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.

Use of trademarks is for informational purposes only and does not constitute product endorsement by the CDC.
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

<table>
<thead>
<tr>
<th>TFV-DP (fmol/specimen)</th>
<th>2-3 doses/wk</th>
<th>4-6 doses/wk</th>
<th>7 doses/wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDF/FTC (3mm punch)</td>
<td>350</td>
<td>700</td>
<td>1250</td>
</tr>
<tr>
<td>TAF/FTC (2x7mm punch)</td>
<td>450</td>
<td>950</td>
<td>1800</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Self-reported ART Use</th>
<th>Total (n=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-positive</td>
<td>ART</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>No ART</td>
<td>1</td>
</tr>
<tr>
<td>HIV-negative</td>
<td>PrEP</td>
<td>37</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>HIV-positive on ART</th>
<th>TFV-DP (fmol/punch)</th>
<th>FTC-TP (fmol/punch)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>258</td>
<td>1923</td>
</tr>
<tr>
<td>Range</td>
<td>(&lt;LOQ – 706)</td>
<td>(1125 – 2455)</td>
</tr>
</tbody>
</table>
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)

<table>
<thead>
<tr>
<th>Analyte Detected</th>
<th>HIV-negative on PrEP (n=37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TFV-DP only</td>
<td>9 (24%)</td>
</tr>
<tr>
<td>FTC-TP only</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>TFV-DP and FTC-TP</td>
<td>22 (59%)</td>
</tr>
<tr>
<td>No TFV-DP or FTC-TP</td>
<td>4 (11%)</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>NRTIs</th>
<th>INSTIs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emtricitabine (FTC)</td>
<td>Bictegravir (BIC)</td>
</tr>
<tr>
<td>Tenofovir (TFV)</td>
<td>Elvitegravir (EVG)</td>
</tr>
<tr>
<td>Lamivudine (3TC)</td>
<td>Dolutegravir (DTG)</td>
</tr>
<tr>
<td></td>
<td>Raltegravir (RAL)</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Regimens*</th>
<th>HIV+ (n=4)</th>
<th>HIV- (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAF/FTC/BIC</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>TAF/FTC/EVG/c + DRV</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>TAF/FTC + DTG</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>TAF/FTC</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>TDF/FTC</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

*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

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

Division of HIV Prevention
Amy Martin
Chuong Dinh
Eric Edwards
Angela Holder
Ayanna Green
Jeff Fountain
Jeff Johnson
Amanda Smith
Robin MacGowan

UNC-Chapel Hill
Lisa Hightow-Weidman

University of Michigan
Rob Stephenson

Emory University
Patrick Sullivan
Ruth Dana
Anandi Sheth
Colleen Kelley
Disclaimer

The findings and conclusions in this presentation are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.
Questions?