Published in final edited form as:

AIDS Res Hum Retroviruses. 2021 October ; 37(10): 744–747. doi:10.1089/AID.2020.0187.

Short Communication: Evaluation of Antiretroviral Drug Concentrations in Minimally Invasive Specimens for Potential Development of Point-of-Care Drug Assays

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Abstract

Point-of-care (POC) tests for antiretroviral drugs (ARVs) could help improve individual adherence. This study sought to define the utility of urine, blood, and buccal swabs as minimally invasive specimens amenable to development of POC tests for ARVs. Urine, dried blood spots (DBS) and buccal swabs were collected from 35 HIV-negative men between 2 and 96 h after a single dose of tenofovir (TFV) alafenamide/emtricitabine (FTC)/elvitegravir (EVG)/cobicistat and darunavir (DRV). ARV concentrations were measured by high-performance liquid chromatography-mass spectrometry. High concentrations of FTC, DRV, and TFV were detectable in urine at least 24 h after dosing. FTC, DRV, and EVG remained detectable in DBS at least 24 h postdose. FTC and DRV were detectable on buccal swabs up to 2 and 24 h postdose, respectively. TFV was not detectable in DBS or buccal swabs collected between 2 and 96 h after dosing. Variable distribution of ARVs in minimally invasive specimens highlights the challenge of developing POC assays for recent ARV exposure.

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Authors' Contributions

R.E.H, A.M., J.F., and C.F.K. designed the research study. L.H. and C.C.-W. recruited study participants and collected data and specimens for analysis. J.F., L.D.L. A.M., M.M., and C.D. performed and analyzed drug measurements. R.E.H., A.M., M.M., and C.F.K. wrote the article with contributions and interpretation of findings from all coauthors. All authors have read and approved the final article.

Disclaimer

The findings and conclusions in this article are those of the authors and do not necessarily represent the official position of the U.S. Centers for Disease Control and Prevention or the Department of Health and Human Services.

Author Disclosure Statement

R.E.H is named in a U.S. Government patent application related to inhibiting HIV seroconversion. A.M., M.M., C.D., J.F., L.D.L., L.H., C.C.-W., and C.F.K. have no competing interests to disclose.

Supplementary Material

Supplementary Data

Keywords

antiretroviral agents; point-of-care test; pre-exposure prophylaxis; dried blood spot; urine; men who have sex with men

Antiretroviral drug (ARV) effectiveness in treatment and prevention of HIV infection is currently dependent on high levels of adherence to daily oral dosing regimens.[1] Behavioral methods to determine adherence, such as self-report and pill count are considered unreliable and rapid point-of-care (POC) tests that indicate levels of ARV exposure could be used to track and improve individual adherence in clinical settings.[2] POC tests for ARVs should use minimally invasive specimens that provide measurable ARV concentrations amenable to rapid analytic techniques. POC tests to assess tenofovir (TFV) concentrations in urine have been developed that allow for rapid determination of recent dosing with tenofovir disoproxil fumarate (TDF)- or tenofovir alafenamide (TAF)-based regimens.[3,4] We examined ARV concentrations in urine, dried blood spots (DBS), and buccal swabs collected from men participating in a study of mucosal drug pharmacology and compared results to plasma to help determine the potential for these minimally invasive specimens to be explored as targets for development of POC tests for ARVs.

Blood, urine, and buccal swabs were collected from 35 HIV-negative male participants between ages 20 and 46 (median age 24 years) at 3 clinic visits between 2 and 96 h after a single oral dose containing TAF, emtricitabine (FTC), elvitegravir (EVG), darunavir (DRV), and cobicistat (COBI) at the Emory Hope Clinic in Atlanta, Georgia. The study was funded by the U.S. Centers for Disease Control and Prevention (CDC) and approved by Emory University and CDC Institutional Review Boards. The trial is registered at clinicaltrials.gov (NCT03472963). Plasma was collected from cell preparation tubes (Becton Dickinson, Franklin Lakes, NJ) after centrifugation. DBS was prepared by transferring 25 lL of whole blood from EDTA tubes (Becton Dickinson) onto a Whatman 903 protein saver card and dried overnight. Urine was collected in sterile specimen containers (Thermo Fisher Scientific, Waltham, MA). Buccal swabs were collected using a polyester swab (Puritan Medical Products, Guilford, ME). All specimens were stored at −70°C before analysis. Concentrations of TFV, FTC, EVG, DRV, COBI, and TAF were measured using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) (Sciex, Foster City, CA; Shimadzu Scientific Instruments, Durham, NC) based on previously described methods (Supplementary Data).[5,6] Plasma standard curve concentrations were created in normal human plasma and DBS standard curve concentrations were generated in whole blood as previously described.[7] The lower limit of quantification for this study was 10 ng/mL for plasma, urine, and DBS, and 10 ng/swab for buccal swabs.

FTC was detectable in all specimen types 2 h after a single observed dose of TAF/FTC/EVG/COBI and DRV (Fig. 1). Although FTC remained reliably detectable in urine and DBS collected from study participants up to 48 and 24 h, respectively, it became undetectable in >50% of buccal swab specimens within 24 h. FTC continued to remain detectable in >50% of urine specimens collected 96 h after a single dose. DRV was also readily detectable in all specimens within 2 h of dosing. DRV was detectable in >50% of

urine and DBS specimens up to 24 and 72 h postdose, respectively. Buccal swab DRV concentrations were detectable in >50% of specimens up to 24 h postdose. TFV was measurable in urine up to 24 h after observed dosing and continued to be detectable in >50% of urine specimens up to 96 h postdose. However, TFV was rarely detected in DBS and buccal swab specimens collected at any time point. TAF was detectable in 10 out of 15 urine specimens collected at 2 h but became undetectable in all specimens within 8 h (data not shown). EVG was routinely detected in DBS collected up to 24 h postdose but was undetectable in >50% of urine and buccal swabs at any point after dosing. COBI was detectable in >50% of DBS, urine, and buccal swabs collected up to 8 h postdose but became undetectable in >50% of specimens collected 24 h after dosing (data not shown).

FTC detection results were concordant between urine and plasma in 67% of paired specimens and concordant between paired DBS and plasma in 82% of paired specimens with 91% of discordant detection results attributable to detection in DBS and urine, but not corresponding plasma specimens (data not shown). DRV detection results were highly concordant between paired urine and plasma specimens (85%) as well as between DBS and plasma specimens (84%). TFV detection was only concordant between urine and plasma in 26% of paired specimens primarily due to the reliable measurement of TFV in urine, but not plasma. Likewise, detection of EVG was highly concordant between paired DBS and plasma specimens (86%), but only concordant between urine and plasma in 43% of paired specimens due to the lack of EVG detection in urine specimens. Measurable urine FTC and DRV concentrations correlated with corresponding plasma drug concentrations (FTC: $r = 0.510$, $p < .001$, DRV: $r = 0.555$, $p < .001$). DBS concentrations correlated with those in plasma for FTC ($r = 0.941$, $p < .001$), DRV ($r = 0.917$, $p < .001$) and EVG ($r = 0.867$, $p < .001$).

Urine TFV assays have been developed and evaluated for persons using TDF/FTC and are being explored for implementation.[3,4,8,9] Our finding that TFV concentrations remain detectable in >50% of specimens up to 96 h after dosing, whereas remaining undetectable in other specimen types highlights the advantage of urine TFV as a surrogate marker of recent dosing. High urine FTC and DRV concentrations persist beyond 24 h after dosing and correlate with plasma drug concentrations indicating the potential for POC tests that do not require high sensitivity and provide a surrogate marker of plasma drug exposure. FTC, DRV, and EVG were readily measurable in DBS suggesting POC assays targeting whole blood collected by fingerstick may provide comparable results to separated plasma. Although a previous study detected TFV and FTC in saliva among men receiving TDF/FTC,[10] TFV was not detectable on buccal swabs collected in our study likely due to low TFV concentrations among persons receiving TAF. Buccal swab FTC and DRV concentrations were low (<500 ng/swab), and rapidly became undetectable suggesting POC tests using buccal swabs would need to be extremely sensitive to provide reliable measures of ARV dosing.

Our study highlights the utility of urine and whole blood in development of POC tests, yet interpretation of these results is limited. Specimens in this study were collected after a single dose of TAF/FTC/EVG/COBI and DRV, not from persons achieving steady-state drug concentrations in these compartments. Drug accumulation in urine and blood after daily dosing may exceed expected concentrations or lead to drug detection beyond the time

frames noted in this study. In addition, drug accumulation after daily dosing may allow for more reliable detection of drugs such as EVG in urine or EVG and TFV in buccal swabs, which remained below the limit of detection in these specimen types. Several factors not explored in this study, such as hydration and body mass index, could affect quantitative ARV concentrations in minimally invasive specimens and will be needed to refine and interpret POC assays for recent dosing. Our study evaluated EVG and DRV in the context of coadministration with COBI; therefore, these results may not reflect concentrations achieved without a pharmacoenhancer. In addition, newer integrase and protease inhibitors do not require coadministration with a pharmacoenhancer and penetration of those newer ARVs into minimally invasive specimen types should be evaluated.

Development of POC tests to detect ARV drugs from minimally invasive specimens may provide opportunities to assess adherence and offer immediate interventions to improve adherence. The data presented in this study highlight challenges to developing POC assays using a single specimen type that effectively assesses adherence to a wide range of multidrug regimens. Our results suggest that POC assay development will need to account for specimen types when targeting ARVs such as TFV and EVG that are reliably detectable in urine and blood, respectively, but not other specimen types.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank the study participants for their time and commitment to this study as well as Walid Heneine for helpful discussions, and Chunxia Zhao and Ashley Butts for technical assistance.

Funding Information

This study was funded by the U.S. Centers for Disease Control and Prevention.

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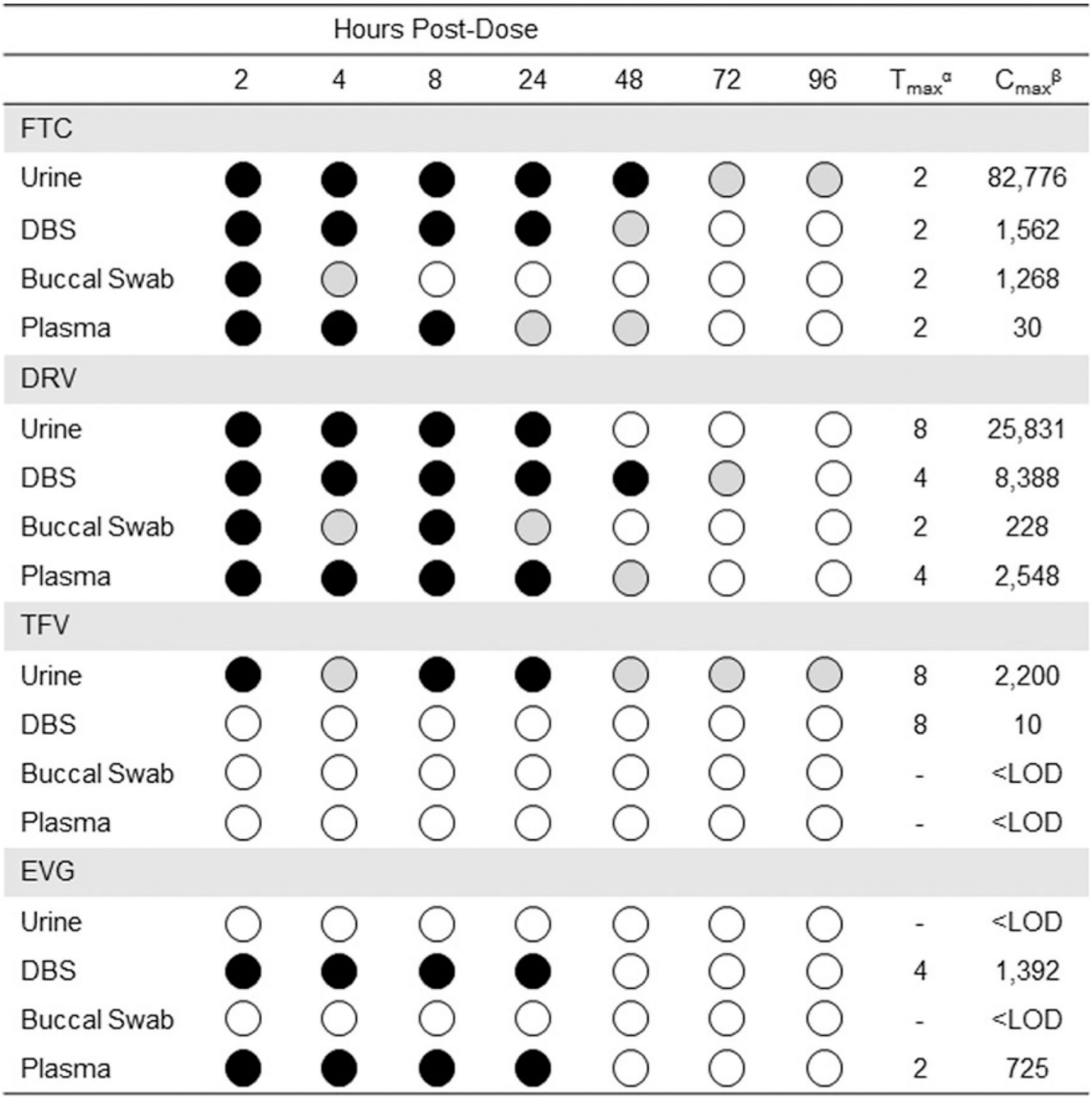

FIG. 1.

Detection of ARVs in urine, DBS, buccal swabs, and plasma. The percentage of specimens with measurable FTC, DRV, TFV, and EVG are indicated by *circles* in each *row* corresponding to the time after dosing (2–96 h). *Black circles* indicate ≥90% of specimens with detectable ARVs, *gray circles* indicate 51%–89% of specimens with detectable ARVs, and *open circles* indicate ≤50% of specimens with detectable ARV concentrations. The LOD was 10 ng/mL for urine, DBS, and plasma, and 10 ng/swab for buccal swabs. $^{\alpha}T_{max}$: time of maximum geometric mean ARV concentration, $^{\beta}C_{max}$: geometric mean concentration at

T_{max}. ARV, antiretroviral drug; DBS, dried blood spots; DRV, darunavir; EVG, elvitegravir; FTC, emtricitabine; LOD, limit of detection; TFV, tenofovir.