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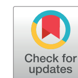
A competitive lateral flow assay for the detection of tenofovir



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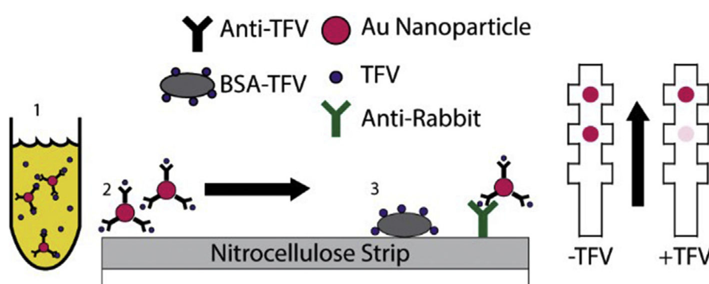
HIGHLIGHTS

- Lateral flow strip is created to test HIV patients for drug adherence to Tenofovir.
- Anti-Tenofovir antibody is raised as a key component of the urine-based test.
- Gold nanoparticle-based competitive assay detects clinically relevant concentrations.
- New assay potentially deployable in resource-limited settings where need is greatest.

GRAPHICAL ABSTRACT



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ABSTRACT

Proper management of an HIV infection requires that a patient be at least 80–95% adherent to a prescribed drug regimen to avoid poor health outcomes and the development of drug-resistant HIV strains. Clinicians generally monitor adherence habits indirectly through patient self-reporting, pill counting, and electronic drug monitoring. While direct measurement of patient samples like urine for monitoring drug levels is possible, it requires specialized equipment and training that is not readily available in resource-limited settings where the need is greatest. In this work we report the development of an antibody that binds to tenofovir (TFV), a key small molecule drug for both the treatment and prevention of HIV, and a competitive lateral flow assay that uses that antibody to monitor urine samples for the presence of the drug. TFV was conjugated to an immunogenic protein and injected into rabbits to raise polyclonal antibodies sensitive to the drug. The antibodies were verified for TFV-sensitivity by immunoprecipitation and HPLC. A gold nanoparticle-based competitive assay was developed to detect the presence of TFV in urine samples with a sensitivity of 1 $\mu g\ mL^{-1}$. This TFV assay could be deployed as a point-of-care device for adherence monitoring in resource-limited settings as a low-cost, accurate, and speedy alternative to current methods to better inform changes in treatment.

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1. Introduction

Tenofovir (TFV) has become a cornerstone of HIV treatment since its approval for use in 2001 as tenofovir disoproxil fumarate (TDF). In 2015, the World Health Organization maintained its recommendation that TDF, which is metabolized into TFV *in vivo*, be part of the preferred first-line regimen for antiretroviral therapy to treat HIV patients [1]. In addition, the WHO recommends pre-exposure prophylaxis (PrEP) therapies containing TFV-derived medications be deployed to prevent the transmission of the virus among high-risk populations in both high- and low-resource settings [2]. As a result of these recommendations and the development of new formulations, such as tenofovir alafenamide (TAF), TFV will likely remain one of the most important tools for the treatment

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and prevention of HIV.

The number of people accessing antiretroviral medications to manage their HIV infections has risen to over 18 million worldwide as of June 2016 [3]. Around 10 million of these people are on treatment regimens containing TFV [4]. Mismanagement of HIV drug regimens routinely results in a heightened risk of transmission, decreased patient health and quality of life, and an increase in the incidence of HIV drug resistance [5]. The WHO cites poor adherence as the main reason for suboptimal clinical benefits managing chronic illnesses such as HIV/AIDS [6]. As a result, it is critical that clinicians monitor the adherence of HIV patients to their prescribed treatment regimens.

To manage HIV infections and keep viral loads low patients must be at least 80–95% adherent to their antiretroviral treatments [7–10], and many populations of patients do not demonstrate adequate adherence rates [11–13]. There are many factors that diminish adherence rates: complexity of regimen, side effects, and patient psychological factors among others [14–18]. Fortunately, there are many interventions that have been shown to improve adherence behaviors and health outcomes [19].

Current methods for tracking adherence behaviors are mostly indirect such as pill counting, electronic drug monitoring, and patient self-reporting [5]. Pill counting and electronic monitoring are limited in their deployment and self-reporting, the most widely used method, is prone to overestimation [20,21]. Current direct methods to measure drug levels in patient samples generally require expensive equipment [22] that is not easily accessible in resource-limited settings where the need is greatest.

We report here the first antibody-based direct measurement of TFV in urine. Using a gold nanoparticle-based competitive lateral flow strip assay, we have detected TFV in spiked urine samples at clinically relevant concentrations. Raising antibodies against small molecule targets like TFV is not straightforward. Conjugation of TFV to a protein substrate is required, and in this case was not trivial (Fig. 1). As such, we have included the details of our approach.

This assay has the potential to facilitate objective monitoring of HIV adherence habits in all settings without the need for expensive equipment or long turnaround times allowing clinicians to intervene in cases of noncompliance and improve overall patient outcomes.

2. Methods

2.1. Materials

TFV was purchased from Ark Pharm, Inc. (Libertyville, IL). Bovine serum albumin (BSA), keyhole limpet hemocyanin (KLH), sulfo-succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), Pierce Protein G agarose beads, CarboxyLink Coupling Gel, Whatman chromatography paper, and pico chemiluminescent substrate were purchased from ThermoFisher (Waltham, MA). Amersham Protran nitrocellulose was purchased from GE Healthcare Life Sciences (Pittsburgh, PA). Anti-rabbit antibody conjugated to horseradish peroxidase (HRP) was purchased from GE Healthcare (Chicago, IL). Goat anti-rabbit antibody was purchased from Abcam (Cambridge, MA). N,N′-Dicyclohexylcarbodiimide (DCC), pyridine, 3-mercapto-1-propanol, silica gel Davisil grade 643, Hi-Flow Plus HF180 nitrocellulose sheets, adenosine monophosphate, Tween 20, sucrose, adenosine monophosphate (AMP), and all solvent and buffers were purchased from Sigma-Aldrich (St. Louis, MO). A 40 nm InnovaCoat Gold Conjugation Kit was purchased from Innova Biosciences (Babraham, England).

2.2. Synthesis of tenofovir-thiol hapten

The synthesis of tenofovir-thiol (TFV-SH) is outlined in Fig. 1a (inset). The synthesis was performed using a modified version of the protocol of Varal et al. [23] for the esterification of the tenofovir phosphonate group. In short, 270 mg of TFV and 389 mg of DCC

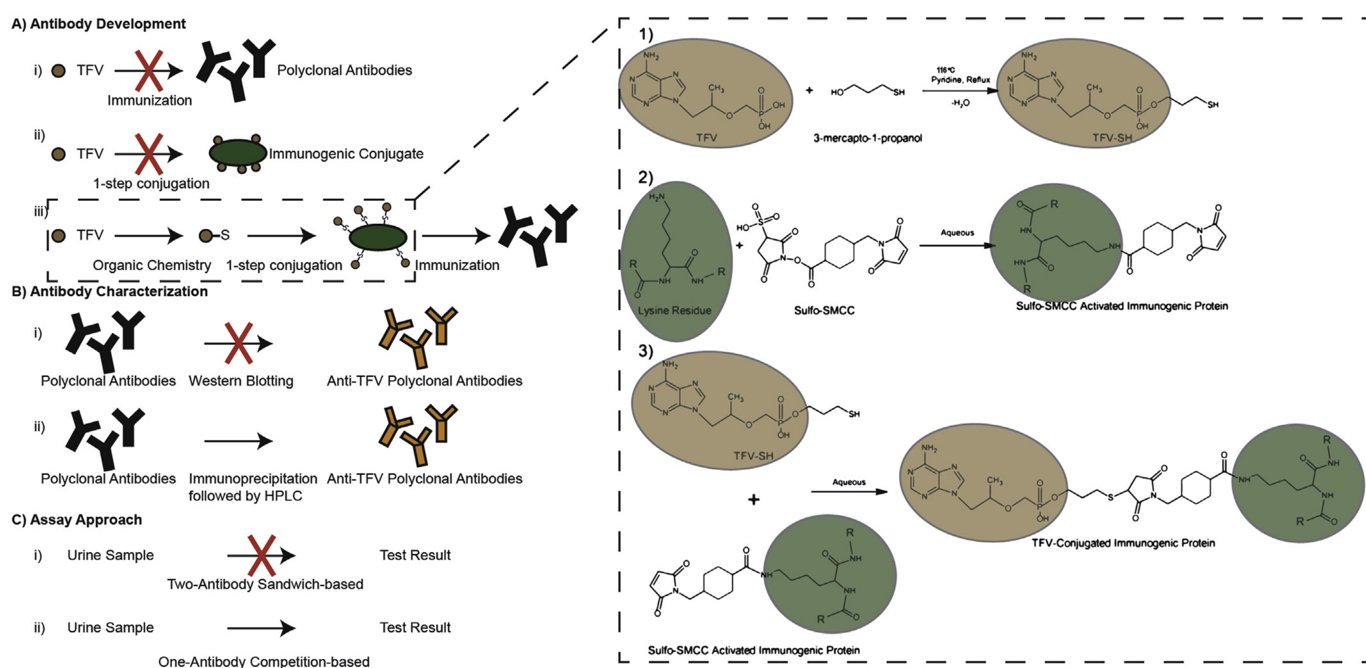

Fig. 1. Technical challenges. A) Antibody development: TFV alone cannot generate polyclonal antibodies (i) and there is not a straightforward method for direct conjugation onto an immunogenic protein (ii). TFV was conjugated to an immunogenic protein (iii). B) Antibody characterization: Antibodies targeting small molecules cannot be characterized by western blotting, the standard method (i). Immunoprecipitation followed by LC-MS analysis was used (ii). C) Assay Approach: A small molecule target is not suitable for standard sandwich-based assays (i). A competition-based assay was designed (ii).

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