innovations for HIV prevention and treatment will result from this proposal. Since TFV-based regimens (TDF or tenofovir alafenamide or TAF) are the backbone of antiretroviral treatment (ART) for HIV infection worldwide, providing a low-cost tool to monitor TFV drug-taking behavior at the clinical point-of-care should launch an entirely new field of adherence intervention for HIV treatment as well as prevention. Not only can real-time feedback on adherence itself improve adherence, but **providing practitioners real-time knowledge on how a patient is adhering to PrEP or ART at a visit can allow for interventions to be triggered before prevention or treatment effectiveness is further compromised.**[144] In the context of treatment, a low-cost POC assay may help avert virologic failure and drug resistance between more expensive viral load measurements.[145-147]

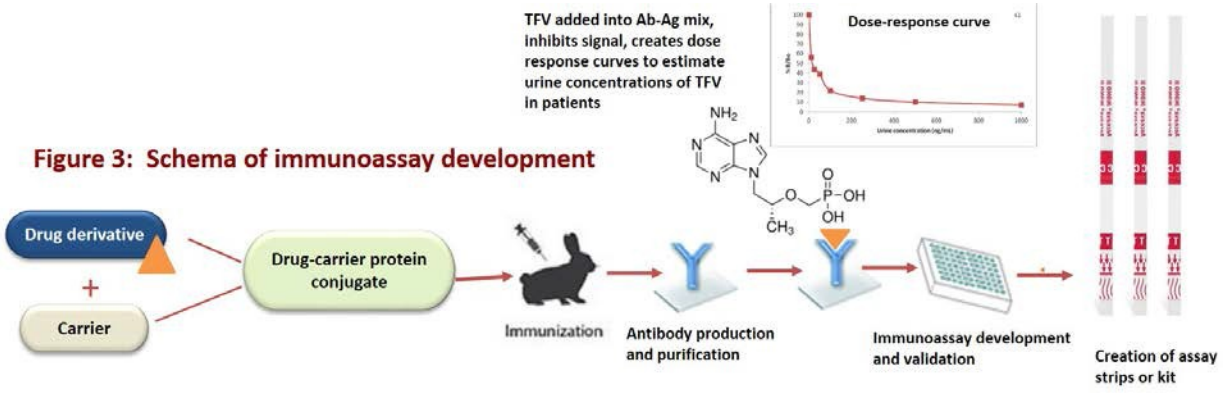
3.3. Preliminary data

Development and validation of the novel urine-based tenofovir lateral flow assay

How lateral flow assays allow for immediate drug detection

Immunoassays use antibodies to detect drug.[116] Lateral flow immunoassays (LFA) can be performed at the POC using a variety of testing platforms, such as a rapid strip test. LFAs are easy-to-perform, provide results quickly, and are cheap compared to standard methods to detect drug, specifically liquid chromatography-tandem mass spectrometry (LC-MS/MS).[118] To develop immunoassays,[153] antibodies are raised in rabbits against derivatives of the drug of interest. Purified antibodies with specific binding to the drug derivative are then isolated. Inhibition of signal needs to then be demonstrated with adding the drug of interest into the antigen-antibody mix to create dose-response curves. The most well-known LFA is the over-the-counter urine pregnancy

test. **Figure 3** illustrates the steps of raising an antibody against tenofovir and creating an immunoassay that can eventually be translated into a lateral flow assay to be used at the point-of-care.



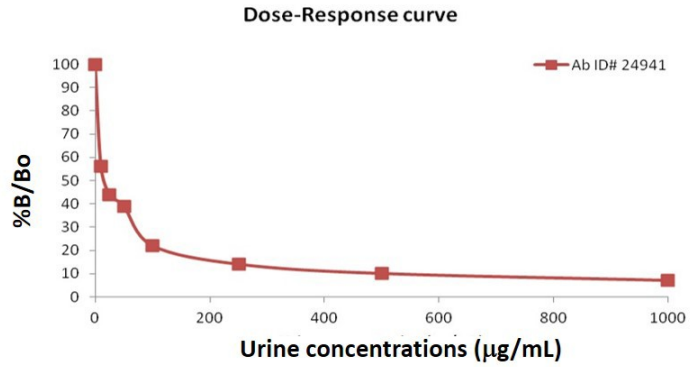
Alere™ Diagnostics (now Abbott Rapid Diagnostic Division (ARDx)) has long-standing experience and expertise in developing point-of-care immunoassays.[118,154-164] ARDx has developed immunoassays for the diagnosis of HIV,[156-160] influenza A/B,[155] tuberculosis,[154] Crohn's disease;[161] for quantification of beta-type natriuretic peptide (BNP),[162] D-dimer,[163] and CD4 cell count;[164] and for the simultaneous screening of multiple therapeutic analgesics and substances in urine.[118] The Hair Analytical Laboratory (HAL) at UCSF and its predecessor (the UCSF Drug Studies Unit), with vast expertise in LC-MS/MS-based methods to analyze drugs in a variety of matrices, including antiretrovirals (ARVs) in hair,[9,10,30] formed a collaboration with ARDx in 2016.

Development of the novel immunoassay for quantification of TFV in urine.

To initiate assay development, scientists from the UCSF HAL[30] and ARDx first scrutinized the molecular structure of TFV to identify unique derivatives with structural distinction from endogenous nucleotides. We then constructed four possible immunogens (TFV derivatives) and each one was injected into three rabbits (12 rabbits total) (**Figure 3**). The rabbits were injected with boosters and bled monthly, with subsequent testing of serum by enzyme-linked immunosorbent assay (ELISA).[165] After 5 months of monthly bleeds, each of the three rabbits injected with one of the immunogens developed a polyclonal antibody demonstrating very specific binding to TFV by ELISA. Inhibition of the signal of the antibody-antigen mix was then seen with the addition of increasing concentrations of TFV to create dose response curves.

Figure 4 shows the dose-response curve for TFV levels in urine developed from the antibody purified from one of the rabbits.[166] The strong inverse relationship between dose and urine concentrations of TFV shows appropriate assay characteristics. Based on these results, the purified antibody was deemed suitable for development into a POC assay.

Figure 4: Dose response curve for quantification of TFV concentrations in urine by immunoassay



*%B/Bo represents the percent of the sample bound to the antibody divided by the maximal possible binding to the antibody; the strong inverse relationship of this dose-response curve indicates a highly suitable antibody for this TFV immunoassay

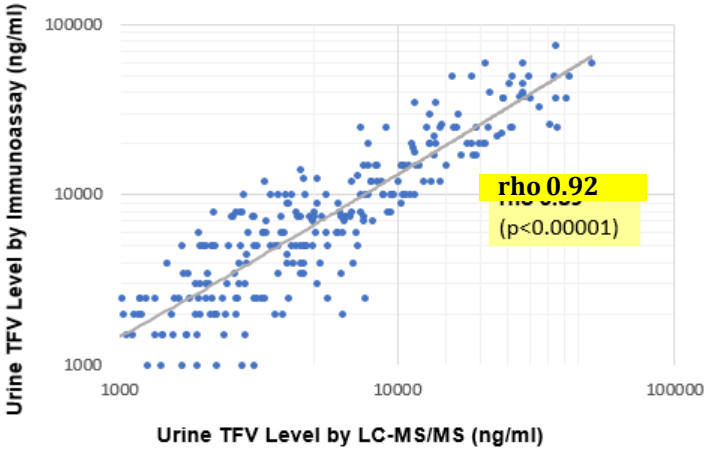
We have now published data showing the excellent performance characteristics of the ELISA-based immunoassay with this antibody compared to the gold-standard method of quantitating TFV levels in urine, specifically LC-MS/MS. Compared to quantification of TFV in urine via LC-MS/MS, the quantification of TFV levels in urine via the ELISA test demonstrate high sensitivity, specificity, accuracy, and precision.[1,2] The high correlation of ELISA-immunoassay results of TFV in urine with those from the gold standard of LC-MS/MS is shown in **Figure 5.**

Development of the TFV assay into a point-of-care LFA platform.

The translation of the plate-based immunoassay to an LFA is a well-established process, and the Abbott Rapid Diagnostics Division is a leading diagnostic company with extensive experience in developing POC assays. The lateral flow assay (LFA) prototype has now been developed by Abbott for testing in this pilot trial.[4] as described below:

The LFA test strip components include a sample pad onto which the test sample (e.g. urine) is applied; a conjugate pad coated with tenofovir specific antibodies conjugated to colloidal gold nanoparticles; a nitrocellulose membrane striped with a test line consisting of a tenofovir antigen and a control line consisting of anti-rabbit antibody; and an absorbent pad designed to draw the sample across the reaction membrane by capillary action. These components are all affixed to inert backing material and packaged within

Figure 5: Correlation of urine immunoassay TFV levels with LC-MS/MS levels in TARGET urine samples with drug detectable in both assays (n=274).

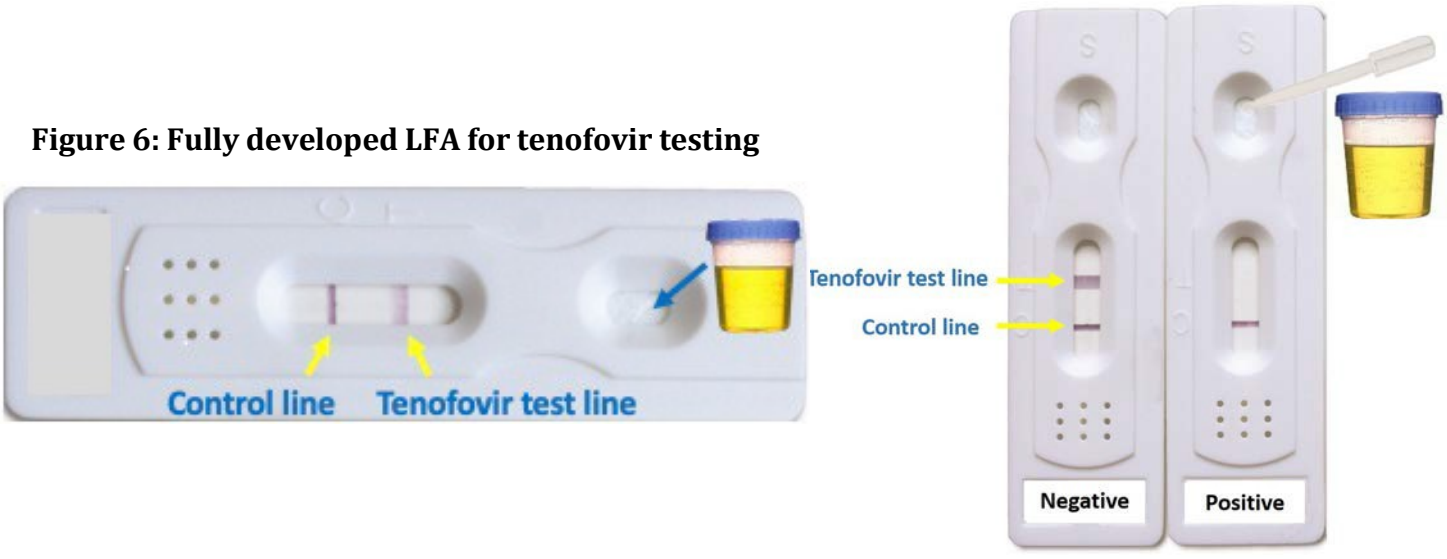


a plastic casing (**Figure 6**).

A photograph of the fully-developed LFA is shown in **Figure 6**. The urine first encounters the colored particles labeled with tenofovir antibody, so free tenofovir in the urine sample will bind the antibodies, preventing them from binding to the antigen line (test line) containing the tenofovir antigen. The presence of tenofovir in the urine, therefore, results in no color signal on the test line. Absence of tenofovir in the urine causes the antibodies to migrate to the antigen pad and bind strongly to the tenofovir antigen, resulting in a dark test line. This competitive assay, therefore, demonstrates a colored line in samples negative for tenofovir and no line in samples positive for tenofovir (**Figure 6**).This LFA requires 2-3 drops of urine to be dropped into the divot at the end and takes <2 minutes to yield results. The test on the left in **Figure 6** shows a urine sample without tenofovir present. The test on the right shows a urine sample taken 8 hours after an HIV-noninfected volunteer was administered TDF/FTC 300mg/200mg. A single test therefore provides qualitative information (yes/no) on recent adherence

As detailed below, laboratory staff at the Thika Clinic will be trained in the interpretation of this test since the presence of a tenofovir test line indicating a "negative results' is counterintuitive. Neither the research provider nor the participant will need to interpret the test. The laboratory staff on site will perform the test, interpret the test, and deliver the results to the research provider for providing feedback to participants in the intervention arm only. Of note, the test shown in **Figure 6** is the prototype of the first test Abbott Rapid Diagnostics has produced. *The final test for marketing (like urine pregnancy tests) will have an overlaid window so that a "positive test" for tenofovir shows up as a "plus sign" and a "negative test" for tenofovir shows up as a "minus sign".*

Figure 6: Fully developed LFA for tenofovir testing



Validation of the point of care tenofovir LFA against gold-standard quantification (LC-MS/MS)

Using urine samples collected from a directly observed therapy (DOT) study (TARGET, NCT0301260)[167] in which 30 HIV-noninfected volunteers from Thailand received directly-observed TDF 300mg/FTC 200mg at 7, 4 and 2 doses per week (to simulate high, moderate and low adherence levels, respectively), we modeled an appropriate adherence benchmark for the lateral flow assay. We found that a urine concentration of 1500 nanograms (ng)/milliliter (mL) of tenofovir in the urine balanced high specificity for adherence with good sensitivity for non-adherence.[2]

To evaluate the performance of the LFA, urine samples were then aliquoted for measurement by both LC-MS/MS and the LFA. For the LC-MS/MS-based method,[1] tenofovir is separated from one thousand-times diluted urine via reverse-phase high-performance LC and quantified by MS/MS using electrospray positive ionization in multiple reaction monitoring mode (TFV, 287.9/175.9 *m/z* (Q1/Q3)). The lower limit of quantification (LLOQ) of the LC-MS/MS-based assay is 500 ng/mL. For the LFA, 2-3 drops of urine are applied from the urine sample on to the LFA and, after approximately two minutes, the lines on the LFA window are read. We then calculated the sensitivity, specificity and accuracy of the LFA compared to LC-MS/MS by cross-tabulating values above/below the 1500ng/mL threshold by the two different assays.[168]

Of 637 urine samples collected among the participants in the TARGET DOT study, 300 were randomly selected to be tested by both the LFA and the gold standard method of LC-MS/MS for validation. The accuracy of the LFA compared to LC-MS/MS was 97% (95% confidence interval (CI): 95% to 99%). Among the 213 tenofovir-negative samples by LC-MS/MS (≤1500ng/mL), 206 were also negative by the LFA, indicating 97% specificity (95% CI: 93% to 99%). Of the 87 tenofovir-positive samples by LC-MS/MS (>1500ng/ml), 86 were also positive by the LFA, indicating 99% sensitivity (95% CI: 94% to 100%). Therefore, our validation results showed high sensitivity and high specificity of the LFA compared to the gold standard method of quantification (LC-MS/MS) at a cut-off of 1500ng/mL of tenofovir in urine.[4]

Validation of the tenofovir LFA against the enzyme-linked immunoassay (ELISA):

This section details the data on the comparison between the LFA and the standard enzyme-linked immunoassay (ELISA) in diverse patient populations.[5] This analysis used stored urine samples from the TRIUMPH study, an ongoing U.S. demonstration project of PrEP in transgender women and men, and the Partners PrEP study, a clinical trial of PrEP among heterosexual men and women. We calculated the sensitivity, specificity and accuracy of the LFA compared to ELISA using urine samples in the two different studies by cross-tabulating values above/below the 1500ng/mL threshold defined for the LFA by the two different assays.

Overall, 684 urine samples were tested from 324 participants in the two cohorts. In Partners PrEP, 454 samples from 278 participants (41% women and 59% men) were tested; the median age was 33 years (interquartile range (IQR) of 28-39). In IBrEATHe, 231 samples from 46 individuals (50% transgender women on estrogen and 50% transgender men on testosterone) were tested; the median age was 31 years (IQR 25-40). Of the 505 samples with tenofovir levels greater than or equal to the cut-off of 1500ng/ml using laboratory-based ELISA, 505 of the POC test results were also positive, yielding 100% sensitivity (one-sided 97.5% confidence interval (CI):99.3%). Of the 179 samples with TFV levels below the cut-off, 176 were negative with the POC test, yielding 98.3% specificity (95% CI:95.2%-99.7%). The accuracy of the POC LFA across the two studies was 99.6% compared to ELISA (95% CI:98.7%-99.9%). Therefore, in a large, diverse sample of men and women (both cisgender and transgender) taking TDF/FTC-based PrEP, the sensitivity, specificity, and accuracy of a the novel LFA were all over 98% when compared to the laboratory-based ELISA method.[5]

Hair levels of tenofovir will serve as the long-term adherence metric in PUMA: prior work by the UCSF HAL examining hair ARV concentrations in the context of HIV treatment and prevention.

Along with acceptability and feasibility, we will also examine the impact of the intervention on hair levels of TFV and FTC as a longer-term metric of adherence. Over the past decade, we have gained extensive expertise in the Hair Analytical Laboratory (HAL) at UCSF in developing methods for the extraction and quantification of ARVs[9-11,15,26,30,31] and anti-TB drugs[169-172] in hair using LC-MS/MS as long-term metrics of adherence.[8] We have found no substantial differences in the median and range concentrations of ARVs in hair from participants of different race/ethnicity or with different hair colors. Experimental work in the HAL found little change in hair levels when performing coloring, permanent or straightening procedures on hair samples. Hair collection is highly acceptable (>95%) in PrEP[32,38] and other studies in Africa/Asia.[12,16-19,22,25,173 39] We have demonstrated a strong linear relationship between adherence to TDF and hair concentrations of TFV,[31] enabling TDF dosing patterns to be estimated from hair levels in a variety of PrEP settings.[32-35,37-39,41-44,46,48] We have also shown a strong correlation between hair TFV levels and DBS levels of TFV-DP, both long-term metrics of adherence.[33] We further demonstrated that hair levels of ARVs increase within individuals following adherence interventions (relevant for this study).[27,28]

3.4. FDA/CDRH and the Nonsignificant Risk (NSR) Designation for this Investigational Device for Tenofovir Testing

We obtained pre-trial consultation advice from the Food and Drug Administration's (FDA's) Center for Devices and Radiological Health (CDRH) regarding the our use of the novel point of care urine tenofovir assay in this newly funded PUMA study (R01 AI143340) through NIAID. The FDA verified that the urine tenofovir assay is not Investigational Device Exempt (IDE) because we are returning test results to participants in the intervention arm of the trial. Per FDA regulations (21

CFR 812), the alternative determinations applicable to our studies are either Nonsignificant Risk (NSR) or Significant Risk (SR).

Under FDA 21 CFR 812.3(m), a Significant Risk (SR) device is defined as an investigational device that "Is intended as an implant and presents a potential for serious risk to the health, safety, or welfare of a subject; is purported or represented to be for use supporting or sustaining human life and presents a potential for serious risk to the health, safety, or welfare of a subject; Is for a use of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and presents a potential for serious risk to the health, safety, or welfare of a subject; or otherwise presents a potential for serious risk to the health, safety, or welfare of a subject." The urine assay does not meet these criteria as it is completely noninvasive and merely tests levels of tenofovir in urine samples. Although test results will be shared with patients, which excludes it from the exempt category, no clinical decisions will be made based on the results. While the assay will be used to measure medication adherence, the collection of adherence information does not present a potential for serious risk to the health, safety, or welfare of the subject. Therefore the urine assay meets the criteria under the IDE Nonsignificant Risk Medical Device study with additional details provided here:

1) The non-invasive urine assay will be used to detect the absence or presence of tenofovir (the metabolite of TDF) in urine to improve adherence counseling. TDF is used in HIV treatment regimens worldwide and is also the only agent (in combination with emtricitabine) approved for pre-exposure prophylaxis (PrEP) worldwide. The threshold of the assay was designed to distinguish between people who have taken TDF within the prior four days ("adherent") versus those who have not ("non-adherent") taken TDF within the prior four days. Participants who are considered "adherent" by the assay in the intervention arm of PUMA will be informed of the result and continue to receive standard adherence counseling. Participants who are considered "non-adherent" by the assay will receive enhanced adherence counseling with positive messaging (detailed below).

2) The antibody that was developed against tenofovir for this novel assay is highly selective. As above, the assay has been demonstrated to have very high sensitivity and specificity in our recently concluded clinical validation studies against both the gold standard of measuring drug levels, LC-MS/MS, and the laboratory-based method of performing immunoassays (enzyme-linked immunosorbent assay or ELISA). Therefore, this test has a very low rate of false positivity and we have designed it to have a very low false negative rate. In the unlikely situation of false positive results (where the results indicate "adherent" when the participant is not taking their medication), participants will continue to receive standard adherence counseling. In the very unlikely event of false negative results (where the results indicate "non-adherent" when the participant is taking their medication) the participant will receive enhanced adherence counseling (which does not meet the

definition of "significant risk") in the intervention arm of PUMA. Therefore, participant access to standard adherence counseling will not be affected by a false positive result. We do not anticipate any adverse consequences for participants who receive enhanced adherence counseling due to being wrongly classified as non-adherent (a very rare event). The feedback and adherence counseling will be delivered in a supportive non-judgemental manner (**Figure 8** and **Section 5.5**).

The FDA confirmed with our study team on October 3, 2019 that they approve the NSR designation of the urine device (Email dated October 3, 2019 from Kellie B. Kelm PhD, Acting Director, Division of Chemistry and Toxicology Devices | OHT7: Office of In Vitro Diagnostics and Radiological Health, Office of Product Evaluation and Quality, CDRH | Food and Drug Administration states "Since the therapies that will be given are approved, given at approved doses and the adherent patients get standard adherence counseling and the non-adherent patients get enhanced adherence counseling with positive messaging, the impact of a false negative and false positive are minimal. We would agree these studies using an investigational device appear to be non-significant risk". This designation of NSR for the urine assay by the FDA is provided in **Appendix VIII.**

Finally, the study staff at Thika Clinic will follow the abbreviated requirements at 21 CFR 812.2(b), which require devices to be clearly labeled as investigational, under study, and marked clearly to specify that no clinical decisions should be based on the outcome of the results. All research participants in PUMA will signed written informed consent forms prior to participation that cite the urine test as investigational (**Appendix I**). The results of this trial will help inform the use of the test in routine clinical care in the future.

3.5. Study Rationale

Women not in serodiscordant couples (SDC) in sub-Saharan Africa (SSA) have demonstrated difficulty adhering to PrEP, as seen in the divergent results between the FEM-PrEP[65] and VOICE[63] trials (where efficacy for women not in SDC was nil) and the Partners PrEP study[58] (where efficacy for women in mutually-disclosed SDC was high). Women in serodiscordant partnerships, as in the Partners PrEP trial, who are aware of the HIV positive status of their partners, have been shown to have higher rates of adherence. Therefore, a focus in the PUMA trial on women not in serodiscordant relationships will allow selection for a possibly more adherence-challenged population, which is helpful when evaluating the effect of adherence interventions.

The reason to conduct this trial in Kenya is that Kenya has one of the highest HIV burdens in SSA, with women accounting for >50% of new infections. **We therefore propose to conduct a pilot RCT to evaluate acceptability, feasibility and impact on PrEP adherence over time of real-time monitoring/feedback via the urine assay among women not in SDC in Kenya.** Of note, the theoretical effect of real-time monitoring of adherence on subsequent adherence in

VOICE and iPrEx OLE was assessed using qualitative methods, but this aim will address a key knowledge gap to assess whether true POC monitoring/feedback has an impact on a quantitative measure (i.e. hair levels). This pilot will provide needed data for a larger trial evaluating the assay's impact on HIV acquisition rates in the context of PrEP.

4. STUDY OBJECTIVES

Our central hypothesis is that providing real-time monitoring/feedback via the POC assay will be acceptable to participants, feasible to research providers, and improve adherence over time.

4.1. Primary Objectives: To test the acceptability to participants, feasibility for providers, and impact on a longer-term metric of adherence among participants (assessed via tenofovir hair levels) of implementing POC urine tenofovir testing and providing real-time feedback and enhanced adherence counseling among women receiving PrEP in Thika, Kenya.

5. STUDY PROCEDURES

5.1. Overview

Given known PrEP adherence challenges among women not in serodiscordant couples and the likely need for daily drug-taking among women for efficacy, we will conduct a pilot RCT by randomizing women on PrEP in Kenya not in serodiscordant couples to standard of care adherence counseling (n=50 - 60) vs real-time adherence feedback using information from the POC urine assay (n=50 - 60). Acceptability, feasibility and the effect of the feedback on long-term adherence over time measured via TFV hair levels will be assessed.

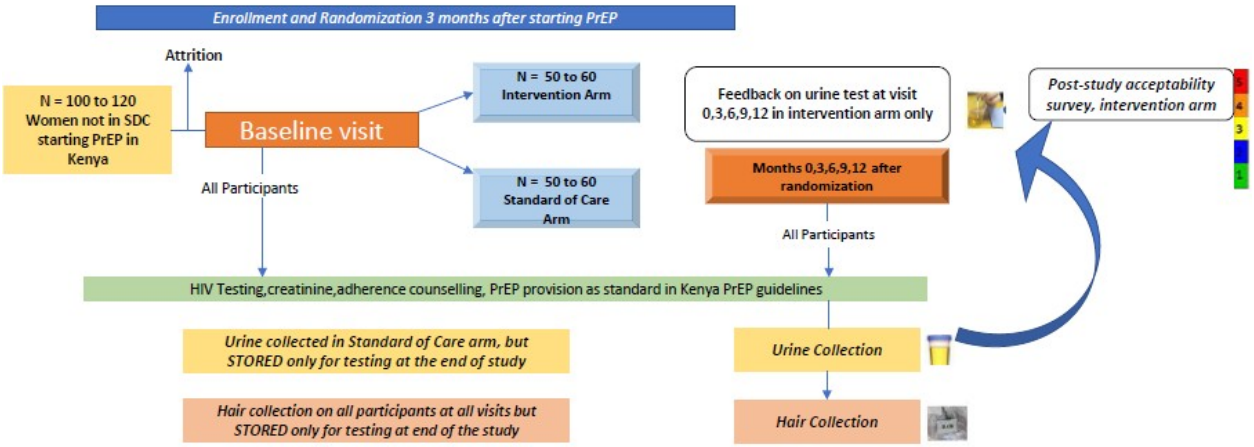
Short-term adherence may improve with feedback from the assay, but hair concentrations do not reflect "white coat" adherence and only increase if overall adherence over time increases. Since hair levels are not susceptible to "white coat adherence", collecting both urine-based metrics of adherence and hair-based metrics (at every visit for every participant, although only the intervention arm participants and their research providers will receive the results of the urine assay) will help provide data on the frequency of "white coast adherence" in this setting. The procedure for hair collection has been described.[14,15,23,32] We have demonstrated high rates of acceptability for hair collection in multiple African-based studies.[12,16-19,22,25,173] Samples are kept at room temperature prior to shipment without biohazard precautions. The assays for measuring TFV/FTC in hair in the UCSF HAL[29,31-42,174,175] have been approved by the Division of AIDS Clinical Pharmacology and Quality Assurance (CPQA) program.[176]

5.2. Study Design

Under the 2016 Kenya PrEP guidelines,[177] all persons initiating PrEP undergo clinical assessment to ask about symptoms of acute HIV infection, receive rapid HIV-1 and creatinine testing (estimated creatinine clearance >60 mL/min is required to start PrEP), and adherence counseling.

Patients who qualify for and initiate PrEP are subsequently seen in 1 month, in 3 months, and then every 3 months for repeat HIV testing, continued risk assessment, and adherence counseling for daily PrEP under the standard of care (SOC). For this pilot RCT, we will recruit women ≥18 years old on PrEP at Thika at the visit that occurs 3 months after PrEP initiation (per the 2016 Kenya PrEP guidelines). Patients will be enrolled and randomized in a 1:1 fashion at that visit

Figure 7: Schedule of Evaluations for Participants in the SOC and Intervention Arm of the Pilot RCT



(month 0 or baseline visit of study) using sealed opaque envelopes to the intervention arm versus the SOC arm (**Figure 7**). A minimum of 100 and not exceeding 120 women will be recruited, with about 50 - 60 randomized to the intervention arm and about 50 - 60 to the SOC arm (**Figure 7**). We anticipate that randomizing established patients (at their second PrEP follow up visit since PrEP visits are to occur 1 and 3 months after PrEP initiation per Kenya guidelines) will minimize attrition during the study.

Upon randomization, study visits will occur at 0 (baseline visit), 3, 6, 9 and 12 months after study enrollment. Each visit will include rapid testing for HIV, pregnancy testing if the participants requests it or is unsure of being pregnant (per standard of care), and creatinine measurement (per 2016 Kenya PrEP guidelines), as well as adherence counseling. As per the 2016 Kenya PrEP guidelines, any other laboratory testing or assessments will be performed per the history and physical examination. Self-reported time since last PrEP dose taken will be collected via a visual analog scale, as will self-reported adherence over the previous 3 and 30 days.[178] Urine samples will be collected at every visit for participants in both arms, but stored only for participants in the standard-of-care arm (to be tested locally, in batches by laboratory staff not involved in daily study conduct). Urine samples will only be tested via the tenofovir assay in real-time for participants in the intervention arm by laboratory staff at the Thika Clinic. The results of the urine assay for intervention arm participants will then be recorded by the laboratory staff and conveyed to the research providers of intervention arm participants who will proceed to immediately provide feedback to participants using counseling guides prepared prior to the trial (and informed by the

results of the formative work to qualitatively assess how to provide supportive counseling message for either yes adherence or no adherence, **Section 3.4**).

Hair samples will be collected at each study visit (months 0, 3, 6, 9 and 12) for storage and testing at the end of the study for all participants. (Please see **Section 3.1** for a discussion of how white-coat adherence patterns can be assessed by collecting both short-term and long-term adherence metrics). Feasibility and acceptability assessments will be conducted at study end for research providers (feasibility) and participants in the intervention arm (acceptability), respectively. Plasma and DBS for TFV and TFV-DP level measurement, respectively, will be collected at each visit in each arm as additional adherence metrics and stored for future analysis in the UCSF Hair Analytical Laboratory (HAL) after seeking independent funding for those concentrations.

Participants will be followed over a 12-month study (baseline visit which is month 0, months 3, 6, 9 and 12) period to assess the study outcome measures. This study will follow all aspects of Kenya's PrEP guidelines, except patients randomized to the intervention arm will receive point-of-care adherence testing by the urine tenofovir assay.

5.3. Location and Setting

The setting is the THIKA Partners in Prevention Research Site and Clinic, THIKA Section 9, Oau Road, next to Thika Nursing Home.The Thika Clinic serves an urban center about 40 kilometers outside of Nairobi and is a center of excellence for PrEP delivery and research in Kenya. Over the past decade, the Thika clinical team established a multi-disciplinary site focused on HIV-1 and sexually transmitted disease prevention research (more than two dozen projects), and on provision of clinical care (HIV-1 testing, HIV-1 comprehensive care, HIV-1 prevention services). The experienced community outreach team at the Thika site has established successful recruitment strategies for research studies, including collaborating with existing HIV-1 testing centers and community-based mobilization for women to engage in HIV-1 prevention studies. The Thika clinic is a center of excellence for PrEP in Kenya and is leading the training of other clinics as part of Kenya's national PrEP scale-up program.

5.4. Population & Eligibility

The eligibility criteria for this study are the following:

- Female
- Adult, age ≥18 years old
- HIV-1 uninfected based on a negative HIV-1 rapid test
- Not currently enrolled in an HIV-1 prevention clinical trial
- Not currently in a serodiscordant relationship
- Already taking PrEP and will be enrolled at the 3-month follow-up visit following PrEP initiation

- Willing to be randomized to point-of-care tenofovir drug testing
- Willing/able to provide informed consent to participate in the study
- No contraindication to use of TDF or FTC
- Note: Women who are pregnant or breastffeeding at screening/enrollment are still eligible

Eligibility criteria are meant to approximate HIV-1 uninfected women at risk who have recently initiated PrEP.

5.5. Services and Intervention

Standard-of-care PrEP arm

Participants will receive HIV-1 counseling, condoms, risk reduction counseling, and syndromic management of sexually transmitted infections according to local guidelines at the baseline enrollment visit and during all follow-up visits. All distribution of PrEP will be done with typical counseling and monitoring during the 12-month study period. The participants in this arm will undergo urine collection for storage only and will NOT receive the point-of-care urine tenofovir assay. Per the standard of care when providing PrEP, creatinine is checked every 6 months, urinalysis every 6 months, and a urine pregnancy test for women is performed as indicated by clinical care.

POC Urine Tenofovir PrEP arm (Intervention)

As with the SOC PrEP arm, participants will receive HIV-1 counseling, condoms, risk reduction counseling, creatinine testing, urinalyses, pregnancy testing, and syndromic management of sexually transmitted infections according to the 2016 Kenya PrEP guidelines (**Table 1**). In addition, participants will have clinic-based point-of-care testing for urine tenofovir performed by the POC urine assay at the baseline visit (month 0) and then at each quarterly visit (month 3, 6, 9, 12) during the 12-month study period. The POC rapid strip test (**Figure 6**) will provide information on YES vs. NO recent PrEP adherence among intervention arm participants.

PrEP medication provision and pharmacy control

Tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) are reverse transcriptase inhibitors that have been approved for the treatment of HIV-1 infection in humans in Kenya, Uganda, and the United States with other antiretrovirals. A fixed-dose, oral co-formulation of FTC/TDF is used in Kenya for PrEP and provided by the Kenya Ministry of Health as part of PrEP roll-out. This drug is not provided as a study drug, but as part of routine care for the participants in this study.

At Thika, TDF/FTC is stored in accordance with the drug manufacturer's recommendations. The pharmacy and storage facility has locked, climate-controlled environments, with controlled humidity and temperature to remain within limits allowed by the manufacturer for drug storage.

Dispensing will be sufficient to last until the next visit (e.g. 90 days) with extra pills (20) dispensed to allow for a generous visit window.

Counseling on TDF/FTC, its side effect profile, how to take the medication, what to do if side effects are experienced, and the importance of not sharing medication to optimize potential efficacy is performed routinely at each PrEP visit as per the 2016 Kenya PrEP guidelines.

Point of care urine monitoring test

The point-of-care urine-based adherence test is shown in **Figure 6** and will be performed for participants in the intervention arm only (urine will be collected and stored without testing until the end of the study for participants in the standard of care arm). The laboratory staff at the Thika Clinic will be trained in the interpretation of this test. As detailed at length in **Section 3.3**, this competitive assay demonstrates a colored line in samples negative for tenofovir and no line in samples positive for tenofovir (**Figure 6**) so the laboratory staff will be carefully trained on the interpretation of the test prior to the start of the trial by the PI of the study, the site PIs, and the laboratory manager of the Thika Clinic. Neither the research provider nor the participant will need to interpret the test. The laboratory technologist on site will aid the provider in urine sample collection, perform the test, interpret the test, mark on the test if it is positive or negative, and deliver the results to the research provider for participants in the intervention arm only. The results will be delivered to the research providers for the intervention arm participants from the trained laboratory staff in the form of “yes” versus “no” for recent adherence to tenofovir. The research providers will then immediately deliver the appropriate messaging and counseling based on the urine test results for participants in the study. Of note, pictures of each urine tenofovir assay for participants in the intervention arm will be uploaded into the research database for quality assurance monitoring. After the picture of the test is uploaded into the research database, the used urine test will be discarded in an appropriate manner.

Feedback to the participants on their tenofovir test results will be delivered in a supportive manner by the research providers. The messages to provide feedback on adherence will be adapted from HPTN082[179] (**Figure 8**), for which Dr. Baeten serves as co-investigator. The counseling messages for this trial will first be piloted in a small group of women on PrEP at Thika for refinement prior to actual implementation.

Enhanced adherence counseling (EAC) to the women in the intervention arm will be delivered by healthcare providers at the Thika Clinic (nurses, doctors, clinical psychologist) with extensive experience in counseling and behavioral interventions. When asking about particular barriers and ways the Thika clinic providers can help in supporting the participant in adherence, counseling messages during EAC will be tailored to the individual's circumstances.

Figure 8: Counseling messages to be delivered based on urine TFV results in real-time in intervention arm (based on HPTN082, *adapted with permission*)

Urine test positive	**Key message:** You are doing really well! You are taking the PrEP. Keep up the good work and remember that taking one PrEP pill every day is needed for strong protection against HIV.
Urine test negative	**Key message**: It looks like you haven't been able to take the PrEP pills, at least recently. Are you still interested in PrEP? If yes, how can we help you take the medication? Talk to us about what makes it hard to take and let us see how we can help

Both study arms

For retention efforts in the study, participants will be contacted every 3 months if they do not appear for their quarterly PrEP visits for follow-up of safety issues, HIV-1 assessment, PrEP refills, and to ask if they want to continue participation in the study. Moreover, all participants will be contacted at the end of the study (even if they did not participate in all study visits) to obtain hair samples for measurement of long-term PrEP adherence and for acceptability interviews if applicable.

5.6. Recruitment and Enrollment

The Thika site has established local recruitment and screening methods that operationalize protocol-specified requirements for eligibility determination in a manner that is tailored to and most efficient for the local study setting and target study population. To reduce any bias towards participants who might be more adherent to PrEP or be amenable to adherence testing, enrollment will target areas with low PrEP uptake.

Recruitment strategies will include partnering with existing voluntary counseling and testing (VCT) centers, outreach workers and community organizations, such as churches, and community mobilization around women's VCT promotion. Recruitment materials will educate women about PrEP. Screening and enrollment may occur on the same day or may be split across days, depending on the preferences of the potential participant. Informed consent for study participation and enrollment in the study may proceed on the same day when eligibility is determined.

A member of the study team will approach individuals within the PrEP clinic at the Thika Clinical research site and at community sites associated with Thika at their 3 month routine follow-up visit

following PrEP initiation. They will describe the study and ask for voluntary participation. They will explain the purpose of the study and review the study eligibility criteria with the participant. They will also address any questions about study participation. The eligible adults interested in participating in the study will be asked to sign the informed consent form to participate (**see Appendix I**). Those people who decide not to participate in the study will experience no detrimental impact on their routine care at the clinic. Those who want to voluntarily participate will be taken to a private area of the clinic to be asked several demographic and clinical questions.

The major talking points will be the following:

- The study is for HIV-uninfected women who recently initiated PrEP.
- The purpose of the study is to investigate whether feedback of urine drug levels may help improve long-term adherence to PrEP.
- If adherence to PrEP is very good, then you will have a lower chance of becoming infected with HIV.
- If you are eligible to participate in the study, then the study team will randomly assign you – by chance (like a coin flip) – to either the "regular care group" or the "intervention group." If you are in the "regular care group", then you will receive all the same treatment that you would normally receive, including the HIV medications for PrEP. If you are in the "intervention group", then you will receive all the same treatment that you would normally receive, including HIV medications for PrEP but in addition, you will have PrEP drug level testing every three months with a simple urine dipstick test.

After enrollment, the participant will be randomized to either the control arm or the intervention arm in a 1:1 fashion.

5.7. Randomization

The study design will be an open-label, randomized, implementation trial with 2 study arms. We will enroll healthy HIV-uninfected adults (≥18 years old). We will enroll a minimum of 100 and not exceeding 120 women receiving PrEP, about 50 - 60 participants in each study arm.

Randomization will be done in variable-sized blocks using opaque envelopes opened at the time of randomization. Randomization will occur in a 1:1 fashion to alternate the intervention arm and the standard of care arm. Randomization will be done at the enrollment visit.

The allocation of participants for the 2 study arms will be the following:

	Standard of Care Arm	Intervention Arm	**Total**
Total	**50 - 60**	**50 - 60**	**100 - 120**

5.8. General Study Procedures

Specific study procedures will take place at screening and enrollment, and then quarterly for both the standard-of-care arm as well as the intervention arm for up to 12 months. At screening, eligibility information will be collected. Subjects who meet the eligibility criteria will then be enrolled in the study.

Once written consent is obtained, a member of the study team will collect demographic and health questions, related to age, birthdate, income, employment history, prior HIV testing, medical conditions, and current symptoms. They will also obtain each participant's phone number, address, and relevant contact information. After obtaining the baseline demographic and clinical data, they will determine the study group randomization of the participant.

A Research Nurse will then meet the participant in the same clinical exam room, and administer a brief clinical questionnaire. The nurse will coordinate the necessary blood draws for the participant, so each participant will only have one blood draw for each clinical visit. As part of PrEP initiation, several standard of care laboratory tests would have been completed prior to trial entry including HIV-1 testing, serum creatinine, Hepatitis B surface antigen, urine pregnancy test. For this study, additional information will be collected on medical history, physical exam findings, and STI syndromic assessment and additional testing will be done as part of standard of care PrEP follow-up.

PrEP delivery will be according to the 2016 Kenya PrEP guidelines, including measurement of renal function (estimated creatinine clearance >60 mL/min to start PrEP and periodic monitoring over time, aligned to the visit schedule of the study), standard clinical assessment to avoid continuing PrEP during acute HIV-1 infection, and adherence counseling. Subjects will then be randomized at enrollment (Month 0 but the 3—month visit on PrEP) in a 1:1 fashion, as described above. Participants in either arm will be provided 3 months of PrEP medication, corresponding to enough to last until the next clinic-based visit. Participants in both arms will be counseled on PrEP discontinuation according to Kenya national guidelines.

At each study visit, we will offer counseling for participants for HIV-1 testing (pre- and post-testing), HIV-1 infection risk reduction best practices, condom promotion and provision, adherence to PrEP medication, as well as other HIV-1 prevention strategies.

Participant contact tracing for retention at all study visits will be done, particularly to establish PrEP continuation and HIV-1 status.

5.9. Standard-of-Care Arm Follow up Procedures

Participants in the standard-of-care arm will be directed to the regular clinical waiting area to be seen and evaluated by the study team every 3 months until the study end (month #12). The study team will prescribe PrEP and additional medications as appropriate, and provide adherence

counseling as appropriate. The standard-of-care includes rapid testing for HIV-1 before dispensing PrEP. If any participants have become HIV-infected, then confirmatory testing will be performed and participants will receive standard HIV care, including initiation of antiretroviral therapy. Urine and hair samples will be collected and stored at the baseline visit and at months 3, 6, 9 and 12 for all standard-of-care arm participants but not tested (until study end).

5.10. Intervention Arm Follow up Procedures

Participants in the intervention arm will receive the same treatment and evaluations as participants in the standard-of-care arm (**Table 1**). In addition, participants will have baseline and quarterly testing for tenofovir by the POC urine assay test. The results of the test will be provided to the participant by the healthcare research provider and will be used to inform enhanced adherence counseling as above. An image of the point-of-care urine adherence assay is shown in **Figure 6**. The laboratory staff at the Thika site will interpret the test (to deliver the information to the research healthcare provider) and then take a picture of each urine test after development and upload to our centralized database.

5.11. Data Collected and Testing during Clinical Visits

We will use structured interviews on HIV-1 testing practices and self-reported PrEP adherence (e.g., frequency, ability, self-rating, missed doses). **Table 1** provides a summary of clinical visits and testing in both study arms. There will be a window of 20 days around study visits to allow for late visits due to life circumstances.

Table 1. Timeline of Visits and Laboratory Testing

	Enrollment	Month 3	Month 6	Month 9	Mont	12
Screening and Enrollment						
Review eligibility criteria	X					
Obtain informed consent	X					
Randomization	X					
Collect sociodemographic information	X					
Research Assistant tasks						
Collect and update contact information	X	X	X	X	X	
Conduct baseline questionnaire	X					
Conduct quarterly questionnaires		X	X	X	X	
Conduct exit study questionnaire					X	
Perform PrEP pill counts	X	X	X	X	X	
Point-of-care and Laboratory Testing						
Hemoglobin (for research study)	X	X	X	X	X	
POC urine tenofovir assay with feedback (Intervention Arm)	X	X	X	X	X	

Urine collected and stored (Standard of Care arm)	X	X	X	X	X
Specimen Collection and Storage					
Stored plasma (10 ml)	X	X	X	X	X
Dried blood spot for TFV-DP	X	X	X	X	X
Hair sample for TFV	X	X	X	X	X

**For the standard of care when providing PrEP, all paticipants in the study will receive a medical history and interval updates, physical examination, rapid HIV-1 testing, PrEP or other drug side effect screen, pill counts for PrEP drugs, PrEP dispensing for a 3-month supply, and standard adherence and risk reduction counseling at every visit. Per the standard of care when providing PrEP, creatinine is checked every 6 months, urinalysis every 6 months, and a urine pregnancy test for women is performed as indicated by clinical care. All research providers at the Thika Clinic are trained clinicians in PrEP provision so they will be performing both the research procedures and the standard-of-care clinical procedures for PrEP.*

For date collection, we will use electronic data capture (REDCap Cloud), which is currently in use at the Thika site.

5.12. Participant Retention and Withdrawal

The Thika site will develop retention methods tailored to and most efficient for the local study setting. Retention activities may include explanation of the study visit schedule and procedural requirements during the informed consent process and re-emphasis at each study visit, collection and updating of locator information, and use of appropriate and timely visit reminder mechanisms (including phone calls and text messages). Visits can be missed, but, to provide complete information at the end of the study, efforts will be made to have a final follow-up visit for each participant. This study does not allow for home visits.

Participants may voluntarily withdraw from the study for any reason, at any time. If the participant decides to leave the study before her last scheduled study visit, the participant will be requested to have a final study visit with the procedures listed in Table 1. The site Investigator also may withdraw participants from the study in order to protect their safety and/or if they are unwilling or unable to comply with required study procedures. Participants may also be withdrawn if the study is stopped or canceled. Reasons for withdrawal will be recorded.

5.13. PrEP Discontinuation and Seroconversion

PrEP Discontinuation. PrEP continuation will be according to Kenya PrEP guidelines. Use of PrEP may be interrupted by the site Investigator due to safety concerns for the participant or if the participant is unable or unwilling to comply with study procedures. All treatment interruptions will be documented.

Seroconversion will be determined by local HIV-1 testing guidelines. For an initially HIV-1 uninfected participant who seroconverts, she will be exited from the study and referred to HIV primary care for confirmation of the positive result and initiation of HIV care.

6. STUDY OUTCOMES AND ANALYSES

6.1. Primary Outcomes

The three primary outcomes for this study will be 1) acceptability of POC urine tenofovir testing among women receiving PrEP in Kenya; 2) feasibility to provide feedback on the POC urine tenofovir assay to participants for healthcare providers, and 3) long-term metrics of adherence as assessed via hair concentrations of TFV and FTC assessed at the 12-month study visit after enrollment.

6.2. Data Collection for Primary Outcomes:

Acceptability for participants taking PrEP: We will conduct a mixed-methods assessment of the intervention arm participants' (all women on PrEP) experiences with real-time monitoring and feedback at the end of the study. A quantitative survey and a qualitative interview guide for in-depth interviews of participants will draw from the Information-Motivation-Behavioral skills (IMB) model.[180-187] Quantitative data collection will occur via standardized interviewer-administered questionnaires. Items to be assessed include the following: 1) Feelings about receiving their PrEP adherence results in real time; 2) Likelihood of participating in other studies using a similar design; 3) Likelihood of wanting to receive results of urine testing outside of a study while they are on PrEP; 4) Concern about the privacy and security of the data regarding their urine results; 5) Grading of the potential impact of knowing their urine TFV results on subsequent medication adherence; 6) Advantages and disadvantages of being told about their adherence in real time; 7) Likelihood of taking PrEP just before later study visits because they knew the urine test was being conducted; 8) Preferences regarding a yes/no assay versus an assay that provides information on "high", "moderate" or "low" adherence. A 5-point Likert item format will be used to assess graded items (such as the likelihood of wanting continued urine testing in the context of PrEP; feelings about the urine testing, ranging from negative to positive; concerns about privacy, ranging from low to high; the potential impact of real-time feedback on subsequent adherence, ranging from low to high). Other items (advantages and disadvantages of being told about adherence results) will provide pre-specified options with one "other" option for open-ended text fields.

Qualitative data collection for the acceptability outcome will include serial in-depth interviews using pre-piloted semi-structured guides (n=20 women from the intervention arm) and focus group discussions (n=4 groups with 5-10 women), conducted by experienced social scientists acting in the roles of facilitator and note taker under Dr. Ngure's supervision. The semi-structured interview guide will elicit feelings about the adherence metric and counseling messages, concerns regarding privacy, advantages and disadvantages of receiving such results, the likely impact of this monitoring test on sustained adherence to PrEP or just short-term adherence. Although the guide will provide a framework for discussion participants will be encouraged to share their own thoughts regarding the feedback from the POC tenofovir assay. The in-depth interview will be

conducted at the end of the study (month 12). The focus group discussions will also be conducted at study exit. Interviews will be conducted using the participants' preferred language and by consensus in the focus group discussions. The sampling for the qualitative interviews to include a range of possible perspectives (stratified-purposive sampling), to allow for stratification by study arm and other relevant factors that may emerge during the interviews. For instance, women will be purposefully sampled based on age group (≤ or > 25 years) given unique adherence barriers among young women.[63,188,189]

Feasibility for research healthcare providers: Under the supervision of Dr. Ngure, who has led qualitative work in Thika for the past decade,[190-196] we will assess the feasibility of the intervention by interviewing research healthcare providers, who will be administering this test at the clinical point of care in the future. The urine test takes 2 minutes and should be feasible in the context of busy clinic visits. We will examine provider perceptions of the assay using in-depth interviews. These key informant interviews (up to 8) will be performed at the end of this study with the healthcare providers (nurses, doctors, counselors) who delivered the counseling messages to intervention arm participants after receiving the results of the POC urine TFV assay from the trained Thika laboratory staff. We aim to understand acceptance, barriers, facilitators, and confidence regarding PrEP adherence testing using this POC assay in the context of PrEP. The semi-structured interview guide will draw from the Unified Theory of Acceptance and Use of Technology (UTAUT) model.{Ventkatesh, #603} This model incorporates factors that influence technology acceptance (in this case, of the POC immunoassay): perceived usefulness (performance expectancy), complexity to use (effort expectancy), stigma/social harm (social influence), and benefits (facilitating PrEP adherence among patients). These interviews will also elicit barriers and facilitators to delivering the TFV assay-informed counseling messages. Suggestions on how to optimize delivery of PrEP adherence testing will be documented.

<u>Storage and Retention of qualitative data sets:</u>

The qualitative discussions for both the Acceptability and Feasibility data collection procedures will be recorded, transcribed, and translated into English by the study team. All source documents including audio-recordings and notes for qualitative data will be stored in secure and lockable cabinets in accordance with the DAIDS Storage and Retention of Clinical Research Records Policy.

Impact on hair concentrations (long-term adherence).

Small hair samples (~50-100 strands) will be collected at study visits using previously described methods.[23] TFV concentrations in hair samples will serve as the metric for cumulative, long-term adherence to PrEP. TFV and FTC are measured in hair samples at the Hair Analytical Laboratory at the University of California San Francisco (UCSF) using validated methods. The Hair Analytical Laboratory (HAL), and its predecessor (the UCSF Drug Studies Unit), have vast expertise (since 1977) in LC-MS/MS-based methods to analyze drugs in a variety of matrices, including

antiretrovirals (ARVs) in hair.[9,10,30] The trial will assess whether TFV levels in hair increase over time with delivery of the feedback results and the opportunity to perform enhanced adherence counseling. Moreover, the combination of short-term adherence metrics (urine tenofovir assay) and long-term adherence metrics (hair TFV assay) in this study will enable us to determine if there are white-coat patterns of adherence.[35,39,41] For example, positive urine TFV assays but low concentrations of TFV in hair (in intervention arm participants or in both arms, as assessed at the end of the study) are suggestive of white-coat adherence patterns. PrEP refills will be measured through data from the clinic's electronic pharmacy system.

6.3. Sample Size and Power

We also assess sample size based on the long-term adherence outcome of this pilot RCT, an increase in hair levels of TFV with the real-time feedback/monitoring in the intervention arm compared to the SOC arm. For the hair level outcome, we consider the simplified situation of estimating difference in hair level changes between intervention and control arms using a single post-intervention level from each person. In this scenario, and assuming the person-to-person variability in TFV hair levels observed in a similar population (women in Africa not in SDC) in VOICE,[35] then the standard deviation for the difference between a baseline and follow-up (log) TFV hair level is 1.2. We estimate the power for detect a difference using a two-sample t-test formula using the standard deviation the formula for two-sample t-test with 50 enrollees per arm, then we can detect a difference of 0.68 (log TFV) between two arm with 80% power and a difference of 0.79 (log TFV) with 90% power on a 0.05 level. This translate to differences of 2.0 fold and 2.2 fold in TFV levels, respectively. Clinical differences could be appreciably smaller so it is not our objective to find statistical significance in hair outcome but, instead, to examine the feasibility of the intervention and to generate suggestive data on effects on long term adherence assessed by hair levels of TFV.

6.4. Statistical analyses

Acceptability

For the questions on the acceptability survey with graded answers, frequency distributions of the Likert scale data will be summarized. The results of the survey question regarding the advantages and disadvantages of real-time monitoring of adherence will be tabulated, including manually reviewing and assigning open-ended responses under the "other" option to appropriate pre-specified options. Qualitative interviews from the participants will be recorded, transcribed, and translated into English by the study team. The transcripts will be reviewed separately by two investigators for completeness and initial theme generation. Coding and analysis will be performed with Dedoose, using an integrated inductive-deductive approach informed by grounded theory.[197,198] Our approach permits us to organize our codes around themes in the semi-structured interview guides, while also leaving room for new codes to emerge. We will then review the results of our coding for consistency of text segmentation and code application with continued

inter-coder agreement. Inconsistent results will be reviewed by the coders until consensus on grouped themes is reached.

Feasibility

The feasibility of this intervention will be assessed by the proportion of women retained in the study at 12 months, the mean number of missed visits, and the proportion of planned urine assessments completed and messages delivered in the intervention arm. Uncertainty around these observed proportions will be described using 95% confidence intervals. For the interview data from providers to assess feasibility, we will use similar qualitative methods to analyze the data as described under *Acceptability.*

Long-term adherence metric

Our analysis (of the adherence outcome) will be a (censored) mixed effects linear regression model, also known as a mixed tobit model, to estimate the effect of the intervention versus SOC on logarithmically transformed levels of TFV in hair. Since large relative differences between very low hair levels are less clinically important than similar relative differences between higher levels, we will censor undetectable levels at the lower detection limit, and we will also add the detection limit to all levels before log-transformation. These steps reduce the influence of minor differences between levels at or near the detection limit, while preserving the approximate interpretation of back-transformed regression coefficients as fold-effects. The predictor variable for intervention will equal zero for everyone at the baseline (0-month) visit (where hair levels reflect pre-randomization baseline) and then will change for those in the intervention arm to equal 1 at all subsequent visits. A random intercept term will account for within-person correlation across the multiple visits; time point will be included as a categorical variable to account for systematic changes over time. We incude the pre-intervention TFV hair levels in the tobit model Correlations between self-reported adherence and pill counts over 30 days and hair levels will also be calculated.

Analyses will be by intention-to-treat. PrEP tenofovir drug levels in hair will be compared between arms using repeated measures mixed effects tobit model with baseline hair TFV included as a baseline covariate. The hypothesis of statistically significantly higher TFV levels will be tested at the month 12 time point. PrEP discontinuation, defined as missing a refill, will be analyzed as a time-to-event outcome using Cox proportional hazards regression with the pre-intervention TFV hair levels as a baseline factor in the model. If PrEP is discontinued by the treating clinician for safety reasons (but not adherence reasons), follow-up thereafter will be censored. Adjusted analyses will be performed to control for potential confounders based on our prior work assessing correlates of PrEP use: demographics (e.g., age, educational level), sexual behaviors (e.g., condom use, outside partnerships), medical status (e.g., depression), and beliefs (e.g., risk perception, PrEP efficacy). Stata package “st” and “metobit” will be used for analyses.

7. PARTICIPANT REIMBURSEMENT

Study participants will be reimbursed for time of visits, inconvenience of study procedures, and transport expenses. The reimbursement per screening or follow-up visit will be 200 Kenyan Shillings (KSh). The reimbursement will be collected after the participant has completed their study visit. The participants will be told, "You will receive a transportation reimbursement and an additional 200 Kenyan shillings for your time and effort."

8. SAFETY

8.1. Risks to Human Subjects

Multinational studies, including the Thika site, which conducted Partners PrEP Study and Partners Demonstration Project demonstrated that PrEP (including FTC/TDF) was safe for use in heterosexual men and women from Kenya and Uganda. There were no statistically significant differences in the frequency of deaths, serious adverse events, adverse events overall, or key laboratory adverse events (specifically, creatinine elevation and phosphorus decrease) for those receiving PrEP compared to those receiving placebo in the Partners PrEP study.

For the purposes of this study, only serious adverse events (SAEs), and adverse events felt related to PrEP adherence testing will be documented. SAEs felt to be related to PrEP will result in temporary hold of PrEP. In the case of temporary holds, the hold will continue until the event is stabilized or resolved. If the event resolves, PrEP may be reinitiated at the discretion of the Investigator, resuming safety monitoring. The severity of clinical symptoms will be scored using the DAIDS Table (July 2017 2.1 Version; https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables) for Grading the Severity of Adult and Pediatric AEs. Reporting on adverse events to the medical officer and relevant IRBs will be according to relevant regulations. Moreover, the study will be alert to any unanticipated problems which might arise in the conduct of the study (e.g. social harms) and take steps to minimize such problems (as detailed in Section 8.2). If any unanticipated problems arise, these will be reported to the relevant IRBs (KEMRI and UCSF) immediately.

Participants may feel pain or discomfort from phlebotomy if selected for a blood sample archive. Participants may become embarrassed, worried, or anxious when answering behavioral or demographic questions. We have trained counselors who are available through the study to help participants deal with any feelings or questions they may have. The study staff will make every effort to protect participant privacy and confidentiality while in the study. However, it is possible that participants' involvement in the study could become known to others, and that social harms may result (i.e., because participants could become known as participating in a trial involving those at risk of HIV). For example, participants could be treated unfairly or discriminated against, or could have problems being accepted by their families and/or communities.

8.2. Adequacy of Protection Against Risks

Recruitment and Informed Consent

The study protocol, consent forms, and project materials have been reviewed and approved by KEMRI Scientific and Research Unit (SERU) in Kenya (KEMRI IRB approval in **Appendix IX)**. All consent forms and participant education materials will be available in English and Swahili, as appropriate. In developing the Swahili consent form, the English document will be translated into Swahili, and a separate person will back-translate the document into English. This process of back-translation of the Swahili consent form will help ensure a satisfactory translation. Throughout the recruitment and informed consent process, we will adhere to ethical norms that are standard to the study setting.

During the course of the study, confidentiality will be preserved and the data will be maintained in a secure cabinet in the research office. The logbook containing the study identification numbers, completed consent forms, and completed data forms will remain on the premises and stored in a locked cabinet. The online data will be stored in REDCap, which is a password protected data management service. All computers with access to the data will be password protected. All data will be analyzed using the participants' study identification number and not their identifiable information.

Protection Against Risk

We will take several steps to minimize risks for participating in the proposed research project. First, the research assistant will emphasize the voluntary nature of study participation and that participants are free to withdraw from the study at any time and without impact to their routine medical care. At any point during interviews, participants (women taking PrEP and healthcare providers) can decide to not answer any question they are uncomfortable answering and may choose to leave the interview or focus group sessions at any time. Second, the study protocol, consent forms, and project materials will be reviewed and approved by the Institutional Review Board in Kenya prior to commencing the study or recruiting participants. Ethical review boards have a Federal-Wide Assurance number. In addition, the consent forms will be translated to Swahili and then back translated into English to ensure accuracy. Third, we intend to minimize any potential breach of confidentiality by conducting interviews in a private space, maintaining study data in a locked office, entering data onto a secure website, and ensuring that all research computers are password protected.

We will take several steps to minimize the clinical risks in the proposed research project. First, to minimize risks of discomfort, bleeding, and infection during the blood draws, we will always use sterile needles and syringes, practicing good technique, and conducting the procedures in a designated space. Second, to mitigate the risks of anxiety related to care and treatment related to HIV risk, we will be able to refer participants to accessible counselors and social workers.

Of note, the study team has extensive experience with counseling about HIV-1 risk and HIV-1 serodiscordancy, PrEP (and ART), and strategies for HIV-1 prevention in general. The risks from the anticipated activities will be no greater than in our previous studies; in fact, given the proven prevention benefits of PrEP and now national roll-out in Kenya, risks are anticipated to be less than in some of our prior studies. We feel the risks associated with the study are small. The benefits are consistent with clinical care benefits and cultural expectations and they follow the established standard with IRB approval in our other studies. We therefore believe the balance of benefit and risk is appropriate

Participants will be asked to inform the clinic staff if they feel they have been injured because of taking part in the study. Injuries may also be identified during laboratory testing, medical histories, and physical examinations. Treatment for adverse events related to study participation will be provided by the treatment clinic. If treatment is required that is beyond the capacity of the clinic, the clinic staff will refer the participant to appropriate services or organizations that can provide care for the injury.

8.3. Potential Benefits of the Proposed Research

Participants will be receiving PrEP as part of the standard of care. Potential benefits to the proposed research include allowing the participant to voice their opinion about the acceptability of this novel point-of-care test. Participants may feel useful to in helping researchers learn potentially new ways to measure and improve adherence to PrEP. Participants and others also may benefit in the future from information learned from this study on this novel urine-based adherence assay. There may be no other direct benefits to participants in this study

8.4. Ensuring patient confidentiality and safety

We will have several mechanisms in place to ensure participant confidentiality and safety. First, we will provide extensive training to the research team regarding the study protocol, consent form, the importance of maintaining participant confidentiality and safety. Laboratory technologists on-site at Thika responsible for interepreting the tenofovir urine assay will be extensively trained. Second, we will be in regular communication with the Thika clinic to ensure that we are not disrupting patient flow or treatment. Third, we will review our data on a regular basis to make certain that our proposed research projects are not causing harm or adverse events. The Principal Investigator will notify the Institutional Review Boards of any breaches in confidentiality, study protocol, or adverse events attributable to this study within ten days of the event. An Independent Study Monitor will review procedures for the study every 3 months with full details provided in Section 12.

8.5. Pregnancy among HIV-negative women

Animal and human date, including from the Partners PrEP Study and Partners Demonstration

Project, suggest safety of FTC/TDF when used by HIV-1 infected women during pregnancy and breastfeeding.{Joseph Davey, 2020 #1219}. For this study, PrEP will not be discontinued when pregnancy is detected. Moreover, per the 2016 Kenya PrEP treatment guidelines, urine pregnancy testing of all women of reproductive age will be conducted at the start of PrEP and when indicated for clinical care **(Table 1,** footnote).

8.6. HIV testing

HIV-1 testing is an essential component of PrEP delivery to protect patient safety – specifically, to prevent the initiation of PrEP among persons who have already acquired HIV-1 and to prevent continuation of PrEP if HIV-1 is acquired while receiving PrEP. In clinical trials and delivery projects of PrEP, the greatest risk of HIV-1 infection is at the time of initiation – either because of unrecognized chronic HIV-1 infection prior to HIV-1 testing as part of PrEP start or recent (acute) HIV-1 infection acquired just prior to PrEP initiation. Such testing will be routine in this study at PrEP initiation and at each PrEP visit (**Table 1**) according to the 2016 Kenya PrEP guidelines. Kenya recommends testing according to the national HIV-1 testing algorithm, which uses third-generation HIV-1 antibody based tests, done in sequence (i.e., if a positive first test, then perform a second test for confirmation, and a third as tie-breaker).

8.7. Social harm considerations for PrEP and PrEP adherence testing

PrEP use at this point is considered one of the most effective biomedical prevention interventions for HIV risk and the benefits far outweigth the risk, which is why PrEP is part of standard clinical guidelines in Kenya and around the world. For this study, we have considered the risk of the adherence counseling provided by the urine-based test. The formative work we are performing now to ask women about how they would like the test information delivered in a supportive, motivating manner (**Section 5.5**) based on how counseling was delivered in HPTN-082 (**Figure 8**) is vital to minimizing any risk of social harm from delivering the information. Moreover, the very low risk of misclassification of test results due to the urine assay's high performance characteristics (low risk of false positives or false positives) is also helpful to minimizing any risk (please see **Section 3.4**).

The 24-hour helpline that Thika clinic has, and which we have used for prior studies, will be available in case of anxiety, social harm, depression, or a positive HIV test. In the event of a clinical need (e.g., side effects, anxiety, symptoms of a sexually transmitted infection), participants will be requested to return to the clinic for care.

9. TIMELINE

The Study Aims will be completed in two years from the initiation of the study, according to the timeline detailed in **Table 2.**

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Table 2. Timeline for Research Activities.

	Year 1				Year 2			
	Q1	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
RCT of PrEP Adherence								
IRB approval, Training & Preparation	**X**							
Enrollment Period		**X**	**X**					
Follow-up Period			**X**	**X**	**X**	**X**		
Laboratory Testing					**X**	**X**	**X**	
Qualitative Acceptability/Feasibility studies								
Data collection					**X**	**X**		
Data analyses and manuscripts						**X**	**X**	X

10. LIMITATIONS

Urine levels, like those in plasma, represent short-term PrEP adherence. Therefore, adherence monitoring via these matrices may be susceptible to "white coat adherence", where adherence improves transiently before a clinic or a study visit.[130]. We have designed this study to capture information on both short-term adherence (via the urine assay) and long-term adherence (via the hair assay) to evaluate for white-coat adherence patterns.[35,39,41] For instance, positive urine TFV assays but low concentrations of TFV in hair are suggestive of white-coat adherence patterns where short-term adherence is high, but long-term adherence is low. In intervention arm participants, short-term adherence may improve with feedback from the assay, but hair concentrations do not reflect "white coat" adherence and will only increase if overall adherence over time increases. Therefore, the careful design of the study and including both short-term and long-term adherence patterns will help us evaluate whether white-coat adherence is observed. Of note, we have elected to collect and store urine samples in the standard-of-care arm participants as well in order to analyze TFV detection at the end of the study in these samples. Hair levels will also be analyzed at the end of the study in SOC arm participants. This will enable us to compare whether the intervention arm participants (who knew urine tenofovir testing would be performed and the information delivered to them) had higher rates of "white coat adherence" than those in the standard-of-care arm.

Another limitation is that we focus only on daily PrEP in this study. Intermittent PrEP has efficacy among MSM,[199,200] although there is no evidence for on-demand PrEP with heterosexual risk in men and in women. Updated CDC guidelines for MSM still recommend daily use.[201] World Health Organization guidelines endorse daily use. Therefore, this study has relevance to the way in which PrEP should be administered in women worldwide.

Finally, the extensive validation data we have now published and summarize in **Section 3.3** shows the high performance characteristics of the assay. Our work evaluating the assay's performance in diverse populations using stored urine samples (e.g. Partners PrEP, manuscript in preparation) verifies its utility across real-world settings. We do not have specific information on the effect of concomitant medications on its performance (e.g. anti-TB drugs), although these are not expected to significantly affect the results of a yes/no tenofovir adherence assay.

11. STUDY LEADERSHIP PLAN

We have assembled an interdisciplinary team for this protocol. **Dr. Monica Gandhi** from UCSF has conducted research on metrics of adherence in HIV treatment and prevention and in HIV and women for over 15 years and is the clinical director of the UCSF HAL. **Dr. Nelly Mugo** has extensive experience in PrEP roll-out in Kenya and in PrEP implementation and clinical trials at Thika Clinic. **Dr. Kenneth Ngure**, an expert in qualitative methods, has led the qualitative work for most of the PrEP and treatment trials conducted at Thika. **Dr. David Glidden**, biostatistician, conducted the analysis of pharmacologic measures for most of the major PrEP trials. **Dr. Jared Baeten** is a world-renowned expert in PrEP, served as the PI of the Partners PrEP clinical trial, is the PI of the Partners Demonstration Project, Partners Scale-UP Project and has vast expertise in HIV prevention interventions in resource constrained settings.

12. EXTERNAL MONITORING PLAN FOR PUMA STUDY- QUALITY ASSURANCE AND QUALITY CONTROL

The International Clinical Research Center (ICRC) at the University of Washington will provide regular study monitoring for the Thika site in the PUMA study. The Study Monitor will be a Senior Research Manager at ICRC. The Research Manager will have Masters in Public Health and/or a Certified Clinical Research Associate (CCRA) certification and will be experienced in site monitoring activities.

12.1. Goals and Objectives: Monitoring is necessary to assure adequate protection of the rights of human subjects and the safety of all participants involved in clinical investigations and the quality and integrity of the resulting data submitted.

The objectives of the monitoring procedures are:

- To ensure that the study is carried out in accordance with the approved protocol
- To identify any problems and suggest / seek solutions

12.2. Clinical Monitoring Activities

The ICRC Study Monitor will conduct on-site monitoring visits twice annually over the course of the 24-month study (12 months of enrollment and 12 months of follow-up). Monitoring visits will start no more than 3-months after study initiation.

The Study Monitor will review standard operating procedures (SOPs) and other study-related documentation on-site and will conduct an in-depth review of a subset of enrolled participants' records. Through this detailed review, monitoring will focus on the following key processes of the study:

- Informed consent process
- Study eligibility criteria met for all participants
- Timely completion of study CRFs
- Sample collection and handling in accordance to Protocol and SOP(s)
- Review of data management procedure
- Reporting of adverse events and protocols violations according to SOP(s)
- Follow up assessments and procedures
- Product accountability
- Regulatory documents on file

12.3. Clinical Monitoring Report

The Study Monitor will submit a written monitoring report to the study PI, the site PIs, the co-I and the Thika research team no more than 14 days after the monitoring visit. In this report, the Study Monitor will assess the following items:

- Regulatory review
- Records review
- Site Operations Assessment
- Status of previous findings
- Action items

This report will be reviewed in detail with the Thika Research Team at the end of the Monitoring Visit. The Study Monitor will request a written response to any identified "Action Items" within 14 days of the report being submitted, which will include how the team responded to any of the Action Items.

13. RESOURCE SHARING & DISSEMINATION PLAN

13.1. Resource Sharing:

For all data generated during the course of this project, we will follow the prevailing standards and guidelines in documenting and depositing data sets. We will make quality-controlled raw data as well as processed data used in publications available. As described in the grant application, protocols and workflows will be implemented exactly as described and documented such that other groups will be able to precisely reproduce results from the raw data.

The study personnel assigned to the project, including KEMRI-based, UCSF-based and University of Washington-based investigators, will disseminate results from this research through presentations at public lectures, scientific institutions and meetings, and/or publication in major journals. The institutions and Principal Investigators will adhere to the NIH Grants Policy on Sharing of Unique Research Resources including the Sharing of Biomedical Research Resources: Guidelines for Recipients of NIH Grants and Contracts on Obtaining and Disseminating Biomedical Research Resources. Study records may be reviewed by the U.S. Office of Human Research Protections.

13.2. Data Sharing Plan

Intellectual property and data generated under this project will be administered in accordance with NIH policies, including the NIH Data Sharing Policy and Implementation Guidance of March 5, 2003. Materials generated under the project will be disseminated in accordance with KEMRI, UCSF, University of Washington, and NIH policies. Depending on such policies, materials may be transferred to others under the terms of a material transfer agreement. Access to databases and associated software tools generated under the project will be available for educational, research, and non-profit purposes. Such access will be provided using web-based applications, as appropriate. Publication of data shall occur during the project, if appropriate, or at the end of the project, consistent with normal scientific practices. Research data that documents, supports, and validates research findings will be made available after the main findings from the final research data set have been accepted for publication. Such research data will be modified to prevent the disclosure of personal identifiers to remain in compliance with the Protection of Human Subjects guidelines.

13.3. Publications

We will disseminate the results from this research as broadly as possible. Of note, drafts of publications will be shared with our NIH program office and medical officer prior to submission. First, we will publish our results in Open Access journals, if appropriate. Second, we will post author PDFs of our manuscripts on our respective websites in accordance with the copyright rules of the journals. Third, we will practice posting our manuscripts on Internet archives (such as arxiv.org) when possible.

13.4. Presentations

We expect that all the research personnel will attend national conferences periodically and present the results from this research to the scientific community. Because of the multidisciplinary nature of the work, different group members will present at various conferences, such as the Conference on Retrovirus and Opportunistic Infections, International AIDS Society, and Kenya-based conferences, which focus on the appropriate aspects of our research.

The study team for this award is committed to public dissemination of results of clinical trial, to trial participants, local stakeholders in Kenya, the global scientific community, and US, Kenyan, and global policymakers. Dissemination of study results will follow principles of good participatory practice. The clinical trial has now been registered with Clinicaltrials.gov prior to initiation (NCT0393546) and results will be updated there in a timely fashion. Results will be published in conference abstracts and peer-reviewed journals. Study results will be disseminated through presentations to local stakeholders and policymakers in Kenya, including the Ministry of Health.

14. ETHICAL CONSIDERATIONS

The study protocol, consent forms, and project materials have been reviewed and approved by the KEMRI IRB (**Appendix IX**). The FDA has reviewed and approved the urine tenofovir test as qualifying for a Nonsignificant risk (NSR) indication for an investigational device (**Section 3.4** and **Appendix VIII**)

All participants will provide written informed consent before participation in the trial or in qualitative interviews in their preferred language. Participants will be informed the purpose of the study, the procedures to be followed and the risks and benefits of participation. The consents forms will be translated into Swahili and then back-translated to ensure accuracy. Specifically the participants will be informed that this novel study will answer a critical questions on acceptability, performance and barriers of objective PrEP adherence testing in the context of implementation of PrEP.

Collection and analyses of socio-demographic information and biological samples taken for clinical purposes will be covered by ethics approval. Informed consent will be obtained from potential participants to participate in the study and to store blood and hair samples. These biological specimens will be handled by trained staff and labeled with a unique identifier for each participant. The results will be transmitted to the clinics in an electronic format but only accessed by authorized and trained clinic staff. Only key members of the data team will have access to the electronic data, which will be password protected. Once enrolled into the study participants will be able to withdraw at any point. The link between participant names and codes, used for samples and data, will be destroyed five years after the study is completed,

15. PROTOCOL REGISTRATION

Prior to implementation of this protocol, and any subsequent full version amendments, each site must have the protocol and the protocol informed consent form(s) approved, as appropriate, by their local institutional review board (IRB)/ethics committee (EC) and any other applicable regulatory entity (RE). Upon receiving final approval, sites will submit all required protocol registration documents to the DAIDS Protocol Registration Office (DAIDS PRO) at the Regulatory Support Center (RSC). The DAIDS PRO will review the submitted protocol registration packet to ensure that all of the required documents have been received.

Site-specific informed consent forms (ICFs) WILL NOT be reviewed or approved by the DAIDS PRO, and sites will receive an Initial Registration Notification when the DAIDS PRO receives a complete registration packet. Receipt of an Initial Registration Notification indicates successful completion of the protocol registration process. Sites will not receive any additional notifications from the DAIDS PRO for the initial protocol registration. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

Upon receiving final IRB/EC 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 RSC. The DAIDS PRO will review the submitted protocol registration packet to ensure that all the required documents have been received. Site-specific ICF(s) 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.

16. REFERENCES

1. Gandhi M, Bacchetti P, Rodrigues WC, et al. Development and Validation of an Immunoassay for Tenofovir in Urine as a Real-Time Metric of Antiretroviral Adherence. *EClinical Medicine (Published by The Lancet); https://doiorg/101016/jeclinm201808004.* 2018.
2. Gandhi M, Bacchetti P, Spinelli MA, et al. Brief Report: Validation of a Urine Tenofovir Immunoassay for Adherence Monitoring to PrEP and ART and Establishing the Cutoff for a Point-of-Care Test. *J Acquir Immune Defic Syndr.* 2019;81(1):72-77.
3. Spinelli MA, Glidden DV, Rodrigues WC, et al. Low tenofovir level in urine by a novel immunoassay is associated with seroconversion in a PrEP demonstration project. *AIDS.* 2019(5):867-872.
4. Gandhi M, Wang G, King R, et al. Development and validation of the first point-of-care assay to objectively monitor adherence to HIV treatment and prevention in real-time in routine settings. *AIDS.* 2020;34(2):255-260.
5. Spinelli MA, Rodrigues WC, Wang G, Vincent M, Glidden DV, Okochi H, Stalter R, Defechereux P, Deutsch M, Grant RM, Ngure K, Mugo NR, Baeten JM, Gandhi M. High accuracy of a real-time urine antibody-based tenofovir point-of-care test compared to laboratory-based ELISA in diverse populations. JAIDS 2020 (in press)
6. Hendrix CW, Andrade A, Bumpus NN, et al. Dose Frequency Ranging Pharmacokinetic Study of Tenofovir-Emtricitabine After Directly Observed Dosing in Healthy Volunteers to Establish Adherence Benchmarks (HPTN 066). *AIDS Res Hum Retroviruses.* 2016;32(1):32-43.
7. Castillo-Mancilla JR, Zheng JH, Rower JE, et al. Tenofovir, emtricitabine, and tenofovir diphosphate in dried blood spots for determining recent and cumulative drug exposure. *AIDS Res Hum Retroviruses.* 2013;29(2):384-390.
8. Gandhi M, Greenblatt RM. Hair it is: the long and short of monitoring antiretroviral treatment. *Ann Intern Med.* 2002;137(8):696-697.

9. Huang Y, Gandhi M, Greenblatt RM, Gee W, Lin ET, Messenkoff N. Sensitive analysis of anti-HIV drugs, efavirenz, lopinavir and ritonavir, in human hair by liquid chromatography coupled with tandem mass spectrometry. *Rapid Commun Mass Spectrom.* 2008;22(21):3401-3409.
10. Huang Y, Yang Q, Yoon K, et al. Microanalysis of the antiretroviral nevirapine in human hair from HIV-infected patients by liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem.* 2011;401(6):1923-1933.
11. Gandhi M, Greenblatt RM, Bacchetti P, et al. A single-nucleotide polymorphism in CYP2B6 leads to >3-fold increases in efavirenz concentrations in plasma and hair among HIV-infected women. *J Infect Dis.* 2012;206(9):1453-1461.
12. van Zyl GU, van Mens TE, McIlleron H, et al. Low lopinavir plasma or hair concentrations explain second line protease inhibitor failures in a resource-limited setting. *J Acquir Immune Defic Syndr.* 2011;56(4):333-339.
13. Prasitsuebsai W, Kerr SJ, Truong KH, et al. Using Lopinavir Concentrations in Hair Samples to Assess Treatment Outcomes on Second-Line Regimens Among Asian Children. *AIDS Res Hum Retroviruses.* 2015;31(10):1009-1014.
14. Gandhi M, Ameli N, Bacchetti P, et al. Protease inhibitor levels in hair strongly predict virologic response to treatment. *AIDS.* 2009;23(4):471-478.
15. Gandhi M, Ameli N, Bacchetti P, et al. Atazanavir concentration in hair is the strongest predictor of outcomes on antiretroviral therapy. *Clin Infect Dis.* 2011;52(10):1267-1275.
16. Cohan D, Natureeba P, Koss CA, et al. Efficacy and safety of lopinavir/ritonavir versus efavirenz-based antiretroviral therapy in HIV-infected pregnant Ugandan women. *AIDS.* 2015;29(2):183-191.
17. Koss CA, Natureeba P, Mwesigwa J, et al. Hair concentrations of antiretrovirals predict viral suppression in HIV-infected pregnant and breastfeeding Ugandan women. *AIDS.* 2015;29(7):825-830.
18. Chawana TD, Gandhi M, Nathoo K, et al. Defining a Cutoff for Atazanavir in Hair Samples Associated With Virological Failure Among Adolescents Failing Second-Line Antiretroviral Treatment. *J Acquir Immune Defic Syndr.* 2017;76(1):55-59.
19. Pintye J, Bacchetti P, Teeraananchai S, et al. Brief Report: Lopinavir Hair Concentrations Are the Strongest Predictor of Viremia in HIV-Infected Asian Children and Adolescents on Second-Line Antiretroviral Therapy. *J Acquir Immune Defic Syndr.* 2017;76(4):367-371.
20. Baxi SM, Greenblatt RM, Bacchetti P, et al. Nevirapine Concentration in Hair Samples Is a Strong Predictor of Virologic Suppression in a Prospective Cohort of HIV-Infected Patients. *PLoS One.* 2015;10(6):e0129100.
21. Gandhi M, Ofokotun I, Bacchetti P, et al. Antiretroviral concentrations in hair strongly predict virologic response in a large HIV treatment-naive clinical trial. *Clin Infect Dis.* 2019;5:1044-1047.
22. Gandhi M, Mwesigwa J, Aweeka F, et al. Hair and Plasma Data Show That Lopinavir, Ritonavir, and Efavirenz All Transfer From Mother to Infant In Utero, But Only Efavirenz Transfers via Breastfeeding. *J Acquir Immune Defic Syndr.* 2013;63(5):578-584.
23. Hickey MD, Salmen CR, Tessler RA, et al. Antiretroviral concentrations in small hair samples as a feasible marker of adherence in rural Kenya. *J Acquir Immune Defic Syndr.* 2014;66(3):311-315.
24. Olds PK, Kiwanuka JP, Nansera D, et al. Assessment of HIV antiretroviral therapy adherence by measuring drug concentrations in hair among children in rural Uganda. *AIDS Care.* 2015;27(3):327-332.
25. Bartelink IH, Savic RM, Mwesigwa J, et al. Pharmacokinetics of lopinavir/ritonavir and efavirenz in food insecure HIV-infected pregnant and breastfeeding women in Tororo, Uganda. *Journal of Clinical Pharmacology.* 2014;54(2):121-132.

26. Gandhi M, Yang Q, Bacchetti P, Huang Y. Short communication: A low-cost method for analyzing nevirapine levels in hair as a marker of adherence in resource-limited settings. *AIDS Res Hum Retroviruses.* 2014;30(1):25-28.
27. Gwadz M, Cleland CM, Applegate E, et al. Behavioral Intervention Improves Treatment Outcomes Among HIV-Infected Individuals Who Have Delayed, Declined, or Discontinued Antiretroviral Therapy: A Randomized Controlled Trial of a Novel Intervention. *AIDS Behav.* 2015;19(10):1801-1817.
28. Hickey MD, Salmen CR, Omollo D, et al. Implementation and Operational Research: Pulling the Network Together: Quasiexperimental Trial of a Patient-Defined Support Network Intervention for Promoting Engagement in HIV Care and Medication Adherence on Mfangano Island, Kenya. *J Acquir Immune Defic Syndr.* 2015;69(4):e127-134.
29. Gandhi M, Gandhi RT, Stefanescu A, et al. Cumulative Antiretroviral Exposure Measured in Hair Is Not Associated With Measures of HIV Persistence or Inflammation Among Individuals on Suppressive ART. *J Infect Dis.* 2018;218:234-238.
30. Phung N, Kuncze K, Okochi H, et al. Development and Validation of an Assay to Analyze Atazanavir in Human Hair Via Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). *Rapid Commun Mass Spectrom.* 2018;Mar 15;32(5):431-441.
31. Liu AY, Yang Q, Huang Y, et al. Strong relationship between oral dose and tenofovir hair levels in a randomized trial: hair as a potential adherence measure for pre-exposure prophylaxis (PrEP). *PLoS One.* 2014;9(1):e83736.
32. Baxi SM, Liu A, Bacchetti P, et al. Comparing the novel method of assessing PrEP adherence/exposure using hair samples to other pharmacologic and traditional measures. *J Acquir Immune Defic Syndr.* 2015;68(1):13-20.
33. Gandhi M, Glidden DV, Liu A, et al. Strong Correlation Between Concentrations of Tenofovir (TFV) Emtricitabine (FTC) in Hair and TFV Diphosphate and FTC Triphosphate in Dried Blood Spots in the iPrEx Open Label Extension: Implications for Pre-exposure Prophylaxis Adherence Monitoring. *J Infect Dis.* 2015;212(9):1402-1406.
34. Gandhi M, Glidden DV, Mayer K, et al. Association of age, baseline kidney function, and medication exposure with declines in creatinine clearance on pre-exposure prophylaxis: an observational cohort study. *Lancet HIV.* 2016;3(11):e521-e528.
35. Koss CA, Bacchetti P, Hillier SL, et al. Differences in Cumulative Exposure and Adherence to Tenofovir in the VOICE, iPrEx OLE, and PrEP Demo Studies as Determined via Hair Concentrations. *AIDS Res Hum Retroviruses.* 2017;33(8):778-783.
36. Saberi P, Neilands TB, Ming K, et al. Strong Correlation Between Concentrations of Antiretrovirals in Home-Collected and Study-Collected Hair Samples: Implications for Adherence Monitoring. *J Acquir Immune Defic Syndr.* 2017;76(4):e101-e103.
37. Gandhi M, Murnane PM, Bacchetti P, et al. Hair levels of preexposure prophylaxis drugs measure adherence and are associated with renal decline among men/transwomen. *AIDS.* 2017;31(16):2245-2251.
38. Koss CA, Hosek SG, Bacchetti P, et al. Comparison of Measures of Adherence to Human Immunodeficiency Virus Preexposure Prophylaxis Among Adolescent and Young Men Who Have Sex With Men in the United States. *Clin Infect Dis.* 2018;66(2):213-219.
39. Abaasa A, Hendrix C, Gandhi M, et al. Utility of Different Adherence Measures for PrEP: Patterns and Incremental Value. *AIDS Behav.* 2018;22(4):1165-1173.
40. Seifert SM, Castillo-Mancilla JR, Erlandson K, et al. Adherence biomarker measurements in older and younger HIV-infected adults receiving tenofovir-based therapy. *J Acquir Immune Defic Syndr.* 2017;Mar 1:77(3):295-298.
41. Baxi SM, Vittinghoff E, Bacchetti P, et al. Comparing pharmacologic measures of tenofovir exposure in a U.S. pre-exposure prophylaxis randomized trial. *PLoS One.* 2018;13(1):e0190118.

42. Markowitz M, Grossman H, Anderson PL, et al. Newly Acquired Infection With Multidrug-Resistant HIV-1 in a Patient Adherent to Preexposure Prophylaxis. *J Acquir Immune Defic Syndr.* 2017;76(4):e104-e106.
43. Thaden JT, Gandhi M, Okochi H, Hurt CB, McKellar MS. Seroconversion on preexposure prophylaxis: a case report with segmental hair analysis for timed adherence determination. *AIDS.* 2018;32(9):F1-F4.
44. Colby DJ, Kroon E, Sacdalan C, et al. Acquisition of Multidrug-Resistant Human Immunodeficiency Virus Type 1 Infection in a Patient Taking Preexposure Prophylaxis. *Clin Infect Dis.* 2018;31:962-964.
45. Koss CA, Liu AY, Castillo-Mancilla J, et al. Similar tenofovir hair concentrations in men and women after directly observed dosing of tenofovir disoproxil fumarate/emtricitabine: implications for preexposure prophylaxis adherence monitoring. *AIDS.* 2018;32(15):2189-2194.
46. Cohen SE, Sachdev D, Lee SA, et al. Acquisition of tenofovir-susceptible, emtricitabine-resistant HIV despite high adherence to daily pre-exposure prophylaxis: a case report. *Lancet HIV.* 2018:doi: 10.1016/S2352-3018(1018)30288-30281. [Epub ahead of print].
47. Murnane PM, Bacchetti P, Currier JS, et al. Tenofovir concentrations in hair strongly predict virologic suppression in breastfeeding women. *AIDS.* 2019;33(10):1657-1662.
48. Velloza J, Bacchetti P, Hendrix CW, et al. Short- and Long-Term Pharmacologic Measures of HIV Pre-exposure Prophylaxis Use Among High-Risk Men Who Have Sex With Men in HPTN 067/ADAPT. *J Acquir Immune Defic Syndr.* 2019;82(2):149-158.
49. Koenig HC, Mounzer K, Daughtridge GW, et al. Urine assay for tenofovir to monitor adherence in real time to tenofovir disoproxil fumarate/emtricitabine as pre-exposure prophylaxis. *HIV Med.* 2017;18(6):412-418.
50. Cottrell ML, Yang KH, Prince HM, et al. A Translational Pharmacology Approach to Predicting Outcomes of Preexposure Prophylaxis Against HIV in Men and Women Using Tenofovir Disoproxil Fumarate With or Without Emtricitabine. *J Infect Dis.* 2016;214(1):55-64.
51. Havlir D, Beyrer C. The beginning of the end of AIDS? *N Engl J Med.* 2012;367(8):685-687.
52. Cohen MS, Chen YQ, McCauley M, et al. Prevention of HIV-1 infection with early antiretroviral therapy. *N Engl J Med.* 2011;365(6):493-505.
53. Cohen MS, Chen YQ, McCauley M, et al. Antiretroviral Therapy for the Prevention of HIV-1 Transmission. *N Engl J Med.* 2016;375(9):830-839.
54. Rodger AJ, Cambiano V, Bruun T, et al. Sexual Activity Without Condoms and Risk of HIV Transmission in Serodifferent Couples When the HIV-Positive Partner Is Using Suppressive Antiretroviral Therapy. *JAMA.* 2016;316(2):171-181.
55. Bavinton B, Grinsztejn B, Phanuphak N, et al. HIV treatment prevents HIV transmission in male serodiscordant couples in Australia, Thailand and Brazil. International AIDS Conference July 2017; Paris, France.
56. Grant RM, Lama JR, Anderson PL, et al. Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. *The New England Journal of Medicine.* 2010;363(27):2587-2599.
57. Thigpen MC, Kebaabetswe PM, Paxton LA, et al. Antiretroviral preexposure prophylaxis for heterosexual HIV transmission in Botswana. *N Engl J Med.* 2012;367(5):423-434.
58. Baeten JM, Donnell D, Ndase P, et al. Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. *The New England Journal of Medicine.* 2012;367(5):399-410.

59. Choopanya K, Martin M, Suntharasamai P, et al. Antiretroviral prophylaxis for HIV infection in injecting drug users in Bangkok, Thailand (the Bangkok Tenofovir Study): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet.* 2013;381(9883):2083-2090.
60. Centers for Disease Control (CDC). Preexposure Prophylaxis for the Prevention of HIV in the United States: A Clinical Practice Guideline - 2017 Update (published April 2018); https://www.cdc.gov/hiv/pdf/risk/prep/cdc-hiv-prep-guidelines-2017.pdf. (Accessed April 23, 2018).
61. World Health Organization (WHO). Updated recommendations on first-line and second-line antiretroviral regimens and post-exposure prophylaxis and recommendations on early infant diagnosis of HIV: interim guidance; http://www.who.int/hiv/pub/guidelines/ARV2018update/en/ (Accessed November 2018).
62. Amico KR. Adherence to preexposure chemoprophylaxis: the behavioral bridge from efficacy to effectiveness. *Curr Opin HIV AIDS.* 2012;7(6):542-548.
63. Marrazzo JM, Ramjee G, Richardson BA, et al. Tenofovir-based preexposure prophylaxis for HIV infection among African women. *N Engl J Med.* 2015;372(6):509-518.
64. Corneli AL, McKenna K, Perry B, et al. The science of being a study participant: FEM-PrEP participants' explanations for overreporting adherence to the study pills and for the whereabouts of unused pills. *J Acquir Immune Defic Syndr.* 2015;68(5):578-584.
65. Van Damme L, Corneli A, Ahmed K, et al. Preexposure prophylaxis for HIV infection among African women. *N Engl J Med.* 2012;367(5):411-422.
66. van der Straten A, Brown ER, Marrazzo JM, et al. Divergent adherence estimates with pharmacokinetic and behavioural measures in the MTN-003 (VOICE) study. *J Int AIDS Soc.* 2016;19(1):20642.
67. Baker Z, Javanbakht M, Mierzwa S, et al. Predictors of Over-Reporting HIV Pre-exposure Prophylaxis (PrEP) Adherence Among Young Men Who Have Sex With Men (YMSM) in Self-Reported Versus Biomarker Data. *AIDS Behav.* 2017.
68. Agot K, Taylor D, Corneli AL, et al. Accuracy of Self-Report and Pill-Count Measures of Adherence in the FEM-PrEP Clinical Trial: Implications for Future HIV-Prevention Trials. *AIDS Behav.* 2015;19(5):743-751.
69. Blumenthal J, Haubrich R. Pre-exposure prophylaxis for HIV infection: how antiretroviral pharmacology helps to monitor and improve adherence. *Expert Opin Pharmacother.* 2013;14(13):1777-1785.
70. Checchi KD, Huybrechts KF, Avorn J, Kesselheim AS. Electronic medication packaging devices and medication adherence: a systematic review. *JAMA.* 2014;312(12):1237-1247.
71. Labovitz DL, Shafner L, Reyes Gil M, Virmani D, Hanina A. Using Artificial Intelligence to Reduce the Risk of Nonadherence in Patients on Anticoagulation Therapy. *Stroke.* 2017;48(5):1416-1419.
72. Bain EE, Shafner L, Walling DP, et al. Use of a Novel Artificial Intelligence Platform on Mobile Devices to Assess Dosing Compliance in a Phase 2 Clinical Trial in Subjects With Schizophrenia. *JMIR Mhealth Uhealth.* 2017;5(2):e18.
73. Burke LE, Zheng Y, Ma Q, et al. The SMARTER pilot study: Testing feasibility of real-time feedback for dietary self-monitoring. *Prev Med Rep.* 2017;6:278-285.
74. Margolis KL, Asche SE, Bergdall AR, et al. Effect of home blood pressure telemonitoring and pharmacist management on blood pressure control: a cluster randomized clinical trial. *JAMA.* 2013;310(1):46-56.
75. Kerby TJ, Asche SE, Maciosek MV, O'Connor PJ, Sperl-Hillen JM, Margolis KL. Adherence to blood pressure telemonitoring in a cluster-randomized clinical trial. *J Clin Hypertens (Greenwich).* 2012;14(10):668-674.
76. Frias J, Virdi N, Raja P, Kim Y, Savage G, Osterberg L. Effectiveness of Digital Medicines to Improve Clinical Outcomes in Patients with Uncontrolled Hypertension and Type 2

Diabetes: Prospective, Open-Label, Cluster-Randomized Pilot Clinical Trial. *J Med Internet Res.* 2017;19(7):e246.

77. Lind M, Polonsky W, Hirsch IB, et al. Continuous Glucose Monitoring vs Conventional Therapy for Glycemic Control in Adults With Type 1 Diabetes Treated With Multiple Daily Insulin Injections: The GOLD Randomized Clinical Trial. *JAMA.* 2017;317(4):379-387.
78. Beck RW, Riddlesworth T, Ruedy K, et al. Effect of Continuous Glucose Monitoring on Glycemic Control in Adults With Type 1 Diabetes Using Insulin Injections: The DIAMOND Randomized Clinical Trial. *JAMA.* 2017;317(4):371-378.
79. Givertz MM, Stevenson LW, Costanzo MR, et al. Pulmonary Artery Pressure-Guided Management of Patients With Heart Failure and Reduced Ejection Fraction. *J Am Coll Cardiol.* 2017;70(15):1875-1886.
80. Patteet L, Morrens M, Maudens KE, Niemegeers P, Sabbe B, Neels H. Therapeutic drug monitoring of common antipsychotics. *Ther Drug Monit.* 2012;34(6):629-651.
81. Schoretsanitis G, Paulzen M, Unterecker S, et al. TDM in psychiatry and neurology: A comprehensive summary of the consensus guidelines for therapeutic drug monitoring in neuropsychopharmacology, update 2017; a tool for clinicians(). *World J Biol Psychiatry.* 2018;19(3):162-174.
82. Hiemke C, Bergemann N, Clement HW, et al. Consensus Guidelines for Therapeutic Drug Monitoring in Neuropsychopharmacology: Update 2017. *Pharmacopsychiatry.* 2018;51(1-02):e1.
83. Gupta P, Patel P, Strauch B, et al. Biochemical Screening for Nonadherence Is Associated With Blood Pressure Reduction and Improvement in Adherence. *Hypertension.* 2017;70(5):1042-1048.
84. Helfer AG, Michely JA, Weber AA, Meyer MR, Maurer HH. Orbitrap technology for comprehensive metabolite-based liquid chromatographic-high resolution-tandem mass spectrometric urine drug screening - exemplified for cardiovascular drugs. *Anal Chim Acta.* 2015;891:221-233.
85. Patel P, Gupta PK, White CM, Stanley AG, Williams B, Tomaszewski M. Screening for non-adherence to antihypertensive treatment as a part of the diagnostic pathway to renal denervation. *J Hum Hypertens.* 2016;30(6):368-373.
86. Lawson AJ, Shipman KE, George S, Dasgupta I. A Novel 'Dilute-and-Shoot' Liquid Chromatography-Tandem Mass Spectrometry Method for the Screening of Antihypertensive Drugs in Urine. *J Anal Toxicol.* 2016;40(1):17-27.
87. Jamison RN, Martel MO, Huang CC, Jurcik D, Edwards RR. Efficacy of the Opioid Compliance Checklist to Monitor Chronic Pain Patients Receiving Opioid Therapy in Primary Care. *J Pain.* 2016;17(4):414-423.
88. Standridge JB, Adams SM, Zotos AP. Urine drug screening: a valuable office procedure. *Am Fam Physician.* 2010;81(5):635-640.
89. Oliva EM, Bowe T, Tavakoli S, et al. Development and applications of the Veterans Health Administration's Stratification Tool for Opioid Risk Mitigation (STORM) to improve opioid safety and prevent overdose and suicide. *Psychol Serv.* 2017;14(1):34-49.
90. Group E-MS. Study protocol of the ESUB-MG cluster randomized trial: a pragmatic trial assessing the implementation of urine drug screening in general practice for buprenorphine maintained patients. *BMC Fam Pract.* 2016;17:24.
91. van der Straten A, Montgomery ET, Musara P, et al. Disclosure of pharmacokinetic drug results to understand nonadherence. *AIDS.* 2015;29(16):2161-2171.
92. Husnik MJ, Brown ER, Marzinke M, et al. Implementation of a Novel Adherence Monitoring Strategy in a Phase III, Blinded, Placebo-Controlled, HIV-1 Prevention Clinical Trial. *J Acquir Immune Defic Syndr.* 2017;76(3):330-337.

93. Mensch BS, Brown ER, Liu K, et al. Reporting of Adherence in the VOICE Trial: Did Disclosure of Product Nonuse Increase at the Termination Visit? *AIDS Behav.* 2016;20(11):2654-2661.
94. Musara P, Montgomery ET, Mgodi NM, et al. How Presentation of Drug Detection Results Changed Reports of Product Adherence in South Africa, Uganda and Zimbabwe. *AIDS Behav.* 2017.
95. Koester KA, Liu A, Eden C, et al. Acceptability of drug detection monitoring among participants in an open-label pre-exposure prophylaxis study. *AIDS Care.* 2015;27(10):1199-1204.
96. Gandhi M, Bacchetti P, Spinelli IM, et al. Validation of a Urine Tenofovir Immunoassay for Adherence Monitoring to PrEP and ART and Establishing the Cut-Off for a Point-of-Care Test. *J Acquir Immune Defic Syndr.* 2019.
97. Gandhi M, Wang G, King R, et al. Development and Validation of the First Point-of-Care Assay to Objectively Monitor Adherence to HIV Treatment and Prevention in Real-Time in Routine Settings. AIDS 2019 (in press).
98. Anderson PL, Glidden DV, Liu A, et al. Emtricitabine-tenofovir concentrations and pre-exposure prophylaxis efficacy in men who have sex with men. *Sci Transl Med.* 2012;4(151):151ra125.
99. Baeten JM, Celum C. Antiretroviral preexposure prophylaxis for HIV prevention. *N Engl J Med.* 2013;368(1):83-84.
100. Anderson PL, Liu AY, Castillo-Mancilla JR, et al. Intracellular Tenofovir-Diphosphate and Emtricitabine-Triphosphate in Dried Blood Spots following Directly Observed Therapy. *Antimicrob Agents Chemother.* 2018;62(1).
101. Liu A, Glidden DV, Anderson PL, et al. Patterns and correlates of PrEP drug detection among MSM and transgender women in the Global iPrEx Study. *J Acquir Immune Defic Syndr.* 2014;67(5):528-537.
102. Donnell D, Baeten JM, Bumpus NN, et al. HIV protective efficacy and correlates of tenofovir blood concentrations in a clinical trial of PrEP for HIV prevention. *J Acquir Immune Defic Syndr.* 2014;66(3):340-348.
103. Musinguzi N, Muganzi CD, Boum Y, 2nd, et al. Comparison of subjective and objective adherence measures for preexposure prophylaxis against HIV infection among serodiscordant couples in East Africa. *AIDS.* 2016;30(7):1121-1129.
104. Hosek SG, Landovitz RJ, Kapogiannis B, et al. Safety and Feasibility of Antiretroviral Preexposure Prophylaxis for Adolescent Men Who Have Sex With Men Aged 15 to 17 Years in the United States. *JAMA Pediatr.* 2017;171(11):1063-1071.
105. Glidden DV, Amico KR, Liu AY, et al. Symptoms, Side Effects and Adherence in the iPrEx Open-Label Extension. *Clin Infect Dis.* 2016;62(9):1172-1177.
106. Grant RM, Anderson PL, McMahan V, et al. Uptake of pre-exposure prophylaxis, sexual practices, and HIV incidence in men and transgender women who have sex with men: a cohort study. *Lancet Infect Dis.* 2014;14(9):820-829.
107. Landovitz RJ, Beymer M, Kofron R, et al. Plasma Tenofovir Levels to Support Adherence to TDF/FTC Preexposure Prophylaxis for HIV Prevention in MSM in Los Angeles, California. *J Acquir Immune Defic Syndr.* 2017;76(5):501-511.
108. Van Damme L, Corneli A. Antiretroviral preexposure prophylaxis for HIV prevention. *N Engl J Med.* 2013;368(1):84.
109. Musinguzi N, Muganzi CD, Boum Y, 2nd, et al. Comparison of subjective and objective adherence measures for preexposure prophylaxis against HIV infection among serodiscordant couples in East Africa. *AIDS.* 2016;30(7):1121-1129.

110. Gross IM, Hosek S, Richards MH, Fernandez MI. Predictors and Profiles of Antiretroviral Therapy Adherence Among African American Adolescents and Young Adult Males Living with HIV. *AIDS Patient Care STDS.* 2016;30(7):324-338.
111. Celum CL, Delany-Moretlwe S, Hosek S, et al. Risk Behavior, Perception and Reasons for PrEP among Young African Women in HPTN 082. Abstract #1049. 25th Conference on Retroviruses and Opportunistic Infections (CROI); March 4-7, 2018; Boston, MA.
112. Rosen EP, Thompson CG, Bokhart MT, et al. Analysis of Antiretrovirals in Single Hair Strands for Evaluation of Drug Adherence with Infrared-Matrix-Assisted Laser Desorption Electrospray Ionization Mass Spectrometry Imaging. *Anal Chem.* 2016;88(2):1336-1344.
113. Simiele M, Carcieri C, De Nicolo A, et al. A LC-MS method to quantify tenofovir urinary concentrations in treated patients. *J Pharm Biomed Anal.* 2015;114:8-11.
114. Calcagno A, Cusato J, Marinaro L, et al. Clinical pharmacology of tenofovir clearance: a pharmacokinetic/pharmacogenetic study on plasma and urines. *Pharmacogenomics J.* 2016;16(6):514-518.
115. Lalley-Chareczko L, Clark D, Zuppa AF, et al. A case study of chewed Truvada((R)) for PrEP maintaining protective drug levels as measured by a novel urine tenofovir assay. *Antivir Ther.* 2017;22(7):639-641.
116. DePriest AZ, Black DL, Robert TA. Immunoassay in healthcare testing applications. *J Opioid Manag.* 2015;11(1):13-25.
117. Pecoraro V, Banfi G, Germagnoli L, Trenti T. A systematic evaluation of immunoassay point-of-care testing to define impact on patients' outcomes. *Ann Clin Biochem.* 2017;54(4):420-431.
118. Bang HI, Jang MA, Lee YW. Evaluation of the Triage TOX Drug Screen Assay for Detection of 11 Drugs of Abuse and Therapeutic Drugs. *Ann Lab Med.* 2017;37(6):522-525.
119. Landovitz R. Personal communication. March 7, 2019.
120. Hunt T, Lalley-Chareczko L, Daughtridge G, Swyryn M, Koenig H. Challenges to PrEP use and perceptions of urine tenofovir adherence monitoring reported by individuals on PrEP. *AIDS Care.* 2019:1-4.
121. Lalley-Chareczko L, Clark D, Conyngham C, et al. Delivery of TDF/FTC for Pre-exposure Prophylaxis to Prevent HIV-1 Acquisition in Young Adult Men Who Have Sex With Men and Transgender Women of Color Using a Urine Adherence Assay. *J Acquir Immune Defic Syndr.* 2018;79(2):173-178.
122. Baeten JM, Palanee-Phillips T, Brown ER, et al. Use of a Vaginal Ring Containing Dapivirine for HIV-1 Prevention in Women. *N Engl J Med.* 2016;375(22):2121-2132.
123. Celum C, Mgodi N, Bekker LG, Hosek S, Donnell D, Anderson P, Dye B, Pathak S, Agyei Y, Fogel J, Marzinke M, Makgamathe K, Kassim S, Mukaka S, Noble H, Adeyeye A, Delany-Moretlwe S. PrEP use in young African women in HPTN 082: Effect of drug level feedback. IAS 2019, 21-24 July, Mexico City, MX.
124. Naderi SH, Bestwick JP, Wald DS. Adherence to drugs that prevent cardiovascular disease: meta-analysis on 376,162 patients. *Am J Med.* 2012;125(9):882-887 e881.
125. Correa NB, de Faria AP, Ritter AM, et al. A practical approach for measurement of antihypertensive medication adherence in patients with resistant hypertension. *J Am Soc Hypertens.* 2016;10(6):510-516 e511.
126. Marrazzo JM, Scholes D. Acceptability of urine-based screening for Chlamydia trachomatis in asymptomatic young men: a systematic review. *Sex Transm Dis.* 2008;35(11 Suppl):S28-33.
127. Hadland SE, Levy S. Objective Testing: Urine and Other Drug Tests. *Child Adolesc Psychiatr Clin N Am.* 2016;25(3):549-565.

128. Drain PK, Kubiak RW, Siriprakaisil O, et al. Urine Tenofovir Concentrations Correlate with Plasma and Relates to TDF Adherence: A Randomized Directly-observed Pharmacokinetic Trial (TARGET Study). *Clin Infect Dis.* 2019.
129. Kearney BP, Ramanathan S, Cheng AK, Ebrahimi R, Shah J. Systemic and renal pharmacokinetics of adefovir and tenofovir upon coadministration. *Journal of Clinical Pharmacology.* 2005;45(8):935-940.
130. Podsadecki TJ, Vrijens BC, Tousset EP, Rode RA, Hanna GJ. "White coat compliance" limits the reliability of therapeutic drug monitoring in HIV-1-infected patients. *HIV clinical trials.* 2008;9(4):238-246.
131. Anderson PL. Pharmacologic Measures of Adherence and Relationship to Drug Response. Conference of Retroviruses and Opportunistic Infections (CROI); Oral symposium; Boston, MA; February 22–25. 2016; Abstract 96.
132. Musinguzi N, Muwonge T, Thomas K, Baeten JM, Bangsberg DR, Haberer JE. Does Adherence Change When No One is Looking? Comparing Announced and Unannounced Tenofovir Levels in a PrEP Trial. *AIDS Behav.* 2016;20(11):2639-2643.
133. Raymond JF, Bucek A, Dolezal C, et al. Use of Unannounced Telephone Pill Counts to Measure Medication Adherence Among Adolescents and Young Adults Living With Perinatal HIV Infection. *J Pediatr Psychol.* 2017;42(9):1006-1015.
134. Pence BW, Gaynes BN, Adams JL, et al. The effect of antidepressant treatment on HIV and depression outcomes: results from a randomized trial. *AIDS.* 2015;29(15):1975-1986.
135. Fredericksen R, Feldman BJ, Brown T, et al. Unannounced telephone-based pill counts: a valid and feasible method for monitoring adherence. *AIDS Behav.* 2014;18(12):2265-2273.
136. Macdonald V, Verster A, Baggaley R. A call for differentiated approaches to delivering HIV services to key populations. *J Int AIDS Soc.* 2017;20(Suppl 4):21658.
137. UNAIDS. Global AIDS update. 2016; http://www.unaids.org/en/resources/documents/2016/Global-AIDS-update-2016. Accessed June 3, 2016.
138. UNAIDS Global AIDS Update 2019. https://www.unaids.org/en/resources/documents/2019/2019-global-AIDS-update.
139. Anderson SJ, Cherutich P, Kilonzo N, et al. Maximising the effect of combination HIV prevention through prioritisation of the people and places in greatest need: a modelling study. *Lancet.* 2014;384(9939):249-256.
140. Abbas UL, Glaubius R, Mubayi A, Hood G, Mellors JW. Antiretroviral therapy and pre-exposure prophylaxis: combined impact on HIV transmission and drug resistance in South Africa. *J Infect Dis.* 2013;208(2):224-234.
141. Celum CL, Delany-Moretlwe S, Baeten JM, et al. HIV pre-exposure prophylaxis for adolescent girls and young women in Africa: from efficacy trials to delivery. *J Int AIDS Soc.* 2019;22 Suppl 4:e25298.
142. Celum C, Mgodi N, Bekker L, et al. PrEP adherence and effect of drug level feedback among young African women in HPTN 082 (Oral Abstract # TUAC0301). International AIDS Society (IAS) Conference on HIV Science; 2019; Mexico City, Mexico.
143. Bekker LG, Roux S, Sebastien E, et al. Daily and non-daily pre-exposure prophylaxis in African women (HPTN 067/ADAPT Cape Town Trial): a randomised, open-label, phase 2 trial. *Lancet HIV.* 2018;5(2):e68-e78.
144. Spinelli MA, Glidden DV, Anderson PL, et al. Brief Report: Short-Term Adherence Marker to PrEP Predicts Future Nonretention in a Large PrEP Demo Project: Implications for Point-of-Care Adherence Testing. *J Acquir Immune Defic Syndr.* 2019;81(2):158-162.

145. Myer L, Dunning L, Lesosky M, et al. Frequency of Viremic Episodes in HIV-Infected Women Initiating Antiretroviral Therapy During Pregnancy: A Cohort Study. *Clin Infect Dis.* 2017;64(4):422-427.
146. Myer L, Redd AD, Mukonda E, et al. ART detection and resistance during viraemic episodes in pregnancy and breastfeeding. Conference on Retroviruses and Opportunistic Infections. 2018; Mar 4-7; Boston, MA. Abstract #140.
147. Nachega JB, Sam-Agudu NA, Mofenson LM, Schechter M, Mellors JW. Achieving Viral Suppression in 90% of People Living with HIV on Antiretroviral Therapy in Low- and Middle-Income Countries: Progress, Challenges, and Opportunities. *Clin Infect Dis.* 2018;Jan 8. doi: 10.1093/cid/ciy008. [Epub ahead of print]
148. Nel A, Bekker LG, Bukusi E, et al. Safety, Acceptability and Adherence of Dapivirine Vaginal Ring in a Microbicide Clinical Trial Conducted in Multiple Countries in Sub-Saharan Africa. *PLoS One.* 2016;11(3):e0147743.
149. Nel A, Van Niekerk N, Van Baelen B, Rosenberg Z. HIV Incidence and Adherence in DREAM: An Open-Label Trial of Dapivirine Vaginal Ring. Abstract #144LB. 25th Conference on Retroviruses and Opportunistic Infections (CROI); March 4-7, 2018; Boston, MA.
150. Brown E, Philips-Palanee T, Marzinke M, et al. Residual dapivirine ring levels indicate higher adherence to vaginal ring is associated with HIV-1 protection. AIDS 2016, Durban, South Africa, July 18-22, 2016; Abstract TUAC0105LB
151. Baeten J, Palanee-Phillips T, Mgodi N, et al. HIgh Uptake and Reduced HIV-1 Incidence in an Open-Label Trial of the Dapivirine Ring. Abstract #143LB. 25th Conference on Retroviruses and Opportunistic Infections (CROI); March 4-7, 2018; Boston, MA.
152. Spence P, Nel A, van Niekerk N, Derrick T, Wilder S, Devlin B. Post-use assay of vaginal rings (VRs) as a potential measure of clinical trial adherence. *J Pharm Biomed Anal.* 2016;125:94-100.
153. Findlay JW, Smith WC, Lee JW, et al. Validation of immunoassays for bioanalysis: a pharmaceutical industry perspective. *J Pharm Biomed Anal.* 2000;21(6):1249-1273.
154. Patel K, Nagel M, Wesolowski M, et al. Evaluation of a Urine-Based Rapid Molecular Diagnostic Test with Potential to be used at Point-of-Care for Pulmonary Tuberculosis: Cape Town Cohort. *J Mol Diagn.* 2017.
155. Trabattoni E, Le V, Pilmis B, et al. Implementation of Alere i Influenza A & B point of care test for the diagnosis of influenza in an emergency department. *Am J Emerg Med.* 2017.
156. Chang M, Steinmetzer K, Raugi DN, et al. Detection and differentiation of HIV-2 using the point-of-care Alere q HIV-1/2 Detect nucleic acid test. *J Clin Virol.* 2017;97:22-25.
157. Murray TY, Sherman GG, Nakwa F, et al. Field Evaluation of Performance of Alere and Cepheid Qualitative HIV Assays for Pediatric Point-of-Care Testing in an Academic Hospital in Soweto, South Africa. *J Clin Microbiol.* 2017;55(11):3227-3235.
158. van Tienen C, Rugebregt S, Scherbeijn S, Gotz H, GeurtsvanKessel C. The performance of the Alere HIV combo point-of-care test on stored serum samples; useful for detection of early HIV-1 infections? *Sex Transm Infect.* 2017.
159. Jani IV, Meggi B, Vubil A, et al. Evaluation of the Whole-Blood Alere Q NAT Point-of-Care RNA Assay for HIV-1 Viral Load Monitoring in a Primary Health Care Setting in Mozambique. *J Clin Microbiol.* 2016;54(8):2104-2108.
160. Hsiao NY, Dunning L, Kroon M, Myer L. Laboratory Evaluation of the Alere q Point-of-Care System for Early Infant HIV Diagnosis. *PLoS One.* 2016;11(3):e0152672.
161. Delefortrie Q, Schatt P, Grimmelprez A, et al. Comparison of the Liaison(R) Calprotectin kit with a well established point of care test (Quantum Blue - Buhlmann-Alere(R)) in terms of analytical performances and ability to detect relapses amongst a Crohn population in follow-up. *Clin Biochem.* 2016;49(3):268-273.

162. Khezri BS, Carlsson L, Larsson A. Evaluation of the Alere NT-proBNP Test for Point of Care Testing. *J Clin Lab Anal.* 2016;30(4):290-292.
163. Helmersson-Karlqvist J, Karlsson B, Fredriksson A, Larsson A. Evaluation of the Alere D-dimer test for point of care testing. *J Thromb Thrombolysis.* 2014;38(2):250-252.
164. Larson B, Schnippel K, Ndibongo B, Long L, Fox MP, Rosen S. How to estimate the cost of point-of-care CD4 testing in program settings: an example using the Alere Pima Analyzer in South Africa. *PLoS One.* 2012;7(4):e35444.
165. The enzyme-linked immunosorbent assay (ELISA). *Bull World Health Organ.* 1976;54(2):129-139.
166. Gandhi M, Rodriguez W, Spinelli M, et al. Development of a Point-of-Care Immunoassay for Detecting Tenofovir in Urine as a Real-Time Metric of PrEP Adherence (oral abstract). 13th International Conference on HIV Treatment and Prevention Adherence 2018. IAPAC meeting; June 8-10, 2018, Miami, Florida.
167. Cressey TR, Siriprakaisil O, Klinbuayaem V, et al. A randomized clinical pharmacokinetic trial of Tenofovir in blood, plasma and urine in adults with perfect, moderate and low PrEP adherence: the TARGET study. *BMC Infect Dis.* 2017;17(1):496.
168. Agresti A., Kateri M. (2011) Categorical Data Analysis. In: Lovric M. (eds) International Encyclopedia of Statistical Science. Springer, Berlin, Heidelberg
169. Gerona R, Wen A, Chin AT, et al. Quantifying Isoniazid Levels in Small Hair Samples: A Novel Method for Assessing Adherence during the Treatment of Latent and Active Tuberculosis. *PLoS One.* 2016;11(5):e0155887.
170. Gerona R, Wen A, Koss C, Bacchetti P, Gandhi M, Metcalfe J. A multi-analyte panel for non-invasive pharmacokinetic monitoring of second-line anti-tuberculosis drugs. *Int J Tuberc Lung Dis.* 2016;20(7):991-992.
171. Metcalfe J, Gerona R, Wen A, Bacchetti P, Gandhi M. An LC-MS/MS-based method to analyze the anti-tuberculosis drug bedaquiline in hair. *Int J Tuberc Lung Dis.* 2017;21(9):1069-1070.
172. Mave V, Kinikar A, Kagal A, et al. Isoniazid concentrations in hair and plasma area-under-the-curve exposure among children with tuberculosis. *PLoS One.* 2017;12(12):e0189101.
173. Tabb ZJ, Mmbaga BT, Gandhi M, et al. Antiretroviral drug concentrations in hair are associated with virologic outcomes among young people living with HIV in Tanzania. *AIDS.* 2018;Feb 12 [Epub ahead of print].
174. Colby D, Kroon E, Sacdalan C, et al. Acquisition of multidrug resistant HIV-1 infection in a patient taking preexposure prophylaxis. Clinical Infectious Diseases 2018 (in press).
175. Thaden JT, Gandhi M, Okochi H, Hurt CB, McKellar MS. Seroconversion On PrEP: A Case Report with Segmental Hair Analysis for Timed Adherence Determination. AIDS 2018 (in press).
176. DiFrancesco R, Tooley K, Rosenkranz SL, et al. Clinical pharmacology quality assurance for HIV and related infectious diseases research. *Clin Pharmacol Ther.* 2013;93(6):479-482.
177. National AIDS & STI Control Programme. Guidelines on the use of antiretroviral drugs for treating and preventing HIV infection in Kenya. In: Ministry of Health, Kenya; 2016.
178. Amico KR, Fisher WA, Cornman DH, et al. Visual analog scale of ART adherence: association with 3-day self-report and adherence barriers. *J Acquir Immune Defic Syndr.* 2006;42(4):455-459.
179. Celum CL, Mgodi N, Bekker LG, et al. Adherence 3 months after PrEP Initation among Young African Women in HPTN 082. Conference on Retroviruses and Opportunistic Infections (CROI), Seattle, Washingon, March 4-7 2019 (abstract 995).
180. Dubov A, Altice FL, Fraenkel L. An Information-Motivation-Behavioral Skills Model of PrEP Uptake. *AIDS Behav.* 2018.

181. Norton WE, Amico KR, Fisher WA, et al. Information-motivation-behavioral skills barriers associated with intentional versus unintentional ARV non-adherence behavior among HIV+ patients in clinical care. *AIDS Care.* 2010;22(8):979-987.
182. Rongkavilit C, Naar-King S, Kaljee LM, et al. Applying the information-motivation-behavioral skills model in medication adherence among Thai youth living with HIV: a qualitative study. *AIDS Patient Care STDS.* 2010;24(12):787-794.
183. Peltzer K, Friend-du Preez N, Ramlagan S, Anderson J. Antiretroviral treatment adherence among HIV patients in KwaZulu-Natal, South Africa. *BMC Public Health.* 2010;10:111.
184. Ware NC, Wyatt MA, Bangsberg DR. Examining theoretic models of adherence for validity in resource-limited settings. A heuristic approach. *J Acquir Immune Defic Syndr.* 2006;43 Suppl 1:S18-22.
185. Starks TJ, Millar BM, Lassiter JM, Parsons JT. Preintervention Profiles of Information, Motivational, and Behavioral Self-Efficacy for Methamphetamine Use and HIV Medication Adherence Among Gay and Bisexual Men. *AIDS Patient Care STDS.* 2017;31(2):78-86.
186. Konkle-Parker DJ, Amico KR, McKinney VE. Effects of an intervention addressing information, motivation, and behavioral skills on HIV care adherence in a southern clinic cohort. *AIDS Care.* 2014;26(6):674-683.
187. Fisher JD, Amico KR, Fisher WA, Harman JJ. The information-motivation-behavioral skills model of antiretroviral adherence and its applications. *Curr HIV/AIDS Rep.* 2008;5(4):193-203.
188. Corneli AL, Deese J, Wang M, et al. FEM-PrEP: adherence patterns and factors associated with adherence to a daily oral study product for pre-exposure prophylaxis. *J Acquir Immune Defic Syndr.* 2014;66(3):324-331.
189. Amico KR, Stirratt MJ. Adherence to preexposure prophylaxis: current, emerging, and anticipated bases of evidence. *Clin Infect Dis.* 2014;59 Suppl 1:S55-60.
190. Ngure K, Heffron R, Curran K, et al. I Knew I Would Be Safer. Experiences of Kenyan HIV Serodiscordant Couples Soon After Pre-Exposure Prophylaxis (PrEP) Initiation. *AIDS Patient Care STDS.* 2016;30(2):78-83.
191. Ngure K, Heffron R, Mugo N, et al. Feasibility and acceptability of HIV self-testing among pre-exposure prophylaxis users in Kenya. *J Int AIDS Soc.* 2017;20(1):21234.
192. Ngure K, Kimemia G, Dew K, et al. Delivering safer conception services to HIV serodiscordant couples in Kenya: perspectives from healthcare providers and HIV serodiscordant couples. *J Int AIDS Soc.* 2017;20(Suppl 1):21309.
193. Ngure K, Mugo N, Celum C, et al. A qualitative study of barriers to consistent condom use among HIV-1 serodiscordant couples in Kenya. *AIDS Care.* 2012;24(4):509-516.
194. Ngure K, Trinidad SB, Beima-Sofie K, et al. The role of male partners in women's participation in research during pregnancy: a case study from the partners demonstration project. *Reprod Health.* 2017;14(Suppl 3):160.
195. Ngure K, Vusha S, Mugo N, et al. "I never thought that it would happen ... " Experiences of HIV seroconverters among HIV-discordant partnerships in a prospective HIV prevention study in Kenya. *AIDS Care.* 2016;28(12):1586-1589.
196. Ngure K, Baeten JM, Mugo N, et al. My intention was a child but I was very afraid: fertility intentions and HIV risk perceptions among HIV-serodiscordant couples experiencing pregnancy in Kenya. *AIDS Care.* 2014;26(10):1283-1287.
197. Glaser BG, Strauss A. The Discovery of Grounded Theory: Strategies for Qualitative Research. Chicago, IL: Aldine Publishing Co, 1967.
198. Charmaz K. Constructing Grounded Theory: A Practical Guide Through Qualitative Analysis. Sage Publications, Thousand Oaks, CA, 2006.

199. Molina JM, Capitant C, Spire B, et al. On-Demand Preexposure Prophylaxis in Men at High Risk for HIV-1 Infection. *N Engl J Med.* 2015;373(23):2237-2246.
200. Antoni G, Tremblay C, Charreau I, et al. On-demand PrEP with TDF/FTC remains highly effective among MSM with infrequent sexual intercourse: a sub-study of the ANRS IPERGAY trial. International AIDS Conference July 2017; Paris, France.
201. Iman N, Carpenter C, Mayer K, Fisher A, Stein M, Danforth S. Hierarchical pattern of mucosal candida infections in HIV-seropositive women. *American Journal of Medicine.* 1990;89:142-146.
202. Baeten JM, Donnell D, Mugo NR, et al. Single-agent tenofovir versus combination emtricitabine plus tenofovir for pre-exposure prophylaxis for HIV-1 acquisition: an update of data from a randomised, double-blind, phase 3 trial. *Lancet Infect Dis.* 2014;14(11):1055-1064.

Appendix I: Enrollment Informed Consent for Clients

Informed Consent for Participants

Study Title: Point-of-care Urine Monitoring of Adherence (PUMA): Testing a Real-Time Urine Assay of Tenofovir in PrEP

Protocol version 6.0
November 11, 2021

INVESTIGATORS

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STUDY SUMMARY

Pre-exposure prophylaxis (PrEP) with oral tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) prevents HIV infection if taken every day. However, previous PrEP trials demonstrated that many women in Africa at risk for HIV who are not in couples (with an HIV-positive partner) need help remembering to take the pill every day. Also, patients on PrEP may not always report their adherence to the pill accurately and "objective" measures of adherence (where PrEP drug levels were measured) are far superior to self-report. We also know that telling a patient his/her PrEP drug levels can be motivating for better adherence in the future. The usual ways to measure PrEP drug levels are very complicated and require expensive equipment and trained laboratory staff. However, our group has now developed a new and innovative way to measure adherence to PrEP using a drug level (specifically tenofovir, TFV) via an antibody-based test ("immunoassay") in urine that can be performed cheaply at the point-of-care (POC) by any healthcare worker (like a urine pregnancy test). A few drops of the patient's urine is dropped into the test and tells the provider "yes" or "no" if the patient has been taking PrEP recently. This protocol is for a pilot trial where we randomize women on PrEP at Thika Clinic to having information from the investigational urine assay delivered to them (or not delivered to them in the control group) in order to provide feedback on adherence from this test in a supportive, motivating manner and enhance adherence

counseling. Women on PreP who are not in a couple with someone who has HIV (called a "serodiscordant couple", where one person has HIV and the other does not) will be enrolled. The pilot trial will assess if the urine tool is acceptable to participants, feasible for the clinic to perform, and if providing feedback on adherence from the urine tool to the participant increases future adherence to PrEP (measured by a longer-term adherence metric with hair levels). Good adherence to PrEP will eventually lead to fewer HIV infections worldwide.

INFORMED CONSENT

We are asking you to participate in a research study. This study is sponsored by the Kenya Medical Research Institute, the University of California, San Francisco (UCSF), the University of Washington, and funded by the National Institutes of Health (USA). If you decide to take part in this study you will be asked to sign this consent form or make your mark in front of a witness. We will give you a copy of this form. This consent form might contain some words that are unfamiliar to you. Please ask us to explain anything you may not understand. A description of this clinical trial will be available on http://www.ClinicalTrials.gov. NCT03935464

CLINICAL TRIALS WEBSITE

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

PURPOSE OF THE STUDY

Studies have shown that the medications used to treat HIV infection can also be used to prevent passing the virus. This concept is called pre-exposure prophylaxis, or PrEP. These studies have also learned that PrEP is safe (meaning that it does not produce significant health problems in persons who take them) when taken by HIV-uninfected men and women. You have now initiated PrEP to help protect you from HIV infection.

Many studies have shown that PrEP has to be taken regularly for it to be effective. It can sometimes be difficult to take PrEP regularly and support is provided at every visit to ask about adherence (or regular PrEP taking) when you are on PrEP. Studies have shown it can be useful for people to see a test that shows that the PrEP drug has entered your system after you take it. We have now developed a new assay that tests your urine to show you that you have taken PrEP recently – the test shows up as "positive" if you have taken PrEP recently. The purpose of this study is to assess the acceptability of this test to measure PrEP drug-taking at your visit. Through a method similar to tossing a coin, you will be assigned to a group that: (1) comes to the clinic every three months and gets PrEP by your health provider in the usual way; (2) comes to the clinic every three months and gets both PrEP and your urine tested for PrEP drug levels (specifically tenofovir) by a health provider. If you are in the second group, you will be shown your PrEP drug level test and you and your provider can talk about how to help you take the drug every

day if you need any support. Approximately, 100 - 120participants who are using PrEP will be enrolled in this study and will be in the study for up to 12 months.

YOUR PARTICIPATION IS VOLUNTARY (YOU MAY DECIDE NOT TO BE IN THE STUDY, OR TO LEAVE THE STUDY AT ANY TIME)

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

- You do not have to be in this study if you do not want to.
- You may decide not to be in the study, or to being in the study at any time, without losing your regular medical care.
- If you decide not to be in the study, you can still join other research studies later, if available and you qualify.
- You may be asked about joining other studies. Due to the time commitment from being in this study, you may not be eligible to join this study if you are in other studies. If you do not agree to join these other studies, you may still take part in this study.
- If you decide to enroll in this study, after your enrollment visit you will be in the study up to 12 months.
- The answers you provide to survey questions will remain confidential and identifying information about you will not be shared.

ENROLLMENT PROCEDURES

The enrollment tests for this study will include questions and tests done from samples of blood and urine. The enrollment procedures will include the following:

- The study staff will ask you where you live and other questions about you and your sexual practices.
- We will counsel you about HIV and other infections passed during sex, and how to avoid these infections including the use of condoms and other contraceptives. We will provide you with free condoms at your screening visit as well as at each visit throughout the study.
- Even if you have recently been tested for HIV, we will need to repeat the HIV test today as part of the study and part of the routine procedures to be on PrEP.
- We will ask you questions about your medical history, you will have a physical examination, and you will undergo an assessment for sexually transmitted infections (STIs)
- You will be asked to give us permission to obtain a urine sample for laboratory tests (although you will receive the results of the tenofovir urine assay only if the coin flip puts you in the arm of the study where you are given feedback on the test)
- You will have blood drawn which is use for both clinical care and for research tests in this study
- Additionally, along with urine, we will ask for a small hair sample (about 50-100 strands, less than you lose from your head each day). We will test for PrEP drug levels in the hair sample

at the end of the study. Your hair will not be stored in any way but destroyed after testing for the PrEP drug levels

- In this research study, you will come to the clinic every 3 months which is standard for anyone on PrEP in Kenya.
- Additionally, you will continue to be offered PrEP to be taken as one pill once each day to prevent HIV infection. The PrEP medication will be provided by this clinic, free of charge.
- If you agree to take part in the study, you will also have a physical examination. During the examination, the study nurse will check for any signs of infection or other illnesses. If you have an active illness that requires medical attention or treatment, then you will be referred to receive the care you need.

FOLLOW-UP PROCEDURES

- You will be asked questions about your health and medical history, including whether you have any clinical symptoms, your sexual practices and other behaviors, and your feelings about taking medications for HIV prevention.
- Risk counseling is part of every PrEP visit. We encourage you to talk with study staff about ways to avoid HIV and other infections passed during sex. You will be offered condoms.
- Get medical care or referrals for medical care and other services if you need them.
- Give updated information on where you live and how to keep in contact with you. The study staff will use this information to remind you of scheduled visits. If you miss a visit, the study staff will try to contact you by telephone. They may try to reach you through the contact people that you list. If they talk to these people, they will not tell them why they are trying to reach you.
- Women will be asked whether they think they may be pregnant. A urine pregnancy test may be done if unsure about being pregnant. Women who are pregnant will not be excluded from the study.
- If your HIV test is negative you can continue to receive PrEP medication. The pharmacy staff will provide you with new bottles with pills to last until your next visit.
- You will be asked to give us permission to obtain a urine sample for laboratory tests (although you will receive the results of the tenofovir urine assay only if the coin flip puts you in the arm of the study where you are given feedback on the test)
- You will have blood drawn which is use for both clinical care (for instance, to check that your kidneys are fine) and for research tests in this study
- Additionally, along with urine, we will ask for a small hair sample (about 50-100 strands, less than you lose from your head each day). We will test for PrEP drug levels in the hair sample at the end of the study. Your hair will not be stored in any way but destroyed after testing for the PrEP drug levels.

At any time in the study:

- If you or the study staff thinks you may have any health problems, you may need to undergo a physical exam and may need to provide blood or other samples for testing.
- You can have extra counseling and testing for HIV if needed between scheduled visits, either with your partner or by yourself.
- If you decide to leave the study before your last scheduled study visit, we will ask you to have a final study visit with all the exams and tests listed above.

Long interviews

At the end of the study for those of you who were in the arm of the study where you got feedback on the urine test, the staff may invite you to answer more questions about the urine test and how you liked it. Some interviews will be done individually and some will be done as a group session with other people who are participating in the study. At any point during interviews, participants (women taking PrEP) can decide to not answer any question they are uncomfortable answering and may choose to leave the interview or focus group sessions at any time. The interviews will be recorded and transcribed, with notes taken during the session, to make sure we remember everything you tell us (removing any mention of names or any identifying information). This sound recording will be stored in a secure lockable cabinet.

Pregnancy

If you become pregnant during this study you will be referred for antenatal care and continue in the study.

IF YOU BECOME HIV INFECTED

During the course of the study we will provide you with condoms, PrEP, and other materials to help prevent getting HIV. However, it is possible to become HIV infected on PrEP although this is very rare if people take their HIV medications regularly.

If you have a positive HIV test during the study:

- The study staff will talk with you about this test result and what this means for you.
- You will stop the taking the PrEP medication
- You will then be referred to an HIV provider who will confirm the positive test result and start HIV care. You will not be in this urine test study anymore.

SPECIMEN STORAGE AND USE OF SAMPLES AND DATA FOR FUTURE STUDIES

The hair samples will be shipped to UCSF for testing of tenofovir levels and then destroyed. The urine samples will be tested at the Thika Clinic and then destroyed (if you are in the part of the study where you will see your urine test, that urine test will be destroyed at the end of the study). Some of the blood taken at study visits will beretained and stored in Thika clinic for future research by us and by other researchers. We will use these samples only for research related to analyzing PrEP medication levels in blood samples. A total of 60 milliliters (4 tablespoons) of blood will be

collected over the course of entire study from you. Before your samples leave the clinic, they will be assigned a code and your name will not be on them. Your name will be linked to the code only at this clinic and only for five years after the study is completed. After that time, the link between your name and the code on your data and samples will be destroyed. An Institutional Review Board or Independent Ethics Committee, which watches over the safety and rights of research participants, must approve any future research studies using your data and samples. If you agree to store your samples, we will keep them for as long as sample remains that can be used for future research. If you do not want to have your samples saved for future research, you can still be in this study and your samples will be destroyed once testing for this study is completed. If you agree to store your samples now, but change your mind before the end of the study, let the study staff know and we will make sure that your samples do not get stored for future research. We will not sell your data or samples. Tests done on your samples may lead to a new invention or discovery. We have no plans to share any money or other benefits resulting from this invention or discovery with you. Future research from this study will never involve genetic sequencing (called “whole genome sequencing”).

PROCEDURE and RISKS

PROCEDURE

This study will involve looking at a new urine assay for adherence and blood draws for research (and also clinical care). Please note that this urine assay is being tested during this study for its utility and is therefore still considered an “investigational” test.

RISKS

You may feel discomfort or pain when your blood is drawn. You may feel dizzy or faint. You may have a bruise where the needle goes into your arm. There is a small risk of bleeding or infection after the blood draw. You may feel slight pulling at your scalp when your hair sample is cut.

The study staff will make every effort to protect your privacy and confidentiality during the entire study period. However, it is possible that others may learn of your participation and as a result, may treat you unfairly or discriminate against you. For example, you could have problems getting or keeping a job, or being accepted by your family or community.

You may become embarrassed, worried, or anxious when talking about your sexual practices, ways to protect against HIV and other infections transmitted during sex, and your HIV test results in the course of usual clinical care on PrEP. You may become worried or anxious while waiting for your test results. If you become infected with HIV, knowing this could make you worried or anxious. Trained counselors will help you deal with any feelings or questions you may have.

You may feel embarrassed, worried, or anxious when talking about your experiences with PrEP adherence testing using the urine assay. Counselors are available through the study to help you deal with any feelings or questions you have. The study staff will make every effort to protect your privacy and confidentiality during the interviews or group discussions. During group discussions, all participants will be reminded for the need for confidentiality, however, group discussions will not be fully confidential since all members of the group discussion will hear the discussions.

For more information about risks of this study, ask the study provider.

BENEFITS

Since this is a pilot study, there are no direct benefits anticipated from the study intervention. PrEP adherence testing using this new urine assay has the potential of improving adherence to PrEP, which will then lower people's risk of becoming infected with HIV. Your participation in this study will contribute to understanding about PrEP adherence testing and counseling in Kenya, which may help others in the future.

NEW FINDINGS

You will be told any new information learned during this study that is important for your health or might cause you to change your mind about staying in the study. You will be told when the results of the study may be available, and how to learn about them.

COSTS TO YOU

There is no cost to you for being in this study. Treatments available to you from the study will be given free of charge.

REASONS WHY YOU MAY BE WITHDRAWN FROM THE STUDY

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

- The study is stopped or canceled.
- The study staff feel that staying in the study would be harmful to you.

ALTERNATIVES TO PARTICIPATION

You can always receive PrEP through the government program even if you are not in this urine assay study. There may be other studies going on here or in the community that you may be eligible for. If you wish, we will tell you about other studies that we know about.

REIMBURSEMENT

You will receive a transportation reimbursement and an additional 200 Kenyan shillings for your time and effort.

CONFIDENTIALITY

Efforts will be made to keep your personal information confidential. However, absolute confidentiality cannot be guaranteed. Your personal information may be disclosed if required by law. Any sample from you or information about you will be identified only by code. The link between your name and code will be kept in a secure location at the clinic only. Any publication of this study will not use your name or identify you personally.

Your study records may be reviewed by study staff and representatives of:

- The University of California, San Francisco
- The University of Washington, including study monitors
- The United States National Institutes of Health
- Kenya Medical Research Institute (KEMRI)
- US Office for Human Research Protections.
- Pharmacy and Poisons Board (PPB-ECCT)
- National Commission for Science, Technology and Innovation (NACOSTI)

In addition, other local, US, and international regulatory entities may review participant records. This research is covered by a Certificate of Confidentiality from the National Institutes of Health. This means that the researchers cannot release or use information, documents, or samples that may identify you in any action or suit unless you say it is okay. They also cannot provide them as evidence unless you have agreed. This protection includes federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The Certificate of Confidentiality does not stop you from willingly releasing information about your involvement in this research. It also does not prevent you from having access to your own information.

RESEARCH-RELATED INJURY

The study staff will monitor your health while you are in this study. If you have any health problems between visits, or if you have a medical emergency that requires immediate care, please contact the study staff.

If you are injured from participating in this study, you will be offered care at the study clinic, free of charge. It is important that you tell the members of the team of researchers at this clinic if you feel that you have been injured because of taking part in this study. There is not a program of monetary compensation through this institution. If you require medical care that the study clinic cannot provide, the study doctors will refer you to the appropriate services or organizations that can provide care for the injury. You do not give up any legal rights by signing this consent form.

The U.S. National Institutes of Health (NIH) does not have a mechanism to provide direct compensation for research related injury.

PROBLEMS OR QUESTIONS

If you ever have any questions about this study, you should contact Dr. Kenneth Ngure at the Thika Study clinic at Tel 06721305/2222561. For research related injury, please call the 24-hour emergency number: 0736464299. If you have questions about your rights as a participant, contact the secretary of the KEMRI Scientific and Ethics Review Unit, P.O. Box 54840-00200, Nairobi, Telephone number 020272-2541, 0722205901, 0733-400003. Email address: seru@kemri.org.

STATEMENT OF CONSENT AND SIGNATURES

I have read this form or had it read to me. I have discussed the information with study staff. My questions have been answered. I understand that my decision whether or not to take part in the study is voluntary. I understand that if I decide to join the study I may withdraw at any time. By signing this form, I do not give up any rights that I have as a research participant.

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

SPECIMEN STORAGE AND USE OF YOUR DATA AND SAMPLES FOR FUTURE STUDIES

Please initial and date one option:

_____ I DO agree to store my data and samples for future research into PrEP adherence

_____ I DO NOT agree to store my data and samples for future research into PrEP adherence

With regard to shipment of my specimens/information to UCSF, in USA (Please initial and date one option)

_____ I DO agree to allow my biological samples to be shipped to UCSF for specialized tests (tenofovir levels in hair) and used for future research tests

_____I DO NOT agree to allow my biological samples to be shipped to UCSF for specialized tests and used for future research tests

With regard to giving permission for audio recording during long interviews (Please initial and date one option)

_____ I DO agree to audio recording during the qualitative interviews

_____I DO NOT agree to audio recording during the qualitative interviews

With regard to giving permission to have my fingerprint taken for purposes of biometric identification (Please initial and date one option)

_____I DO agree to have my finger print taken

_____I DO NOT agree to have my fingerprint taken

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

Appendix II: CONSENT FORM FOR INTERVIEWS OF HEALTH CARE PROVIDERS

Study Title: Point-of-care Urine Monitoring of Adherence (PUMA): Testing a Real-Time Urine Assay of Tenofovir in PrEP

Protocol version 6.0
November 11, 2021

INVESTIGATORS

Investigator	Title	Institution	Telephone
Kenneth Ngure	MPH, PhD	Jomo Kenyatta University of Agriculture and Technology	067 22561
Nelly Mugo	MBChB, MMed, MPH	Kenya Medical Research Institute	020 2736744
Monica Gandhi	MD	University of California, San Francisco	001 415-260-6709
Jared Baeten	MD, PhD	University of Washington	001 206-520-3808

24 HOUR EMERGENCY TELEPHONE NUMBER: Tel: 0736464299

Study overview

Researchers at the University of California, San Francisco and the Kenya Medical Research Institute (KEMRI) are conducting a study to understand what health care providers think about a new test to monitor PrEP adherence during clinic visits. You are being asked to take part in this study because you are a health care provider who works with PrEP clients at the Thika clinic and you contributed to the pilot trial where women on PrEP were provided with urine tenofovir adherence assay results. Please note that the utility of this new urine assay is being examined in this study and the urine assay is still considered an investigational tool. Researchers from KEMRI are now conducting in-depth interviews with PrEP providers at the Thika clinic at the end of the PUMA trial in order to understand their perceptions of this new test.

The study interviewer will explain the study to you. Research studies include only people who choose to take part. You are not required to take part in the research study. However, your participation is appreciated as it will contribute to the development of an intervention that may providers help their patients adhere to PrEP. Please take your time to make your decision about participating.

Why is this study being done?

The purpose of this study is to understand what providers think about a new point-of-care test that can be used to monitor PrEP adherence during clinic visits. We are interested in learning what providers think of this test after seeing it being administered during the PUMA pilot trial, how they would use this test in their practice, and whether they think it would help clients improve their PrEP adherence. This information will be used to address providers' concerns and preferences as this test is rolled out.

How many people will take part in this study?

Approximately 8 health care providers at the Thika clinic, will participate in this study.

Your participation is voluntary

Before you learn about the questions you will be asked in this study, it is important that you know the following:

- You do not have to be in this study if you do not want to.
- If you choose not to be in this study, it will not affect your work at Thika clinic.
- The answers you provide will remain confidential and identifying information about you will not be shared.
- You may decide not to answer the questions, or to stop the questions at any time, without penalty.
- If you decide not to answer the questions, you can still provide clinical guidance to our research studies later, if available and if you qualify.

What will happen if I take part in this research study?

IN-DEPTH INTERVIEW:

- The interview should last no more than 30-60 minutes and will take place in a private space at the Thika clinic.
- The interviewer will audio record the interview and take notes to make sure we remember and understand everything you tell us. After the interview, someone will transcribe the recorded interview and remove any mention of names or other identifying information. The recordings will be stored under locked conditions accordance to the DAIDS Policy on Storage and Retention of Clinical Research Records policy, following the strictest of any applicable laws, regulations, policies or other requirements for record retention.
- The interviewer will show you a picture of the new test that was used to measure PrEP adherence in the study and remind you how it can be used to provide women with feedback on their adherence during their regular clinic visits. You will have seen this test during the study. The test tells you if a patient took her PrEP recently (yes/no). The interviewer will ask for your thoughts about this test. Specifically, we are interested in knowing what you think about the test itself, whether you thought it helped clients on PrEP adhere to their medication, and how you might use it in your own practice in the future.

What side effects or risks can I expect from being in the study?

You may feel embarrassed, worried, or anxious when talking about your opinions about this new test to the study staff. The study staff will make every effort to protect your privacy and confidentiality while you are answering the questions

What are the benefits to taking part in this study?

There will be no direct benefit to you from participating in this study. However, the information you provide will help inform the development of an intervention that may improve clients' adherence to PrEP and help them protect their health.

What are the costs of taking part in this study?

There are no costs associated with participation in this study and there is no payment for participation in the study.

Will information about me be kept private?

We will do our best to make sure that the personal information gathered for this study is kept private. The KEMRI study team will conduct the interviews. The researchers will keep information about you as confidential as possible, but complete confidentiality cannot be guaranteed. On rare occasions, your personal information may be disclosed if required by law. Also, sometimes the law makes us report certain things to appropriate authorities, especially if we learn that you plan to hurt yourself or others. In that case, we have to do what the law says. While our questions may not ask you specifically about those things, please keep this is mind as you choose what to share with us.

We will share data collected during this study in aggregate form only, and any data shared will not include your name or identifying information. Your answers will have no impact on your work at Thika clinic. All of the information you provide will be kept in a locked cabinet (for papers) or a password-protected and encrypted computer folder (for electronic files). Your name will not appear on any interview materials. Instead, we will keep track of your information with a unique numbered code. The link between your name and this code will be destroyed approximately five years after the study is completed. The transcript of your interview/group discussion will not have your name on it and will be used only in analysis together with all the other transcripts.

Your responses to the questions may be reviewed by study staff and representatives of:

- The University of California, San Francisco
- The University of Washington, including study monitors
- The United States National Institutes of Health
- Kenya Medical Research Institute (KEMRI)
- US Office for Human Research Protections.
- Pharmacy and Poisons Board (PPB-ECCT)

- National Commission for Science, Technology and Innovation (NACOSTI)

We plan to apply the lessons we learn from the interviews to the development of an intervention to improve PrEP adherence among clients at risk for HIV. We will also share the lessons we learn at conferences and meetings, as well as in written articles. We will report results about the group as a whole and never about you individually. Your identifying information or the identifying information of any other participant will never appear in any of these materials.

Who can answer my questions about this study?

If you ever have any questions about this study, you should contact Kenneth Ngure at the Thika Partners in Health, Research and Development clinic at Tel 06721305/2222561.

If you have questions about your rights as a research participant, you should contact the Secretary of the KEMRI Ethics Review Committee, P. O. Box 54840-00200, Nairobi. Telephone number: 020272-2541, 0717719477, 0722-205901, 0733-400003. Email address: seru@kemri.org

STATEMENT OF CONSENT AND SIGNATURES

I have read this consent form or had it read to me. I have discussed the information with study staff. My questions have been answered. I understand that my decision whether or not to take part in this study is voluntary. I understand that if I decide to answer the questions, I may withdraw at any time. By signing this form, I do not give up any rights that I have as a research participant.

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

PERMISSION FOR AUDIO RECORDING

(Please check the appropriate response below)

□ I give permission for the long interview and group discussions that I will participate in to be recorded.

□ I do NOT give permission for the long interview and group discussions that I will participate in to be recorded.

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

KENYA MEDICAL RESEARCH INSTITUTE ETHICS REVIEW COMMITTEE
P.O.BOX 54840-00200
NAIROBI
TELEPHONE 2722541

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO HUMAN SUBJECTS DIVISION
3333 CALIFORNIA ST., SUITE 315
SAN FRANCISCO, CA, 94143
BOX 0962
TELEPHONE 011-1-415-476-1814

PERMISSION FOR AUDIO RECORDING

(Please check the appropriate response below)

□ I give permission for the long interview and group discussions that I will participate in to be recorded.

□ I do NOT give permission for the long interview and group discussions that I will participate in to be recorded.

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

KENYA MEDICAL RESEARCH INSTITUTE ETHICS REVIEW COMMITTEE
P.O.BOX 54840-00200
NAIROBI
TELEPHONE 2722541

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO HUMAN SUBJECTS DIVISION
3333 CALIFORNIA ST., SUITE 315
SAN FRANCISCO, CA, 94143
BOX 0962
TELEPHONE 011-1-415-476-1814

Appendix III: QUALITATIVE GUIDE FOR IN-DEPTH INTERVIEWS FOR WOMEN

Qualitative component: Exploring HIV adherence testing experiences among HIV-1 uninfected women.

***Target group*:** Participants enrolled in the intervention arm of the PUMA Study at Thika.

***Objectives*:**

1. Learn opinions of HIV-uninfected participants on PrEP adherence testing.
2. Gain an understanding of how participants performed the PrEP adherence testing including any challenges experienced.

Approach:

Focus group discussions will consist of HIV-uninfected women not in serodiscordant relationships; Four focus group discussions of 5-10 participants each will be conducted.

INTRODUCTION

What kinds of PrEP adherence tests are you aware of?

Probe:

- Clinic based PrEP adherence testing?
- How is PrEP adherence testing performed?

Topic 1: **Acceptability**

What do you think about Urine testing for PrEP drugs? What are the advantages? What are the disadvantages?

Possible probes:

- What would be the advantages or benefits?
- What would be the challenges or disadvantages?
- Are there any reasons that would make you not perform a test?
- Possible concerns with urine testing for PrEP drugs?

Topic 2: **Usability**

How was your experience with urine drug-level testing?

Possible probes:

- What challenges did you experience in performing urine drug-level testing?
- Have you shared your results with others?
- Have you used the 24 hour helpline?

Topic 3: **PrEP adherence testing preferences**

What would you prefer urine drug testing at home or clinic-based testing?

Possible probes:

- What would you prefer, adherence testing at home or clinic based testing? What are your reasons?
- What are the things that would make you prefer PrEP adherence testing?
- How did you find the every 3 month testing schedule?

Topic 4: **Closing discussion**

Possible probes:

- When people test for adherence, what are some ways to help them have better PrEP adherence?
- Probe about helping via phone calls, packaging inserts that could provide helpful messages, what kind of preparation should people have before they have adherence testing to prepare for the results, etc.
- What would make it easier for you or others to perform adherence testing?
- Do you like the test that says yes/no on adherence or would you prefer a test that gives you an idea of high, moderate or low adherence?
- Any other thoughts or suggestions on adherence testing that you would like to share?

Appendix IV: Focus Group Discussion Guide for Clients

Introduction

Discussion leader will introduce herself and the moderator [and anyone else present from the study team] to the group.

Thank you for coming today to participate in this focus group. We have asked you to participate in this discussion because you are currently taking PrEP for the prevention of HIV. We're conducting this focus group to get your input on a point-of-care test that measures a person's adherence to PrEP by measuring the amount of tenofovir found in the person's blood, urine, or saliva. Tenofovir is one of the drugs found in your PrEP. The goal of this focus group is to understand your feelings about this type of test.

We really appreciate you taking the time to talk with us today. The focus group should take about 1 hour. We are going to audio record the conversation in order to capture all the details and summarize them later on. For that reason, please do not use your name, other people's names, or organization names, including clinic names, to ensure we maintain the confidentiality of the focus group. We will also be taking notes during the discussion to use as a reference. After we've transcribed the focus group discussion, we will delete the audio recording. Please remember that participation in the focus group is completely voluntary, and you may decide to not answer any question as you wish, and you can leave at any time.

Please feel free to think out loud and answer questions as honestly as possible. I encourage all of you to participate in the conversation. And remember that there are no right or wrong answers.

Are there any questions before we begin?
Do I have everyone's permission to record this discussion? *[If everyone answers yes, turn on audio recorder.]*
Does anyone *not* want to participate? If you would like to leave, please do so at this time.

Discussion Guide

1. Has your provider ever talked to you about the need to take your PrEP regularly? Do you ever bring it up? How are those conversations?
2. What's it like to answer questions about taking PrEP? Do you think your answers are pretty accurate? What are some reasons why you might not tell the truth?
3. What are some of the strategies your provider uses in the clinic to monitor or assess your adherence to your PrEP? What are the strengths and weaknesses of the existing strategies?

4. What happens in the clinic when your provider learns that your adherence is less than perfect?

Now, I'd like to give you a little more information about the test that we'll be discussing today. *[Project image of test strips onto screen]* This is a simple screening test that would measure whether a person has enough tenofovir in their system that would meet the threshold for protection from acquiring HIV. The test would just indicate a positive or negative result, indicating that a person is adherent to their medication or *not* adherent to their medication. The test strips shown on the screen are an example of what the test would look like. They would be used on urine samples, and results would appear within 5 minutes. Does everyone understand how this test would work before we continue?
[Ensure the group understands how the test works before moving on.]

5. What are your initial thoughts about a test like this?
6. Would you want to use this test if it were optional and free at your clinic? Would you want it to be part of your care? If yes, how would it fit in with care for you or others on PrEP? What if your doctor asked you to use it? Some of you had this testing performed and how did you like it or feel about it?
7. How would you feel about using this test on your own (for example, in your home or another private location) instead of using it in the clinic with your provider present? Think about the differences between the two in terms of convenience, privacy, client-provider relationship, effects on your mental health, etc. If you were to use this test on your own, under what circumstances would you use it (e.g., when and why would you use it?)?
8. Do you think there would be any benefits or negative effects if your doctor tested you for adherence as a part of routine care? If yes, what are they and why?
9. Do you think there are some types of clients who might benefit more from this type of test? If yes, what types? Are there some types of clients who might be negatively affected by being tested for adherence? If yes, what types?
10. If you're interested in being tested for adherence, how frequently would you want to be tested? Why?
11. If you were tested and your results showed that you had low adherence, how would it affect your pill-taking behavior and your relationship with your provider? How do you think it would affect your mental health?
    [Probe: Think about both the short-term and the long-term effects on your behavior and your relationship.]
12. Overall, what are your final thoughts on being tested for adherence?
13. Do you like this yes/no test for adherence or would you want a test that tells you if you have had high, medium or low adherence?
14. Do you have any other concerns or thoughts about this test that we have not yet discussed?

Closure

Discussion leader will close the focus group around 55 to 60 minutes after it began. She will ask for any further points from the participants that were not discussed.

Finally, the discussion leader will conclude the focus group and thank the participants for their time.

Appendix V: Qualitative Guide for In-Depth Interviews (IDIs) for Providers

Introduction

[~5 minutes]

Interviewer will introduce herself to the provider.

Thank you for coming today to participate in this interview. We have asked you to participate in this discussion because you provide care and treatment services for individuals who are at higher risk for acquiring HIV. We're conducting this interview to get your input on a point-of-care test that we used in the PUMA study. As you know, this test can measure a person's adherence to pre-exposure prophylaxis (PrEP) by measuring whether tenofovir is found in the person's urine. The goal of this interview is to understand your feelings about this test and to discover potential applications and use cases for this test.

We really appreciate you taking the time to talk with us today. The interview should take about 30-60 minutes. We are going to audio record the conversation in order to capture all the details and summarize them later on. For that reason, please do not use your name, other people's names, or organization names to ensure we maintain the confidentiality of the interview. We will also be taking notes during the discussion to use as a reference. After we've transcribed the interview, we will delete the audio recording. Please remember that participation in the interview is completely voluntary, and you may decide to not answer any question as you wish, and you can leave at any time.

Please feel free to think out loud and answer questions as honestly as possible. And remember that there are no right or wrong answers.

Are there any questions before we begin?
Do I have your permission to record this discussion? *[If provider answers yes, turn on audio recorder.]*

Discussion Guide

1. To begin, please tell me about your role in the PrEP program.
   Probes:
   a. What do you do on a day-to-day basis?
   b. How do you interact with patients on PrEP?
2. How long have you been in this role? (years/months)
3. How do you monitor or assess PrEP adherence at Thika clinic? If at all?
4. How easy or difficult is PrEP adherence for your PrEP clients?
   a. Would you say non-adherence is common?

5. How comfortable are women in reporting adherence challenges with PrEP to you or other providers in the clinic? Please explain.
6. Does the clinic provide counsel or support around PrEP adherence- trying to take PrEP every day? Please tell me about that.
    a. Please give some examples of the support services are provided. (For example, do providers spend time counselling patients on the importance of taking PrEP every day? If so, how often is this done?)
    b. What do you think about the quality of support provided to PrEP clients to improve their adherence? Is it helpful? Are clients responsive to this support?

II. <u>Present the urine assay strip picture</u>

[Interviewer to bring out the rapid strip urine picture below and show it to the provider]

Interviewer: *This is a new test that was developed that can be used to measure people's adherence to PrEP during their clinic visit. We used this in the PUMA study and half of the clients received counseling based on the results of this test and half of the clients received standard-of care counseling.*

You saw that all the client has to do was to provide a urine sample, and the provider then dips this strip into the urine sample (up to the test line indicated) and the strip will tell the provider the results at that moment (within about 5 minutes).

The strip tells the provider whether the client has taken their PrEP drugs (yes/no) in the last 4 days. A colored line at the top of the test when dipped into urine means no (has NOT taken PrEP drugs in the last 3 days) and no colored line means YES (has taken PrEP drugs in the past 3 days).

- *If the test shows no colored line, it means that person has taken PrEP in the past 4 days (YES)*
- *If the test shows a colored line, this means that the person hasn't been able to take their PrEP pills for a while (NO)*
- *Please note that this is the <u>opposite of other rapid tests</u> that we are familiar with, such as pregnancy tests.*

III. <u>Elicit feedback about the test</u>

General thoughts on clarity of test: *Now I'm going to ask you about what you thought about this new test.*

1. What were you first thoughts about this test?
    a. What do you like about it?
    b. What do you dislike about it?
2. How clear were the results to you? Or was it confusing? What makes these results clear or confusing?

Applications of PrEP Adherence Testing

1. Do you think there would be any benefits or negative effects of using this test on your HIV-negative clients? If yes, what are they and why?
2. Would you consider using this test on all of your HIV-negative clients or just a subset? If just a subset, which ones and why? *[Probe: If you think the test would be best for only a subset, how would you minimize stigmatization of that subset of clients?]*
3. For the HIV-negative clients whom you would use this test on, how frequently would you test them and why?
4. If you received test results for an HIV-negative client that showed less than perfect PrEP adherence, how would you use the results to support your clinical decision making? Would you do anything differently than what you are currently doing for poor adherence? Why?

Feasibility and Acceptability of PrEP Adherence Testing

5. Do you believe implementing this type of test would be feasible in your clinic or in another setting? Why or why not?
6. Do you think providers need training to deliver these test results? Why/why not?
7. How do you think this test could be implemented? That is, who would do the test? Where would the test be done? Who would give the results? When would the results be given?

IV. <u>Conclusion</u>

8. We have reached the end of our interview. Do you have any other concerns or thoughts about this test that we have not yet discussed? Is there anything that we didn't ask that we should have asked?

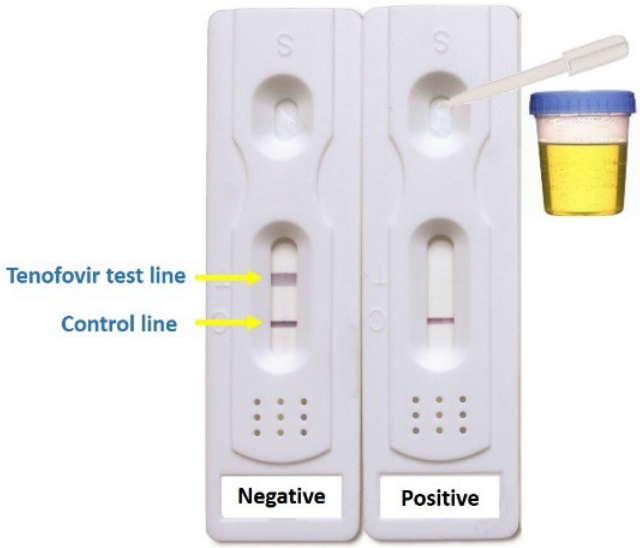
[Interviewer]: *Thank you again for participating in our study!*

Appendix VI: SOP for Performing and Interpreting Urine Tenofovir Adherence Assay

SOP for Performing and Interpreting Urine Tenofovir Adherence Assay

1. Present client with a cup and ask them to please provide a urine sample. Of note, there is no need to clean the urethra prior to urine collection. The client can just urinate into the cup (about 5-10mL). Please ask them to urinate into the toilet if they have excess urine after filling the cup to the line.
2. Please ask the client to screw the lid on to the urine cup after collection and bring it back out to the provider.
3. The provider (using gloves) should use the dropper provided with the urine test to drip 3-4 drops of urine from the urine cup into the divot of the urine test strip (indicated by blue arrow on below picture).
4. After putting the urine drops on the urine strip test, please lay it on a paper towel and let it develop for 3-5 minutes
5. Please take the sheet provided (table shown below) and write in the participant ID in the first column.
6. The control line should turn positive no matter what the adherence line shows. Please mark the sheet provided in the second column with a "Y" for yes if the control line turns positive. If the control line does not turn positive, please mark "N" for no and use another test.

Urine rapid strip assay picture



Participant ID number	Dark control line present (Y/N)	Test line present (Y/N)

7. The test line will appear if the client is NOT taking his/her PrEP and be absent (not appear) if the client is taking his/her PrEP. Please mark the sheet provided in the third column with a "Y" for yes if the test line turns positive. If the control line does not turn positive, please mark "N" for no.
8. Please take a picture of the rapid strip test with the participant ID label next to it so that we can record the results for the future. (Pictures for QC will be eventually emailed to the UCSF Hair Analytical Laboratory at Karen.kuncze@ucsf.edu).
9. Please discard the urine test strip after marking the sheet and taking the picture

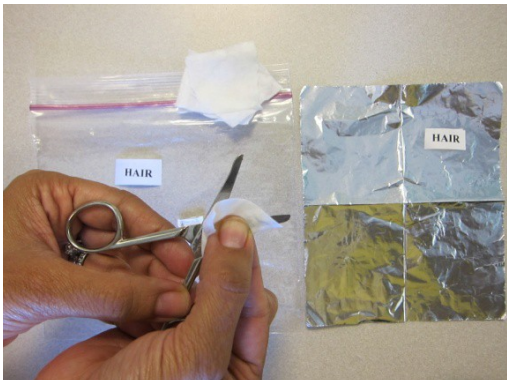
Appendix VII: SOP for Collection and Storage of Hair Samples

Materials: Scissors, alcohol wipe, piece of aluminum foil (folded), tape, participant ID labels, and Ziplock bag (or any sealable plastic bag).

Video demonstration on how to collect hair for study:
Collecting 50-100 hairs, http://www.youtube.com/watch?v=F1Fd0b2IlaQ

1. Ready your scissors, the hair collection packet (aluminum foil envelope inside Ziplock bag), the thin piece of tape for labeling the hair sample, and the participant ID label for outside the envelope.
2. Clean the scissor blades using the alcohol wipe prior to each use and allow the blades to completely dry.
3. Lift up the top layer of hair from the occipital region of the scalp (back of the scalp). You may want to use a clip to hold up the top layer of hair.
   NOTE: Hair samples from other parts of the body cannot be used.
4. Isolate a thatch of hair (approximately 100 strands of hair) from *underneath* this top layer of hair from the occipital region with your fingers.
5. Cut the hair sample off the subject's head *as close to the scalp as possible.*
6. Unfold the piece of aluminum foil and place the cut thatch of hair inside the piece of foil keeping your fingers over the distal end of the hair sample so that you know which end to label (the distal end is the end farthest from the scalp).
7. Use tape or a thin label to secure the DISTAL end of the hair thatch to the inside of the aluminum foil, leaving the proximal end free (the proximal end is the end closest to the scalp).
   NOTE: Please make sure the label is on the very edge of the hair, leaving most of the hair sample free.
   NOTE: Thin hair and short hair may become dislodged. You may choose to mark the distal end of the hair with nail polish or white-out as an extra precaution if you think the hair might be dislodged. Or a hairpin to pin down the distal end of the hair. It is very important to know which side is which for the laboratory (which side is farthest from the scalp and which side is closest) so labeling is important when possible!
   NOTE: If the distal end of the hair sample cannot be labeled, such as if the hair is very short and/or curly, then let the hair fall directly into the piece of foil and no need to label the distal end.
8. Refold the foil over to completely enclose the thatch of hair.
9. Place a participant label on the outside of the folded piece of foil or a label with the patient's ID number, date of collection and study name.
10. Place the folded piece of foil inside the Ziplock bag and seal the bag.
11. Place another participant ID label on the outside of the Ziploc bag.
12. Pictures of the hair collection process for different styles of hair are shown below.
13. Hair samples should be stored at room temperature and in a dark place at each processing lab prior to shipment to the testing lab.

Materials required: Scissors, piece of tin foil, patient labels (2), Ziplock bag, and alcohol swabs.
Suggest making these "hair kits" ahead of time.



Step 1: Clean the blades of a pair of scissors with an alcohol pad and allow blades to completely dry.
Clean off blades of scissors between patients.

Step 2: Lift up the top layer of hair from the occipital region of the scalp. Isolate a small thatch of hair (~50-100 fibers of hair) from underneath this top layer.
Can use hair clip to keep top layer of hair away if easier. Can also cut from more than one place if the participant would like that, although 50-100 strands of hair is not cosmetically noticeable.

Step 3: Cut the small hair sample as close to the scalp as possible

STRAIGHT HAIR

CURLY HAIR

SHORT HAIR

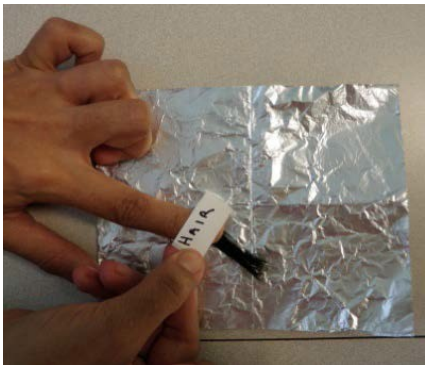
Can let hair fall directly into piece of tin foil when very short/cropped (no need to label end since too short)

BRAIDED HAIR

Cut hair thatch from in-between braids or dread locks

Step 4: Keep your fingers on the part of the hair that was FURTHEST away from the scalp and put the hair sample down on an unfolded piece of tin foil.

Step 5: Put a thin label over the end of the hair sample that was FURTHEST away from the scalp.
If hair very short just let it fall into the piece of tin foil and no need to label the distal end. Make sure the label is thin so you don't cover the hair sample and put it at the very end.



Step 6: Refold the foil over to completely enclose the hair and place a participant D label on the folded piece of foil

Step 7: Place the folded piece of foil inside the plastic (e.g. Ziplock) bag and seal the bag. Put another patient ID label on the outside of the bag

Appendix VIII: FDA DESIGNATION OF THE URINE TENOFOVIR ASSAY AS NSR

From: Kelm, Kellie <Kellie.Kelm@fda.hhs.gov>
Sent: Thursday, October 3, 2019 6:36 PM
To: 'Paul K. Drain' pkdrain@uw.edu; Morton, Tia M (NIH) <frazierti@niaid.nih.gov>; Gandhi, Monica <Monica.Gandhi@ucsf.edu>; Zhang, Hao (NIH) <hazhang@niaid.nih.gov>; Andrade, Adriana S (NIH) <adriana.andrade@nih.gov>; Lin, Leyi (NIH) <leyi.lin@nih.gov>
Subject: Approval for NSR designation for urine test by FDA

Dr. Gandhi

Since the therapies that will be given are approved, given at approved doses and the adherent patients get standard adherence counseling and the non-adherent patients get enhanced adherence counseling with positive messaging, the impact of a false negative and false positive are minimal. We would agree these studies using an investigational device appear to be non-significant risk.

Good luck on your studies, and if you're interested in distributing the device in the US, please look into our free pre-submission program where we can give you feedback on regulatory pathway, and clinical and analytical study protocols.

Regards,
Kellie Kelm

Kellie B. Kelm, Ph.D.
Acting Director
Division of Chemistry and Toxicology Devices | OHT7: Office of In Vitro Diagnostics and Radiological Health
Office of Product Evaluation and Quality
CDRH | Food and Drug Administration
White Oak, Bldg. 66, Rm. 3512 |10903 New Hampshire Avenue | Silver Spring, MD 20993
Tel: 301-796-6145
FDA U.S. FOOD & DRUG ADMINISTRATION

APPENDIX IX: KEMRI IRB APPROVAL OF PUMA TRIAL DATED 10/16/19

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel: (254) (020) 2722541, 2713349, 0722-205901, 0733-400003, Fax: (254) (020) 2720030
Email: director@kemri.org, info@kemri.org, Website. www.kemri.org

RECEIVED
25 OCT 2019

KEMRI/RES/7/3/1 **October 16, 2019**

TO: **DR. NELLY MUGO**
PRINCIPAL INVESTIGATOR

THROUGH: **THE DIRECTOR, CCR**
NAIROBI

22/10/2019

Dear Madam,

Re: **KEMRI/SERU/CCR/0126/3921** ***(RESUBMISSION OF INITIAL SUBMISSION)*****: POINT-OF-CARE URINE MONITORING OF ADHERENCE (PUMA): TESTING A REAL-TIME URINE ASSAY OF TENOFOVIR IN PREP. PILOT TRIAL TO EXAMINE THE FEASIBILITY, ACCEPTABILITY AND IMPACT OF AN INTERVENTION USING A NEW URINE-BASED TENOFOVIR ADHERENCE ASSAY.** ***(VERSION 3.0 DATED SEPTEMBER 23, 2019)***

Reference is made to your letter dated October 01, 2019. The KEMRI Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised study documents on October 04, 2019.

This is to inform you that the issues raised during the 290th Committee C meeting of the KEMRI Scientific and Ethics Review Unit (SERU) held on **August 29, 2019** have been adequately addressed.

Consequently, the study is granted approval for implementation effective this day, **October 16, 2019** for a period of **one (1) year**. Please note that authorization to conduct this study will automatically expire on **October 15, 2020.** If you plan to continue with data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **September 03, 2020**.

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise SERU when the study is completed or discontinued.