Published in final edited form as:

Curr HIV/AIDS Rep. 2020 October ; 17(5): 487–498. doi:10.1007/s11904-020-00512-3.

Point-of-care and near real-time testing for antiretroviral adherence monitoring to HIV treatment and prevention

Paul K. Drain^{1,2,3}, Ashley R. Bardon^{1,3}, Jane M. Simoni^{1,4,5}, Tim R. Cressey^{6,7,8}, Pete Anderson⁹, Derin Sevenler¹⁰, Ayokunle O. Olanrewaju¹¹, Monica Gandhi¹², Connie Celum^{1,2}

¹Department of Global Health, Schools of Medicine and Public Health, University of Washington, Seattle, USA ²Department of Medicine, School of Medicine, University of Washington, Seattle, USA ³Department of Epidemiology, School of Public Health, University of Washington, Seattle, USA ⁴Department of Psychology, University of Washington, Seattle, USA ⁵Department of Gender, Women, and Sexuality Studies, University of Washington, Seattle, USA ⁶PHPT/IRD 174, Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Thailand ⁷Harvard T.H. Chan School of Public Health, Harvard University, Boston, USA ⁸Department of Molecular and Clinical Pharmacology, University of Liverpool, United Kingdom ⁹Department of Pharmaceutical Sciences, University of Colorado, Aurora, CO, USA ¹⁰Center for Engineering in Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, USA ¹¹Department of Mechanical Engineering, University of Washington, Seattle, USA ¹²Department of Medicine, University of California, San Francisco, San Francisco, USA

Abstract

Purpose of Review—In this report, we review the need for point-of-care (POC) or near real-time testing for antiretrovirals, progress in the field, evidence for guiding implementation of these tests globally, and future directions in objective antiretroviral therapy (ART) or pre-exposure prophylaxis (PrEP) adherence monitoring.

Recent Findings—Two cornerstones to end the HIV/AIDS pandemic are ART, which provides individual clinical benefits and eliminates forward transmission, and PrEP, which prevents HIV acquisition with high effectiveness. Maximizing the individual and public health benefits of these powerful biomedical tools requires high and sustained antiretroviral adherence. Routine monitoring of medication adherence in individuals receiving ART and PrEP may be an important component in interpreting outcomes and supporting optimal adherence. Existing practices and subjective metrics for adherence monitoring are often inaccurate or unreliable, and therefore, are generally ineffective for improving adherence. Laboratory measures of antiretroviral

Correspondence to: Dr. Paul K Drain , 325 Ninth Ave, UW Box 359927, Seattle, WA 98104-2420, pkdrain@uw.edu, Fax: 206-520-3831; Phone: 206-520-3820.

Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

Human and Animal rights and Informed Consent

This article does not contain any data from studies with human or animal subjects that was performed by any of the authors and has not already been published elsewhere.

concentrations using liquid chromatography tandem mass spectrometry have been utilized in research settings to assess medication adherence, although these are too costly and resource-intensive for routine use.

Summary—Newer, less costly technologies such as antibody-based methods can provide objective drug level measurement and may allow for POC or near-patient adherence monitoring in clinical settings. When coupled with timely and targeted counseling, POC drug level measures can support adherence clinic-based interventions to ART or PrEP in near real-time.

Keywords

HIV; adherence; antiretroviral therapy (ART); pre-exposure prophylaxis (PrEP); point-of-care test; tenofovir

INTRODUCTION

Over 20 million people living with HIV (PLHIV) are receiving antiretroviral therapy (ART), and millions more are expected to start in the coming years (1). Newer ART regimens that include potent HIV integrase strand transfer inhibitors (INSTIs) with a high barrier to resistance may reduce the prevalence and impact of HIV drug resistance (2). The Joint United Nations Programme on HIV/AIDS (UNAIDS) has set an ambitious goal of initiating 95% of all PLHIV on ART by 2030 (3). The feasibility of ending the HIV/AIDS epidemic will in part depend on achieving near-universal coverage of knowledge of HIV status, ART for all PLHIV, and maintaining suppressed HIV viral load (VL), which requires ongoing adherence, but prevents onward HIV transmission (4–6).

Recent treatment as prevention trials, however, highlight the challenges of reaching near universal coverage of ART and the continued importance of primary prevention (7). When taken with good adherence, either daily or on an event-driven basis (8), PrEP significantly reduces the risk of HIV acquisition and is an important tool for HIV prevention (9–11). The World Health Organization (WHO) recommends all HIV-uninfected persons in areas with high risk of HIV acquisition (defined as ≥3% HIV incidence) receive pre-exposure prophylaxis (PrEP) to prevent HIV acquisition (12). Globally, millions of people at risk of HIV acquisition, including approximately 1.2 million individuals in the United States (U.S.), qualify for PrEP (13). However, the estimated number of actual PrEP users may be far less.

The efficacy of both ART and oral PrEP are highly dependent on patients maintaining adequate adherence (14). Ending HIV/AIDS will require improvements in HIV medication adherence from current levels to prevent further acquisition and transmission and to improve HIV-related outcomes. Therefore, routine monitoring of medication adherence may have a crucial role in supporting individuals receiving ART or PrEP. All PrEP and approximately 90% of ART regimens include tenofovir disoproxil fumarate (TDF), or tenofovir alafenamide (TAF), co-formulated with lamivudine or emtricitabine (FTC). In this review, we present the need for point-of-care (POC) or near real-time adherence testing, technologies currently available for real-time, objective monitoring of tenofovir-based ART and PrEP adherence, evidence for implementing these technologies globally, and future directions in objective HIV adherence measurement.

NON-PHARMACOLOGIC MEASURES OF ADHERENCE

For the millions of PLHIV receiving ART and those at risk of HIV acquisition who are on PrEP, adequate adherence remains critical to effectiveness (15,16). An accurate assessment of adherence that is available during a clinical encounter allows for immediate clinical decision-making and provides an opportunity for timely, accurate, and strategic provider-patient communication around medication-taking. Appropriate counseling and interventions that can be conducted in real-time during the patient's visit may help address adherence barriers and lead to more optimal treatment outcomes (17,18).

In routine clinical practice, however, obtaining an accurate account of real-time adherence to HIV treatment or PrEP has proven challenging. Clinicians often rely on clinical measures of therapeutic impact of ART, most often HIV RNA VL, or decreasingly, CD4 lymphocyte counts or presence of opportunistic infections. However, the latter indicators are not relevant for PrEP, are more often indicative of long-standing adherence problems to ART, and are not often available in real-time in routine care. Delayed recognition of adherence problems can mean missed opportunities for more timely counseling and interventions prior to the onset of prevention failure, virologic failure, or immunosuppression. Moreover, waiting for these indicators can be particularly problematic since adherence patterns are established early in the course of treatment, and early attrition in ART programs remains high (19).

Several subjective measures of adherence, such as self-reported adherence, clinician assessments, pill counts, pharmacy refill records, and clinic visit attendance, have been widely used to assess adherence at the time of a clinical encounter. These measures are convenient, inexpensive, and offer flexibility in the mode of administration and timing of assessment to provide detailed data on patterns of medication-taking (20). However, they all have serious shortcomings for both validity and reliability. Self-reports are susceptible to social desirability and affirmation biases, as well as inaccurate recall, and concerns about their tendency to overestimate adherence in comparison to more direct measures, such as drug levels, are well-documented (20–24). Clinician assessment is notoriously flawed and generally not better than chance predictions (25). Pill counts have been associated with other measures of adherence, including electronic monitoring devices, self-report, and concurrent (yet not reliably correlated) viral suppression (26). However, pill counting is subject to inaccuracies from sharing of medications, forgetting to bring pills to clinics, and intentional pill dumping, and therefore, has been poorly correlated with drug levels (27,28). Pharmacy refill records, which need to be comprehensive, systematically recorded, readily retrievable, and facilitated by a universal health care system, do not reliably correlate with viral suppression, especially in low- and middle-income countries (LMICs) and adolescents (29–32). Clinical attendance, especially timeliness of attendance, can be an indicator of self-reported medication adherence and immune function, but existing studies have not demonstrated reliable associations with HIV VL (33–35).

Electronic drug monitoring, such as the medication event monitoring system (MEMS), WisePill, along with newer technologic advances, offer more objective estimates of adherence, yet they are expensive, cumbersome, and subject to non-use, which is difficult to distinguish from non-adherence (17,36). With the rapid increase of PLHIV initiating ART

globally and the desired uptake in PrEP, there is an urgent need to redesign models of antiretroviral monitoring and management, while not increasing the burden to HIV providers, laboratories, and health systems (37–40), especially in LMICs. Objective data and counseling on recent medication adherence provided at regular intervals and in real-time may open the window for more accurate and strategic communication about medication-taking during routine ART and PrEP visits.

ANTIRETROVIRAL DRUG LEVELS IN BIOLOGICAL SAMPLES AS AN OBJECTIVE ADHERENCE MEASURE

Pharmacologic measurements of antiretroviral drugs or their metabolites have been routinely used to assess adherence in multiple PrEP studies and clinical trials (41). Biological samples for adherence assessments are generally not timed samples at “peaks” or “troughs” after dosing, but rather, obtained at “random” times after dosing during a clinical visit. However, pharmacologic measurements for PrEP and ART adherence assessment can be grouped into “recent dosing” and “cumulative dosing” metrics (Table 1) (41). Because TDF and TAF are commonly used for both ART and PrEP, and co-formulated with lamivudine or FTC, we will focus mostly on tenofovir (TFV)-based regimens and their metrics.

Short-term metrics of adherence

Recent dosing assessment includes measuring TFV and/or FTC in plasma (42), urine (43–45), and saliva samples (46), as well as emtricitabine-triphosphate (FTC-TP) in red blood cells (RBCs) (47). The interpretation of recent dosing adherence is a function of the drug half-life and/or pharmacokinetic variability of the drug moiety and can provide useful information about the timing of the last dose. For example, TFV has a plasma terminal half-life of approximately 15 hours, which results in minimal accumulation with repeated daily doses (42,48). Thus, TFV concentrations following a single dose are similar to concentrations following repeated daily doses, which is a major limitation of short-term metrics since recent dosing may not reflect typical patterns of dosing. Sensitive TFV plasma assays (e.g., with lower limit of quantification [LLOQ] of 0.3 ng/mL) can detect TDF ingestion within the prior seven days, whereas less sensitive TFV plasma assays (e.g., with LLOQ of 10 ng/mL) detect TDF dosing within the preceding two days (9,42). However, these short-term metrics are subject to “white coat” adherence bias, where an individual may take a dose or doses just prior to a clinic visit or drug level testing (49). Nevertheless, placebo-controlled PrEP efficacy trials demonstrated a strong relationship between plasma TFV levels and PrEP efficacy (14,50), and a recent study in South Africa found plasma TFV levels to be good predictors of viral suppression among women receiving ART (51,52).

Other biological samples, including urine, fingerprick whole blood, and oral swabs, may be more accessible and less invasive for a POC and near real-time test. Urine TFV concentrations are much higher and more variable than plasma TFV concentrations, but TFV exhibits a similar half-life in urine to plasma (43–45). Sensitive urine assays (e.g., 50 ng/mL) can detect TDF ingestion as far back as 10–14 days following drug cessation (43,44). Saliva and oral fluid TFV concentrations are lower and more variable compared with plasma, but are easier to collect and have potential for self-testing, as demonstrated by

several HIV self-tests (53). In two studies, testing saliva and oral fluid was preferred by both adult and adolescent patients over the collection of fingerprick blood, but these results may not be generalizable (54,55). Detection of TFV in saliva poses the additional challenge that TFV concentrations are only 2–3% of the plasma TFV concentration, or roughly 0.5 ng/mL to 5 ng/mL (45,53). However, this is not beyond the limits of detection for small molecule drugs by POC diagnostic immunoassays since several drugs of abuse are detectable at these low levels with existing technologies (56).

Finally, FTC-TP in dried blood spots (DBS) is a metabolite with a short half-life (~31 hours) and is highly correlated with recent dosing of TDF/FTC and TAF/FTC (47,57). However, blood from fingerprick or venipuncture must be spotted onto cards to form a DBS within 60 minutes after collection to allow for the accurate measurement of intracellular FTC-TP (47). Blood resting at room temperature prior to spotting will allow any FTC from plasma (if present) to load RBCs during this time, causing a spuriously high result for FTC-TP (58).

Long-term metrics of adherence

Longer-term metrics of adherence include tenofovir-diphosphate (TFV-DP) in peripheral blood mononuclear cells (PBMCs) and RBCs (DBS), and TFV and FTC in hair (59–61). The longer half-life of these drugs or metabolites in their respective biological samples results in drug accumulation, which can provide an indication of averaged adherence over longer time periods. Long-term metrics, therefore, are less susceptible to “white coat” adherence (61–63). To date, directly-observed therapy (DOT) trials have shown strong linear relationships between patterns of TFV-based drug dosing (e.g., two, four, and seven doses per week) and TFV or TFV-DP concentrations and a reduced risk of HIV acquisition in the context of PrEP (14,62,64,65). Intracellular TFV-DP in PBMCs has a half-life of approximately four days, and accumulation of approximately five- to eight-fold with daily TDF dosing (42,48). Efficacy thresholds or ‘benchmarks’ were estimated for TFV-DP in PBMCs, including 40 fmol/10⁶ cells and 83 fmol/10⁶ cells (freshly lysed cells) as the EC90 and EC99 for adults receiving PrEP, respectively (62). These concentrations represented approximately two to three TDF doses per week and seven TDF doses per week on average over the preceding weeks. However, PBMC collection is laborious and expensive, and therefore, not practical for routine clinical settings. TFV-DP in DBS exhibits a 17-day half-life and 25-fold accumulation, which provides a measure of cumulative daily TDF adherence over one to two months (61). In the iPrEx open label extension (OLE) study of daily TDF/FTC in 1,225 men who have sex with men (MSM), none of the 28 observed HIV infections occurred in PrEP-taking participants who had TFV-DP 700 fmol/punch, corresponding with 100% efficacy (95% confidence interval [CI]: 84–100%) (65). An equivalent threshold for TFV-DP and effectiveness among women has yet to be established, although directly-observed dosing of TDF/FTC has established comparability and dose-proportionality of DBS TFV-DP measurements in women and men (62). TFV-DP 700 fmol/3mm punch is commensurate with 4 doses per week on average according to DOT studies with TDF and 950 fmol/two 7mm punches for TAF (61,62). Strong associations were also observed between TFV-DP in DBS and virologic outcomes in those receiving ART (66,67).

Hair samples have been tested for TFV and FTC as another measure of cumulative dosing (59). Testing can be conducted on small hair samples (100–200 strands) using hair strands proximal to the scalp and cut down to 1 to 1.5 cm, representing about four to six weeks of hair growth. Hair levels can reflect dosing out to many weeks, depending on how long the hair sample is and how the hair is cut (68). TFV and FTC hair concentrations are highly correlated with TFV-DP and FTC-TP levels in DBS, respectively, and higher TFV concentrations in hair are associated with greater increases in creatinine on PrEP (69,70). Drug concentrations can be assayed along the length of the hair shaft, providing an estimate of adherence over different time periods in the past (71–73). Segmental hair analysis, therefore, can provide granularity in assessing patterns of adherence, which is helpful to overcoming some of the limitations of longer-term metrics. Finally, concentrations of antiretroviral drugs in hair have been associated with virologic outcomes on ART (74). Hair collection is noninvasive, and hair can be stored and shipped at room temperature and without biohazard. As with other long-term metrics of adherence, development of POC tests have been hampered by the need for relatively complex measurement by tandem mass spectrometry (75).

POINT-OF-CARE URINE TENOFOVIR TESTING

Several POC tests intended to measure TFV for monitoring adherence are either in development, have been developed, or are currently being evaluated in clinical studies. Recently, several groups have been developing and validating POC lateral flow immunoassays to detect TFV in urine (76–79). These assays use antibody-based technology and provide results within minutes, similar to a urine-based pregnancy test. This type of POC test can provide information on the actual ingestion of a TFV-based compound directly, allowing for objective and real-time measurement of adherence during a routine clinical visit. Currently, however, these tests have not yet been approved for routine clinical use.

The existing POC TFV tests are single antibody inhibitive immunoassays that utilize an immunoglobulin G (IgG) or M (IgM) antibody that selectively binds to the phosphonate or amine group of the TFV molecule. A POC antibody-based TFV test can be developed as a semi-quantitative test or as a qualitative (yes/no) test for a pre-specified drug concentration threshold. Determining a threshold for a qualitative test will introduce differences in diagnostic sensitivity and specificity in relation to actual pill ingestion and clinical adherence. Setting a higher drug concentration threshold will increase diagnostic sensitivity and decrease specificity, which can lead to more false positive results. For example, one POC antibody-based TFV test set a detection threshold of 1,500 ng/mL, which was estimated to provide 98% accuracy for detecting TDF ingestion within the preceding 24 hours (78,80). Another immunoassay set a detection threshold of 1,000 ng/mL, based on clinical data from observed cross-sectional mass spectrometry data (43). While the test may be as simple to use as an over-the-counter urine pregnancy test, all short-term metrics of adherence will remain susceptible to issues of “white coat” adherence.

The development of these novel POC TFV-based metrics of adherence will present several challenges and opportunities for delivering ART or PrEP, particularly in LMICs. An ideal test to monitor ART or PrEP adherence would be low cost, have a fast turnaround time, and

be simple to both administer and interpret results (Table 2). The POC tests in development are low cost and easy to administer. However, while urine collection is generally more acceptable than blood collection, its collection also requires some personal privacy, which is not always achievable in a busy clinical setting. Several studies (detailed below) are currently being planned to determine the feasibility, acceptability, impact, and cost-effectiveness of implementing urine POC TFV testing for monitoring ART or PrEP adherence in LMICs.

POINT-OF-CARE TESTING FOR ADHERENCE MONITORING IN HIV CARE

As new technologies for HIV care emerge, the potential for improvements in the HIV care cascade and HIV-related outcomes may broaden. POC tests intended for use in HIV care settings include HIV diagnostic tests, CD4 cell counts, and HIV VL tests. Since 2007, the U.S. Centers for Disease Control and Prevention (CDC) and the WHO have recommended that primary care settings integrate POC diagnostic tests into routine HIV screening and diagnostics (81–83). Newer POC TFV tests may improve HIV outcomes by allowing timely, actionable counseling and interventions for PLHIV who present with poor ART adherence. Therefore, integrating POC TFV testing into HIV care could have a significant impact on HIV-related outcomes.

Many studies have demonstrated the positive impacts of POC HIV self-testing platforms, as well as POC VL and CD4 testing, in the context of HIV screening and care, but the potential impact of POC urine TFV-based adherence testing on care outcomes for PLHIV is currently unknown. Real-time, POC urine TFV testing may improve adherence monitoring and offers the opportunity for earlier, better-informed adherence counseling and support for PLHIV receiving ART (23). The STREAM-HIV ([Clinicaltrials.gov NCT04341779](https://clinicaltrials.gov/ct2/show/study/NCT04341779)) and Standing Tall studies are planned to assess the feasibility and acceptability of POC urine TFV monitoring in varying settings in South Africa, as well as its effect on ART adherence, retention in care, viral suppression, drug resistance, and cost of care (84). Additionally, these studies will evaluate the potential effects of integrating POC urine TFV testing into care with other POC tests, such as a POC VL test. A qualitative study of providers, PLHIV receiving ART, and people receiving PrEP in Seattle found that POC urine TFV testing was acceptable and potentially useful for better informed clinical decision-making (85).

Overall, POC tests in the context of HIV screening and care may identify new infections earlier, link newly diagnosed PLHIV to care more rapidly, improve HIV-related outcomes, reduce costs, and improve health care efficiencies by reducing turnaround time for test results and reducing time in clinic for PLHIV receiving ART. Rapid HIV screening at the point of care has the potential to yield higher volumes of HIV diagnoses (86), while HIV self-testing in various settings has been found to increase uptake and frequency of testing (87).

A meta-analysis of studies that integrated POC CD4 cell count into care found improvements in projected life expectancy and reduced costs when CD4 count was conducted in real-time at the clinic as compared to laboratory-based testing (88). With more recent universal test-and-treat guidelines, countries have shifted away from routine CD4

testing in favor of treating all PLHIV regardless of CD4 count and have instead placed more emphasis on VL testing to monitor ART adherence (89). However, POC CD4 count at diagnosis may continue to play a role in HIV care, as WHO guidelines recommend intensive follow-up and care for those with a low CD4 count (90).

Several POC tests for measuring HIV VL are currently available, and have proven to be effective for facilitating rapid ART initiation and improving retention in care for infants, as well as increasing viral suppression rates and improving retention in care for adults (91–93). POC HIV VL test results may also prompt earlier interventions, such as ART regimen switches, when needed (92,94). POC VL monitoring costs are similar to laboratory-based VL testing, but POC VL monitoring may be more cost-effective when accounting for improvements in clinical care (95–97).

Integrating POC urine TFV testing into HIV care with other POC tests may prove effective in earlier identification of those at risk of defaulting from care, stopping treatment, viremia, or developing ART drug resistance. POC adherence testing could lead to targeted and expedited enhanced adherence counseling or necessary ART regimen switches. More research is still needed to elucidate the role of POC urine TFV monitoring in care and how it may be used alongside other POC tests (Figure 1), such as a VL test, to improve outcomes along the HIV continuum of care and efficiencies within the health care system.

TENOFOVIR AND TENOVOFIR-DIPHOSPHATE DRUG LEVELS FOR PrEP ADHERENCE COUNSELING

The use of antiretroviral drug levels was essential to understanding a strong dose-response relationship between PrEP dosing (as measured by plasma TFV levels) and observed efficacy in several PrEP clinical trials (51). In the era of PrEP implementation, using drug levels to guide PrEP adherence counseling is a nascent field, and few studies have used TFV-DP levels to provide feedback. Qualitative research among 59 MSM who participated in the iPrEX open label extension study indicated that detectable/undetectable drug level feedback was highly acceptable, and half the participants considered the information useful (98). In the VOICE PrEP trial, a subset of women who received feedback about their drug levels found the information to be useful and suggested that real-time drug level monitoring and feedback would promote more honest discussions about PrEP use (99). In a single-arm longitudinal study, a brief intervention based on results from drug level monitoring may have increased PrEP adherence among self-identified MSM and transgender women in Los Angeles (100).

The HIV Prevention Trials Network (HPTN) 082 study was designed to evaluate initiation and adherence to PrEP among African adolescent girls and young women (AGYW). AGYW were randomized to either a standard adherence support package (two-way SMS, adherence clubs, and brief counseling) versus enhanced adherence support, which also included antiretroviral drug level monitoring (via TFV-DP levels in DBS) with feedback and appropriate counseling. The drug level categories used a threshold of 500 fmol/punch at week four and 700 fmol/punch at week eight for the high adherence category, based on the threshold of TFV-DP that was associated with 100% effectiveness of PrEP among MSM in

iPrEx OLE (65). At study conclusion, adherence at six months was similar between AGYW in the two adherence intervention arms (101). However, almost 40% of AGYW in the intervention arm did not receive drug level counseling because of shipping and laboratory turnaround time, missed visits, or incorrect counseling about adherence due to transcription errors (101).

Two studies will soon be starting to evaluate the acceptability, feasibility, and impact of implementing POC urine TFV adherence testing and counseling for PrEP. First, the PUMA trial will be a pilot randomized clinical trial to determine whether POC TFV adherence testing with subsequent feedback improves long-term PrEP adherence (measured via TFV levels in hair) among Kenyan women ([Clinicaltrials.gov NCT03935464](https://clinicaltrials.gov/ct2/show/study/NCT03935464)) (102). Second, the PrEP SMART study will compare the effectiveness of a scaled approach to PrEP adherence support strategies among South African AGYW ([Clinicaltrials.gov NCT04038060](https://clinicaltrials.gov/ct2/show/study/NCT04038060)) (103). In the PrEP SMART trial, young women initiating PrEP will be randomized to either two-way SMS or WhatsApp peer support groups, and those who have moderate or low PrEP drug levels after two months of use (intracellular TFV-DP levels <500 fmol/punch) will undergo a secondary randomization to more intensive adherence support, either quarterly drug level feedback based on detectable urine TFV via the POC assay or monthly adherence counseling sessions. These studies will inform the effectiveness of real-time adherence counseling based on urine TFV monitoring on subsequent PrEP adherence. Comparative acceptability, effectiveness, and cost-effectiveness studies of TFV assays that provide a measure of a longer window of PrEP use, such as TFV-DP levels in DBS or TFV levels in hair, are needed in diverse populations of PrEP users.

FUTURE DIRECTIONS IN POC ANTIRETROVIRAL TESTING

Existing POC urine TFV immunoassays are limited by the half-life of TFV in urine, which limits information on adherence to the prior week or less, informing drug level feedback and counseling accordingly. A POC test in development for measuring intracellular TFV-DP (which allows for longer-term adherence monitoring) may be an important tool in the future of adherence and therapeutic drug monitoring for ART and PrEP. New technologies for near real-time measurement of TFV-DP using benchtop platforms would provide timely, informative, and actionable information for patients and providers in a range of clinical settings. Drug measurements may also be useful for newer long-acting drug formulations, particularly during the long pharmacokinetic tail of long-acting drugs (104).

Benchtop liquid chromatography-mass spectrometry for near real-time drug testing

Liquid chromatography tandem mass spectrometry (LC-MS/MS) is widely used to measure TFV and TFV-DP to determine ART and PrEP adherence in research settings (44,62). Portable mass spectrometers have been developed for applications in forensics, environmental monitoring, and clinical diagnostics (105–108). LC-MS/MS technology may be possible to apply for near real-time drug level testing, and instruments could be adapted to detect a wide variety of biomarkers (109). While complex sample preparation and throughput are major barriers, newer portable instruments are compatible with complex sample matrices, easy-to-use disposable cartridges, and improved software for automated

analysis (107). Perhaps the largest obstacle to widespread deployment of these instruments for HIV treatment and PrEP adherence monitoring are the unit and per-sample costs, which are still not competitive with POC antibody-based diagnostic tests. Nevertheless, clinical use of LC-MS/MS-based technology continues to increase for laboratory medicine and for diagnosing infections in clinical settings (109–111). The DBS assay developed by Anderson *et al* is now in development for near real-time use (62). Increasing miniaturization, as well as broader incorporation of LC-MS/MS technology into different areas of medicine, may converge to enable near real-time adherence testing in the setting of HIV treatment and prevention (112).

Detection of tenofovir-diphosphate by competitive immunoassay

A competitive immunoassay to measure TFV-DP could be developed into a POC lateral flow assay, but poses some additional challenges. Unlike TFV, which is freely circulating in urine or serum, measurement of intracellular TFV-DP requires cell lysis before detection. The clinical concentration range of TFV-DP in blood lysate is 15–170 fmol/10⁶ RBCs or about 75 nM–850 nM in whole blood, which is two- to five-fold higher than the clinical range of TFV in plasma (17 nM–170 nM), and 20-fold lower than TFV in urine (1.7 µM–170 µM) (44,61). In addition, cell lysate contains high concentrations (0.1–1.5 mM) of adenosine triphosphate, which has a similar molecular structure to TFV and may lead to non-specific binding to the detection antibody (113). Cell lysis solutions can be integrated into the lateral flow consumable for POC diagnostic tests (114,115). A POC diagnostic test for TFV-DP will be more complex, but will also provide more clinical information than a POC TFV test alone. New immunoassay formats may also be able to use fingerprick blood samples since they are compatible with small sample volumes (<5 µL), and previous studies have established correlations between HIV drug metabolites in venous and fingerprick blood draws (116).

Detection of tenofovir diphosphate by enzymatic assay

In contrast to an immunoassay, TFV-DP could also be detected by an enzymatic assay that measures the drug's pharmacological activity (117). DNA synthesis by reverse transcriptase (RT), the enzyme targeted by TFV-DP, can be measured using DNA templates, nucleotides, and fluorescent dyes to estimate TFV-DP concentration in a patient sample. A theoretical model to predict fluorescence output of the assay as a function of TFV-DP levels was developed and validated using TFV-DP spiked in whole blood to demonstrate the assay operates in clinically relevant samples (118). The assay distinguished TFV-DP levels corresponding to low and high PrEP adherence. The approach may be suitable as a POC test, since it is fast (30-minute assay time), requires a small volume of blood that can be collected from a fingerprick, and has a simple sample preparation step (dilution in water). Enzymatic assays monitoring activity of HIV drugs and their metabolites are currently being validated with clinical samples and benchmarked against drug level measurement by LC-MS/MS.

CONCLUSIONS

Newer technologies and assays are emerging that allow for POC and near real-time antiretroviral drug level testing for monitoring adherence to HIV treatment and prevention.

These novel POC tests may improve real-time adherence counseling and interventions to reduce barriers and improve outcomes. Short-term metrics of adherence (such as TFV levels in urine) are more amenable to POC, but are more fallible to “white coat” dosing than long-term adherence metrics (80). Several POC urine TFV tests have been developed and will be evaluated in upcoming clinical trials to determine acceptability, feasibility, costs, and impact on subsequent ART or PrEP adherence. Furthermore, measurement of drug pharmacokinetics from POC tests may allow personalized assessment of drug metabolism and enable patients and providers to tailor ART dosing schedules and PrEP delivery.

As the antiretroviral drug landscape evolves for both ART and PrEP, identifying and assessing other potential drugs/metabolites to assess recent and/or cumulative adherence will be important. In addition, more research will be needed on the methodology transfer from sophisticated lab-based scientific instruments to quantify drug concentrations to less costly POC tests that enable providers to counsel about adherence at the same visit. Future POC and near real-time tests should be suitable for newer formulations of TFV, such as tenofovir alafenamide (TAF), but may need to be adapted for newer drug classes, including INSTIs (119). As efforts accelerate to end the HIV/AIDS epidemic, POC adherence monitoring may be an essential tool for both achieving viral suppression for PLHIV and preventing new infections with PrEP.

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Figure 1. Integration of POC testing in the HIV continuum of care.

*Should a POC resistance test become available

Table 1.

Antiretroviral drug concentration thresholds and interpretations.

Drug Metabolite	Threshold Concentration	Interpretation	Limitations
<i>Short-term metrics of adherence</i>			
TFV urine (44,78)	1,000–1,500 ng/mL	Ingested dose in the preceding 24 hours	Recent dose only
FTC urine (45)	1,844 ng/mL	Ingested dose in the preceding 24 hours	Recent dose only
TFV plasma (42)	35 ng/mL	Ingested dose in the preceding 24 hours at steady state	Recent dose only; requires obtaining and processing venous blood; requires LC-MS/MS *
FTC plasma (42)	49 ng/mL	Ingested dose in the preceding 24 hours at steady state	Recent dose only; requires obtaining and processing venous blood; requires LC-MS/MS *
FTC-TP (DBS) from F/TDF (63)	0.125 pmol/3 mm punch	Ingested dose in the preceding 24 hours at steady state	Recent dose only; requires obtaining and processing venous blood; requires LC-MS/MS *
<i>Long-term metrics of adherence</i>			
TFV-DP (freshly lysed PBMC) (14)	40 fmol/10 ⁶ cells	2–3 doses/week on average; 90% effective concentration for MSM	Specialty test; impractical and too costly for widespread use; requires venipuncture; requires LC-MS/MS *
TFV-DP (DBS) from TDF (62,65)	700fmol/3mm punch	4 doses/week on average; >90% efficacy in MSM	Cumulative dosing only; requires LC-MS/MS *
TFV-DP (DBS) from TAF (58)	950 fmol/two 7 mm punches	4 doses/week on average	Cumulative dosing only; requires LC-MS/MS *
TFV scalp hair (59,69)	0.023 ng/mg	4 doses/week on average	Requires LC-MS/MS *

* LC-MS/MS limitations include expense and need for proper equipment and laboratory personnel

Table 2.

Target product profile for a POC TFV test.

Test Attributes	Minimum Acceptable	Ideal
Qualitative vs. quantitative	Qualitative	Semi-Quantitative
Specimen	Plasma or whole blood from venipuncture	Urine, saliva, or fingerprick blood
Specific analyte	Short-term metabolite (TFV)	Long-term metabolite (TFV-DP)
Power	Simple battery	None required
Cost	<\$20 per test	<\$2 per test
Capacity	1 sample	Multiple samples
Analysis time	<60 minutes	<10 minutes
Stability of result	30 minutes after analysis	180 minutes after analysis
Equipment/reader	Plate reader (if quantitative results)	Lateral flow readout (visual)
Sample preparation required	Low technological level that could be completed by a health worker	None required
Shelf life/stability	1 year at room temperature	2 years at room temperature
Sensitivity	90%	98%
Specificity	90%	98%