Clinical Accuracy of an Enzymatic Reverse Transcriptase Test for Monitoring Antiretroviral Adherence

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BACKGROUND

- In 2024, ~31.6M people received ART and ~3.5M accessed PrEP worldwide, with **tenofovir (TFV) - based oral regimens** continuing as the backbone.
- Although cost-effective and widely available, **daily oral ART and PrEP remain challenging** for many to sustain adequate adherence and sufficient drug concentrations.
- **Adherence monitoring using drug concentrations** can identify those needing adherence support or transition to long-acting injectables.
- The **RE**verSe **TR**anscr**I**ptase **C**hain **T**ermination (**RESTRICT**) assay measures tenofovir diphosphate (**TFV-DP**) concentrations in blood by quantifying the enzymatic inhibition of reverse transcriptase.
- **This study evaluated the clinical accuracy of RESTRICT against liquid chromatography tandem mass spectrometry (LC-MS/MS), for monitoring real-world, long-term adherence to TFV-based oral ART and PrEP.**

METHODS

- **Design & setting**: Cross-sectional study at two ART/PrEP clinics in Seattle.
- **Participants**: Adults on stable TFV-based oral ART or PrEP for ≥ 3 months
- **Specimens**: Fresh whole blood tested by RESTRICT; Dried blood spots (DBS) tested by LC-MS/MS.
- Receiver Operating Characteristics analysis:
  - *Adherence classified by TFV-DP from LC-MS/MS*
  - *Separate benchmarks for tenofovir disoproxil fumarate (**TDF**) vs. tenofovir alafenamide (**TAF**)*
  - *Poor adherence : < 4 doses/wk)*
  - *Suboptimal adherence : < 7 doses/wk*

RESULTS

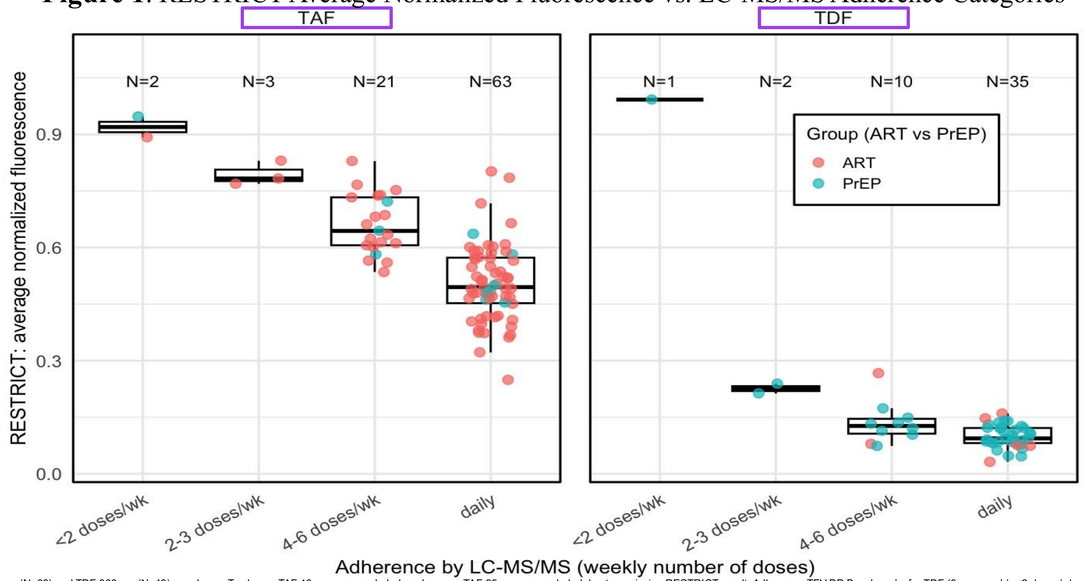
- **Participant characteristics**: 150 adults enrolled (100 on ART, 50 on PrEP): median age at 53.8 years; 92% male; 63.3% white; 12 on 10mg TAF-based ART excluded from the following analysis
- **48 on TDF-based regimens** (300mg): 40 PrEP clients(83%); 8 ART patients(17%).
- **90 on TAF-based regimens** (25mg): 10(11%) PrEP clients; 80(89%) ART patients.
- **Adherence** (TFV-DP in DBS by LC-MS/MS): 72% consistent with **daily dosing**, 23% **4-6 doses/wk**, 5% **<4 doses/wk**.
- **Correlation**: **RESTRICT fluorescence** vs **DBS TFV-DP by LC-MS/MS**:
  - TDF: Pearson r = –0.92 (95% CI: –0.95 to –0.86)
  - TAF: Pearson r = –0.69 (95% CI: –0.78 to –0.57)

Table 1: Sensitivity and specificity of the RESTRICT assay against LC-MS/MS*

		Sensitivity		Specificity	
Non-adherence		n/N_{pos}	% (95% CI)	n/N_{neg}	% (95% CI)
< 7 doses/wk					
	TDF	8/13	62%(38%-85%)	30/35	86%(74%-97%)
	TAF	22/26	85%(69%-96%)	55/63	87 %(79%-95%)
< 4 doses/wk					
	TDF	3/3	100%(37%-100%)	44/45	97.78%(93%-100%)
	TAF	5/5	100%(55%-100%)	81/84	96.43%(92%-100%)

**One removed due to missing RESTRICT*

Figure 1. RESTRICT Average Normalized Fluorescence vs. LC-MS/MS Adherence Categories*



* wk= week. Only participants on TAF 25 mg (N=89) and TDF 300 mg (N=48) are shown. Twelve on TAF 10 mg were excluded, and one on TAF 25 mg was excluded due to a missing RESTRICT result. Adherence TFV-DP Benchmarks for TDF (3mm punch): <2 doses/wk: <450 fmol/punch; 2-3 doses/wk: 450-899 fmol/punch; 4-6 doses/wk: 900-1599 fmol/punch; daily: ≥ 1600 fmol/punch. Adherence TFV-DP Benchmarks for TAF (2 x 7mm punches): <2 doses/wk: <450 fmol/punch; 2-3 doses/wk: 450-949 fmol/punch; 4-6 doses/wk: 950-1999 fmol/punch; daily: ≥ 2000 fmol/punch.

CONCLUSIONS

- The RESTRICT assay demonstrated high accuracy for detecting poor long-term adherence to TDF-based PrEP and ART.
- With simpler preparation, faster turnaround, and widely available lab equipment, the RESTRICT assay shows strong potential as a practical alternative to LC-MS/MS.
- Further refinement is needed to optimize RESTRICT calibration and adherence benchmarks for TAF-based regimens.

LIMITATIONS

- Long-term adherence assessment assumed steady-state drug levels, excluding individuals on TFV-based regimens for <3 months.
- 10 mg TAF formulations were excluded, limiting generalizability to all TAF dosing strategies.
- Potential effects of drug-drug interactions and pharmacologic boosters were not evaluated and warrant further study.

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