# UTILITY OF MINIMALLY INVASIVE SPECIMENS TO INFORM ARV ADHERENCE TEST DEVELOPMENT

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## Background

Antiretroviral drug (ARV) efficacy in treatment and prevention of HIV infection is currently dependent on high levels of adherence to daily oral dosing regimens (1,2). Behavioral methods to determine adherence such as self-report and pill count are considered unreliable (3,4) and rapid objective measures of ARV concentrations indicating levels of adherence could provide opportunities for interventions that improve ARV adherence for treatment or prevention.

Rapid point of care (POC) tests that indicate levels of ARV exposure could be used to track and improve individual adherence in clinical settings. POC tests for ARVs should use minimally invasive specimens that provide measurable ARV concentrations amenable to rapid analytic techniques. POC tests to assess TFV concentrations in urine have been developed that would allow for rapid determination of recent dosing with tenofovir-based regimens (5,6). While much focus has been placed on developing adherence assays for TDF/FTC for PrEP, less attention has been given to other ARVs that may be used for HIV treatment or future PrEP regimens.

We sought to define the pharmacology of ARVs in men following a single oral dose containing TAF and FTC as well as the integrase inhibitor elvitegravir (EVG), the protease inhibitor darunavir (DRV) and the pharmacoenhancer cobicistat (COBI) to provide information regarding the utility of urine, dried blood spots (DBS), and buccal swabs as minimally invasive specimens amenable to POC test development for ARV adherence.

## Methods

### Study Design

Thirty-five male participants between ages 18-49 were sequentially assigned to study arms to provide specimens at 3 clinic visits following a single observed oral dose of TAF/FTC/COBI/EVG and DRV. Participants provided specimens 2, 24, and 72 hours post dose (n=15); 4, 48, and 96 hours post dose (n=15); or 8, 24, and 48 hours post dose (n=5). Blood specimens were collected in cell preparation tubes (CPT) and EDTA tubes (Becton Dickinson, Franklin Lakes, New Jersey, USA). Plasma aliquots were collected from CPT tubes following centrifugation. DBS were prepared by transferring 25 µL of whole blood from EDTA tubes onto a Whatman 903 card and dried overnight. Urine was collected in sterile specimen containers (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Buccal swabs were collected using a polyester swab (Puritan Medical Products, Guilford, Maine, USA).

### Laboratory Measurements

TFV, FTC, EVG, DRV and COBI concentrations were measured using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) (Sciex, Foster City, CA, Shimadzu Scientific Instruments, Durham, NC). Standard curves for plasma and urine specimens were generated in normal human plasma and urine, respectively. Standard curves for DBS were constructed using spiked whole blood to make DBS of appropriate whole blood concentrations (7). Standard curves for buccal swabs were generated by spiking ARVs onto dry swabs in a range of 0.5-2000 ng/mL. The lower limit of quantification (LLOQ) and limit of detection for this study was 10 ng/mL for plasma and urine, 10 ng/swab for buccal swabs, and 10 ng/mL for DBS. Measurements below the LLOQ were assigned a value of one-half the LLOQ for statistical calculations.

## Results

- Urine FTC, TFV and DRV are measurable in urine specimens beyond 24 hours post dose.
- Urine FTC, DRV and COBI concentrations correlate with plasma concentrations.
- EVG is not detectable in urine or buccal swabs after dosing.
- TFV is routinely detectable in urine, but not other specimens following TAF dosing.
- DBS FTC, DRV and EVG concentrations correlate with plasma concentrations.
- ARVs are only detected on buccal swabs shortly after dosing.

| | Hours Post-Dose | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2 | 4 | 8 | 24 | 48 | 72 | 96 |
| **TFV** | | | | | | | |
| Plasma | | | | | | | |
| DBS | | | | | | | |
| Urine | ● | ○ | ● | ● | ○ | ○ | ○ |
| Buccal Swab | | | | | | | |
| **FTC** | | | | | | | |
| Plasma | ● | ● | ● | ○ | ○ | | |
| DBS | ● | ● | ● | ● | ○ | | |
| Urine | ● | ● | ● | ● | ● | ○ | ○ |
| Buccal Swab | ● | ○ | | | | | |
| **EVG** | | | | | | | |
| Plasma | ● | ● | ● | ● | | | |
| DBS | ● | ● | ● | ● | | | |
| Urine | | | | | | | |
| Buccal Swab | | | | | | | |
| **DRV** | | | | | | | |
| Plasma | ● | ● | ● | ● | ○ | | |
| DBS | ● | ● | ● | ● | ● | ○ | |
| Urine | ● | ● | ● | ● | | | |
| Buccal Swab | ● | ○ | ● | ○ | | | |
| **COBI** | | | | | | | |
| Plasma | ● | ● | ● | | | | |
| DBS | ● | ● | ○ | | | | |
| Urine | ○ | ○ | ● | | | | |
| Buccal Swab | | ○ | ○ | | | | |

**Figure 1:** Detection of antiretroviral drugs (ARVs) in minimally invasive specimens. The percentage of specimens with measurable TFV, FTC, EVG, DRV or COBI are indicated by circles in each row corresponding to the time following dosing (2-96 hours). Closed circles indicate ≥90% of specimens with detectable ARVs, open circles indicate 51-89% of specimens with detectable ARVs and no circle indicates ≤50% of specimens with detectable ARV concentrations. The limit of detection was 0.010 µg/mL for plasma, urine and DBS and 0.010 µg/swab for buccal swabs.

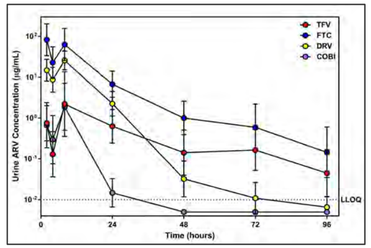



**Figure 2:** Antiretroviral drug (ARV) concentrations in urine following a single oral dose of TAF/FTC/EVG/COBI and DRV. Geometric mean and 95% confidence intervals are presented for urine TFV (red), FTC (blue), DRV (yellow) and COBI (purple) concentrations from 2 to 96 hours following dosing. The dotted line indicates the lower limit of quantification (LLOQ) for ARV measurement in urine (0.010 µg/mL).

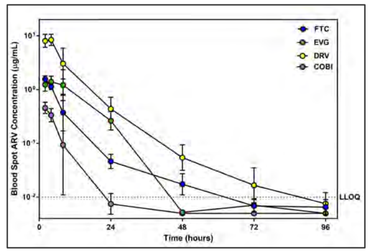



**Figure 3:** Mean antiretroviral drug (ARV) concentrations in dried blood spots (DBS) following a single oral dose of TAF/FTC/EVG/COBI and DRV. Geometric mean and 95% confidence intervals are presented for urine FTC (blue), EVG (green), DRV (yellow) and COBI (purple) concentrations from 2 to 96 hours following dosing. The dotted line indicates the lower limit of quantification (LLOQ) for ARV measurement in DBS (0.010 µg/mL).

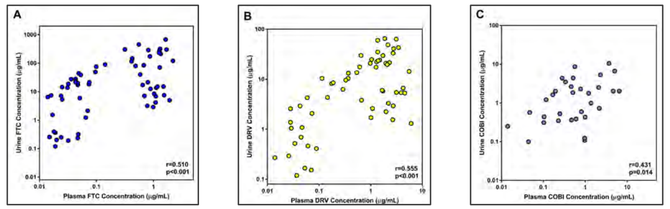



**Figure 3:** Correlation between urine and plasma antiretroviral (ARV) drug concentrations. Urine and corresponding plasma ARV concentrations are presented for FTC (A), DRV (B) and COBI (C). Correlation coefficients (r) and p-values were calculated using the Spearman correlation test.

## Conclusions

- High urine TFV, FTC or DRV concentrations that persist beyond 24 hours following dosing indicate potential for rapid POC tests for adherence.
- FTC, EVG or DRV in DBS provides a reliable correlation to plasma drug concentrations suggesting DBS or whole blood could be used to develop rapid POC tests for adherence.
- Buccal swabs are unlikely to provide a reliable marker of ARV adherence.
- Heterogeneity in drug detection between drug classes and specimen types will make development of a single POC adherence assay challenging.

## References

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