Evaluation of self-collected Microtainer™ specimens for measuring antiretroviral drugs

Richard Haaland
Division of HIV Prevention
Centers for Disease Control and Prevention
Atlanta, Georgia

I have no competing interests to disclose.

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HIV treatment and prevention

- **Antiretroviral drugs (ARVs) are key component of biomedical interventions to treat and prevent HIV infection**
  - Suppress viral loads in HIV-positive individuals
    - Antiretroviral therapy (ART)
  - Prevent infection in HIV-negative individuals
    - Pre-exposure prophylaxis (PrEP)
    - Post-exposure prophylaxis (PEP)

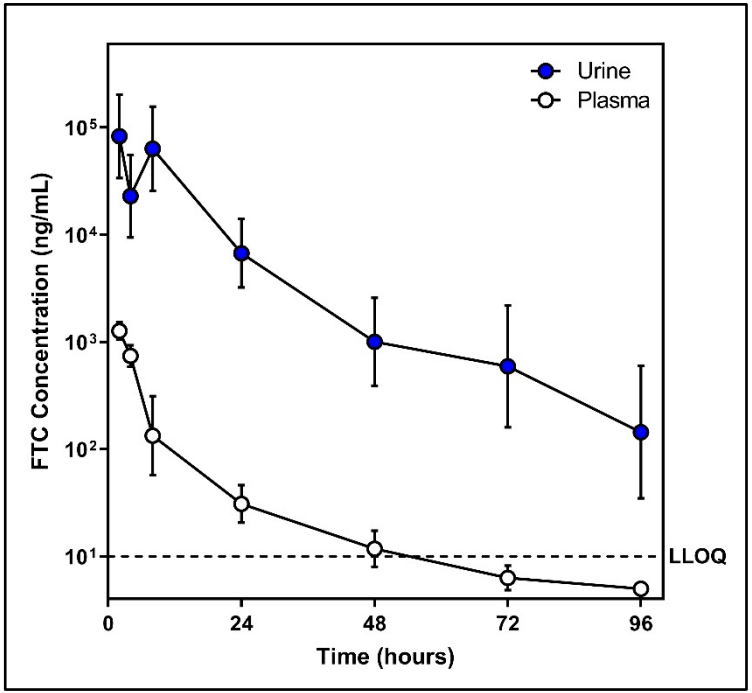
- **Dosing modalities**
  - Oral dosing (daily and on-demand)
  - Long-acting formulations

Antiretroviral drug measurements

- **Objective marker of ARV use**
  - Clinical trial adherence
    - Determine which participants are adherent to study medication
  - Surveillance
    - Distinguish aware and unaware HIV-positive respondents
    - Assess adherence to PrEP
  - Clinical care management
    - Identify persons in need of additional support to ART or PrEP
  - Measurements by chromatography-mass spectrometry (LC-MS/MS)
    - Typically performed in centralized and specialized laboratory

Recent PrEP Adherence

- **Specimens determine interpretation**
- **Plasma and urine drug concentrations reflect recent dosing**
  - Limited drug accumulation with daily dosing
  - Rapid decline to undetectable concentrations
  - Not all ARVs excreted in urine



Cumulative PrEP Adherence

- **Tenofovir-diphosphate (TFV-DP) concentrations in DBS reflect cumulative dosing**
  - TFV-DP accumulates in red blood cells during daily dosing with tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide (TAF)
  - Provide estimate of dosing over previous 2-3 months
    - Analysis dependent on regimen

	TFV-DP (fmol/specimen)		
	2-3 doses/wk	4-6 doses/wk	7 doses/wk
TDF/FTC (3mm punch)	350	700	1250
TAF/FTC (2x7mm punch)	450	950	1800

Anderson, PL et al. *Antimicrob Agents Chemother.* 2018.
Yager, J et al. *J Acquir Immune Defic Syndr.* 2020.

Microtainer™ self-collection

- **Microtainers™**
  - Offer potential for self-collection of whole blood in non-clinical settings
  - Whole blood can be processed using existing analysis pipelines
    - Dried blood spots (DBS) or whole blood
    - Plasma
    - Cells

- **Objective**
  - Examine utility of self-collected Microtainers™ for ARV measurements

ARV measures in self-collected Microtainers™

- **Microtainers™ collected as part of iSTAMP study**
  - HIV-positive participants
  - HIV-negative participants on PrEP
  - Specimen self-collection to laboratory receipt: median = 2 days
- **Whole blood spotted on DBS**
  - Dried overnight
  - Analyzed one 6mm punch for TFV-DP and FTC-TP by LC-MS/MS
    - LLOQ (TFV-DP: 100 fmol/punch)
    - LLOQ (FTC-TP: 500 fmol/punch)

Specimens	Self-reported ART Use	Total (n=42)
HIV-positive	ART	4
	No ART	1
HIV-negative	PrEP	37

*ART or PrEP regimen not defined

ARV measures in self-collected Microtainers™

- **HIV-positive participants**
  - 1 participant self-reported no ART
    - TFV-DP and FTC-TP: <LOQ
  - 4 participants self-reported on ART
    - FTC-TP suggests recent dosing in previous few days prior to specimen collection
    - TFV-DP suggests dosing with TAF-based regimens

HIV-positive on ART	TFV-DP (fmol/punch)	FTC-TP (fmol/punch)
Median	258	1923
Range	(<LOQ – 706)	(1125 – 2455)

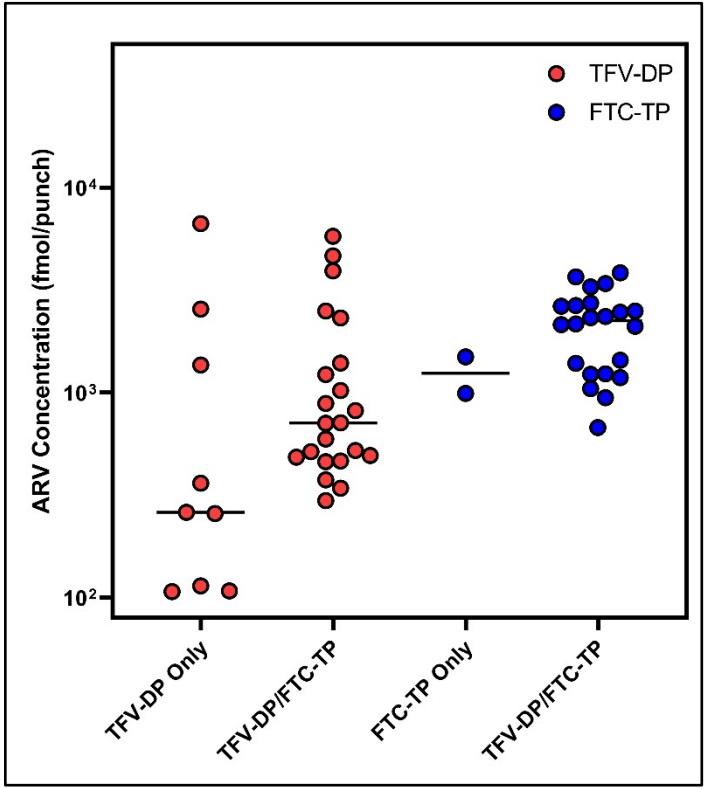
ARV measures in self-collected Microtainers™

- **HIV-negative participants**
  - 37 participants self-reported PrEP use
    - Regimens not disclosed
  - Most participants had ARV markers supporting PrEP use (33/37)

Analyte Detected	HIV-negative on PrEP (n=37)
TFV-DP only	9 (24%)
FTC-TP only	2 (5%)
TFV-DP and FTC-TP	22 (59%)
No TFV-DP or FTC-TP	4 (11%)

ARV measures in self-collected Microtainers™

- **HIV-negative participants**
  - 37 participants self-reported PrEP use
    - Regimens not disclosed
  - Most participants had ARV markers supporting PrEP use (33/37)
- **PrEP dosing estimates based on TFV-DP concentrations**
  - Daily dosing: 15
  - Less than daily dosing: 18
    - 9 participants suggest <4 doses/week

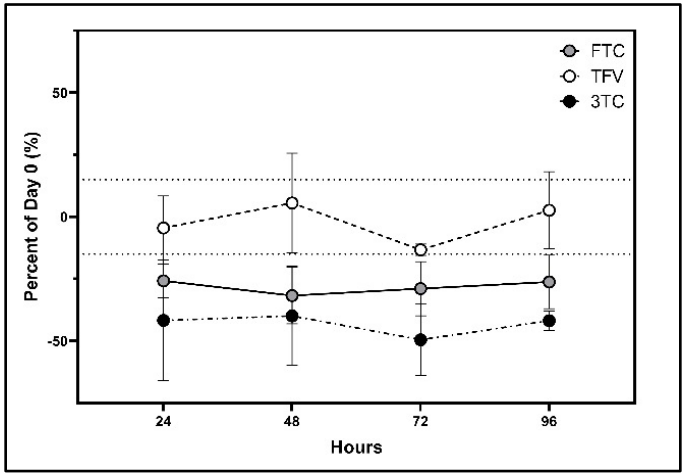


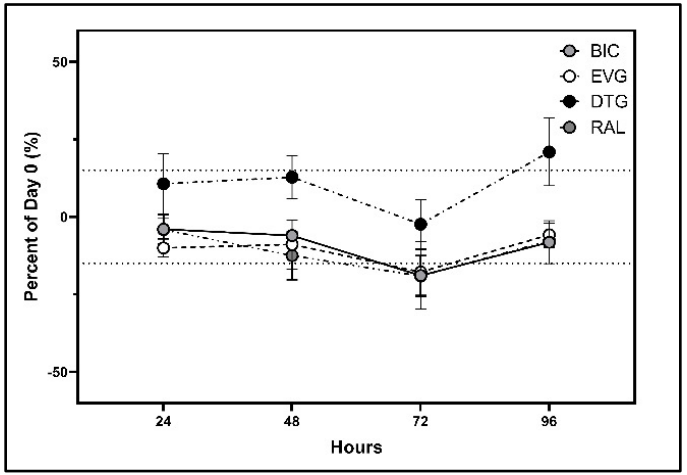
ARV stability in Microtainers™

- **Spiked ARVs into blood and stored up to 96 hours (4 days) in Microtainers™**
  - Nucleoside reverse transcriptase inhibitors (NRTIs) and integrase inhibitors (INSTIs)
  - 25, 100 and 1000 ng/mL
  - Measured by HPLC-MS/MS with lower limit of quantification (LLOQ) of 10 ng/mL
  - Measurements compared to Day 0 with <15% difference considered acceptable

NRTIs	INSTIs
Emtricitabine (FTC)	Bictegravir (BIC)
Tenofovir (TFV)	Elvitegravir (EVG)
Lamivudine (3TC)	Dolutegravir (DTG)
	Raltegravir (RAL)

Microtainer™ ARV stability





- **FTC and 3TC measurements decline within 24 hours**
  - FTC and 3TC concentrations remained measurable
  - TFV concentrations remain stable up to 96 hours in MCTs
- **INSTIs generally stable in MCTs**
- **Results remained consistent across concentrations (25 – 1000 ng/mL)**

ARV stability in Microtainers™

- **Whole blood specimens collected from HIV+ persons on ART and HIV- persons on PrEP**
- **Blood placed in Microtainers™ and stored up to 96 hours (4 days) after collection**
  - Collected 2-4 measurements/participant
  - Measurements compared to Day 0

Regimens*	HIV+ (n=4)	HIV- (n=2)
TAF/FTC/BIC	2	
TAF/FTC/EVG/c + DRV	1	
TAF/FTC + DTG	1	
TAF/FTC		1
TDF/FTC		1

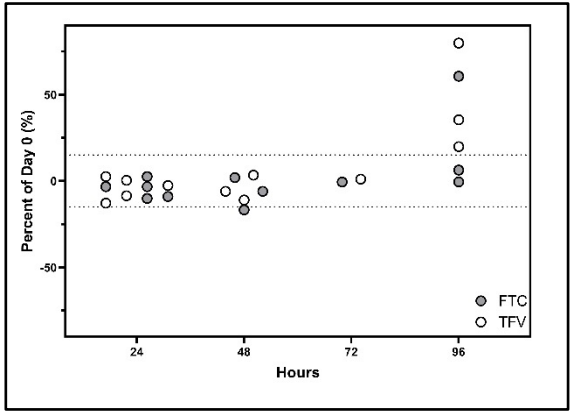
*ART and PrEP regimens self-reported

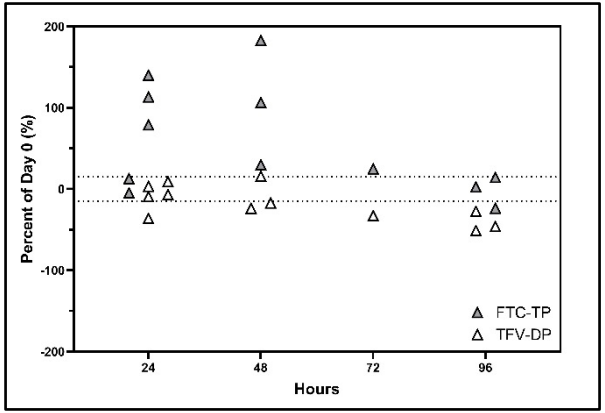
ARV stability in Microtainers™

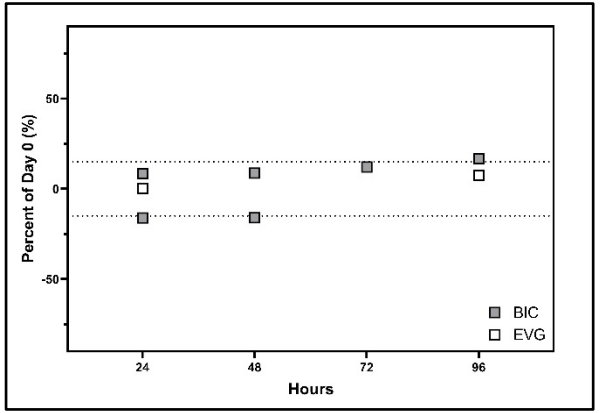
- **Examine ARV stability for shipping and analysis**
- **Darunavir (DRV) and DTG not detected**
- **No ARVs detected for 1 participant**
  - TAF/FTC + DTG

Day 0 Concentrations	Median (Range)
FTC	1329 (99 – 2030) ng/mL
TFV	58 (13 – 227) ng/mL
TFV-DP	2546 (465 -14,975) fmol/punch
FTC-TP	3828 (2848 – 6465) fmol/punch
BIC	(882 – 1060) ng/mL
EVG	961 ng/mL

Microtainer™ ARV stability







- **NRTIs (TFV/FTC; TFV-DP, FTC-TP) more variable**
  - Variability likely due to continuing drug metabolism
  - NRTIs remain detectable

- **INSTIs generally stable in Microtainers™ up to 4 days after collection**

Conclusions

- **ARV measurements in self-collected Microtainers™ can provide quantitative or qualitative measures of adherence**
  - Assessment determined by ARV regimen and analyte

- **Microtainers™ provide opportunity to examine ARV use in self-collected specimens**
  - Allow for combined ARV, viral load and HIV testing with single specimen
  - Allow for testing using existing methodology for DBS and/or plasma

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Questions?