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REVIEW ARTICLE

Tenofovir Urine Assay to Monitor Adherence to HIV Pre-exposure Prophylaxis

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Abstract: Tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide (TAF) are prodrugs of tenofovir and have excellent long-term efficacy and tolerability for the treatment of HIV. An objective marker of adherence to tenofovir-based therapy could be clinically useful in supporting adherence to TDF-based HIV pre-exposure prophylaxis (PrEP) in populations in whom, self-report has been shown to be unreliable, and could play a role in resource-limited settings to support HIV and hepatitis B treatment adherence. A semi-quantitative high-performance liquid chromatography-mass spectrometry method for tenofovir quantification of urine samples was developed. This assay detects tenofovir concentration in $\log_{10}$ levels between 1 and 10,000 ng/mL, and was shown to distinguish between recent adherence and low/non-adherence to both TDF and TAF, with a concentration of >1000 ng/mL, highly predictive of medication ingestion in the last 24-48 hours. This assay was validated relative to other markers of adherence including dried blood spot and self-report in a highly adherent population of PrEP patients, and tenofovir was shown to be stable at room temperature in urine for at least 14 days. The assay was successfully used in a clinical setting to maintain high PrEP adherence and retention in care of 50 young men who have sex with me (MSM) over 48 weeks, to assess PrEP adherence in youth with mental health conditions, and to monitor drug levels relative to plasma levels in a case study of chewed TDF/FTC (tenofovir/emtricitabine) for PrEP. Further studies are underway to implement the tenofovir urine assay to monitor adherence and pre-exposure prophylaxis, nationally and internationally.

Keywords: Tenofovir disoproxil fumarate (TDF), tenofovir alafenamide (TAF), HIV, pre-exposure prophylaxis (PrEP), liquid chromatography-mass spectrometry, urine samples.

1. INTRODUCTION

Pre-exposure prophylaxis (PrEP) has a great potential to reduce the incidence of new HIV infections. Consistent use of tenofovir/emtricitabine (TDF/FTC) as PrEP is ≥ 90% effective in preventing HIV [1, 2], and up to 99% effective when taken daily for at least one week [3]. PrEP is recommended by the Centers for Disease Control and Prevention in the United States [4], and the World Health Organization globally [5]. However, monitoring adherence to PrEP in real-time is critical for its success. Tenofovir disoproxil fumarate (TDF) is a prodrug of tenofovir (TFV, Fig. **1**), a nucleoside monophosphate analog, which is rapidly converted into TFV, and is further metabolized intracellularly to its active anabolite, tenofovir diphosphate [6]. Tenofovir is primarily cleared by renal elimination and tubular secretion.

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Several approaches such as dried blood spot (DBS) analysis, hair analysis and plasma analysis have been evaluated for monitoring adherence toPrEP in patients in research settings [7, 8]. However, these assays require sample collection procedures that may not be acceptable to patients outside the clinical trials and are associated with delays in reporting that prevent the timely implementation of effective interventions, while DBS and hair analysis provide information that does not reflect recent PrEP use. Plasma TFV concentrations have been measured in clinical trials but can only provide information about adherence of the last 24 to 36 hours [7]. Based on the pharmacokinetic profile of tenofovir, a urine-based test would address many of these concerns. Recently, the development and utilization of a urine assay to measure the concentration of TFV to objectively monitor adherence to PrEP in a clinical setting were reported [9].

2. TENOFOVIR URINE ASSAY

A high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) urine assay with high sensitivity

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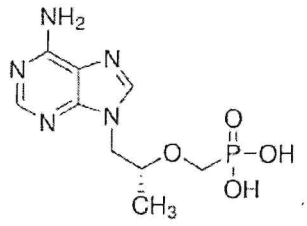

Fig. (1). Tenofovir.

and specificity for tenofovir (TFV) was developed as a semi-quantitative test (*i.e.* values are read as <10, >100, >1000, >10,000 ng/mL. This assay was robust in the determination of urine TFV concentrations in a small volume of urine samples (2 μL), and was 100% concordant with plasma urine TFV analysis. Diluted urine samples (50-fold in blank human plasma) were extracted and tenofovir was separated by reverse-phase chromatography and detected with tandem mass spectrometry [9]. This assay was demonstrated to be fast, simple, specific and robust and was successfully implemented for clinical sample analysis [9, 10].

3. CLINICAL APPLICATION

Recruitment, enrollment, and study visits were conducted at Philadelphia FIGHT, a community-based organization that provides comprehensive care to patients living with and at risk for HIV infection (http://fight.org/). This study was approved by the Institutional Review Boards at Philadelphia FIGHT.

The first large scale, post-validation application of the urine TFV assay occurred between February 2015 and July 2016 during which, 50 young (mean age=22.1 years) men who have sex with men (MSM) and transgender women (TGW) of color were enrolled into a 48-week, prospective observational trial aimed at achieving high rates of retention (at least 70% of participants who picked up at least 50% of PrEP as prescribed) and high levels of adherence to daily TDF/FTC for PrEP. Urine samples were collected monthly with paired plasma samples collected every 24 weeks[10]. Participants on PrEP or deemed clinically eligible for PrEP by their providers were enrolled from a community drop-in center co-located with a multidisciplinary, youth-focused

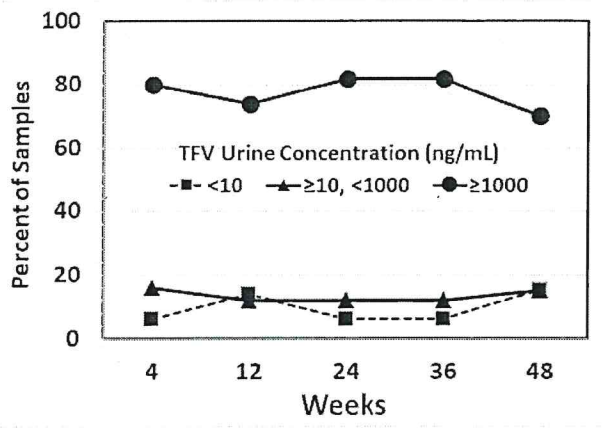

Fig. (2). Urine TFV concentrations over 48 weeks in a prospective observational cohort study of 50 men who have sex with men (MSM) taking pre-exposure prophylaxis (PrEP).

health center located in Philadelphia, Pennsylvania. Participants were routinely made aware of when urine and plasma testing would be conducted for objective adherence monitoring, and participants received feedback on urine TFV results as soon as results were available. The turn-around time, as this test was not yet commercially available at the time of this study, was typically between 1-4 weeks. The study staff provided a standard of care adherence counseling to all participants at each visit[1] regardless of the results of urine TFV testing. However, patients with urine TFV levels consistent with recent nonadherence or suboptimal adherence received enhanced adherence support by the PrEP retention coordinator through motivational interviewing and, if deemed necessary by the PrEP retention coordinator, a separate counseling session with the prescribing physician or nurse practitioner. TFV values over time are depicted in Fig. **2**, and demonstrate that those study participants maintained high adherence (measured by both plasma and urine TFV levels) to TDF/FTC throughout the study period. Additionally, urine TFV levels were entered into the participants' electronic medical record (EMR) to facilitate communication both between participants, their primary care clinicians, and study staff (Fig. **3**).

Edit | Export	Help	08/11/2015	08/28/2015	09/08/2015	09/14/2015	09/21/2015
Tenofovir Level		0 ng/mL (Not	0-1000 ng/mL	>1000 ng/mL		>1000 ng/mL
HIV viral load						
HIV-1 RNA by PCR						
log10 HIV-1 RNA						
HIV testing						
HIV Rapid Test					Negative	

Fig. (3). Participants' electronic medical record.

Since the completion of study, the urine TFV test is being promoted and advanced to the public health sphere by a social venture, UrSure, Inc. The American Medical Association (AMA) granted the urine TFV test a Proprietary Laboratory Analyses (PLA) CPT code of 0025U, effective from January 1, 2018. In September 2018, the Centers for Medicare & Medicaid Services (CMS) made a preliminary determination to crosswalk PLA 0025U to one time (1x) G0480, an existing Healthcare Common Procedure Coding System (HCPCS) billing code, as part of its upcoming calendar year 2019 Clinical Laboratory Fee Schedule (CLFS). Reimbursement must be attainable through Medicaid/Medicare that started from 2019. The urine TFV test is currently being run out of a commercial lab (Synergy Medical Laboratory, Manalapan, NJ) using the same methodology as utilized at CHOP, and results are obtained within 3 days.

CONCLUSION

Currently, the urine TFV assay is being utilized as a means of therapeutic drug monitoring for patients taking PrEP within the adolescent and adult medicine practices of an urban Federally Qualified Health Center in Philadelphia, Pennsylvania. TFV monitoring is voluntary, samples are sent to and processed at the commercial laboratory, and the results are communicated to clinicians and PrEP retention coordinating staff and are (for now) manually entered into our EMR. As in the aforementioned clinical trial, urine TFV values are used as a point of conversation in the larger context of retention and active engagement in care, and to help patients determine how to best match periods of high adherence with periods of potential increased risk of HIV acquisition. Urine TFV testing has been highly acceptable to our patients [9]. Promising opportunities exist in the development, validation and implementation of point of care testing for monitoring the urine levels of TFV in clinical samples [11-15].

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

Dr. Helen Koenig has served as a consultant to and has received a research grant from Gilead, the manufacturer of Truvada™.

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