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PHARMACOLOGY

Evaluation of a Multidrug Assay for Monitoring Adherence to a Regimen for HIV Preexposure Prophylaxis in a Clinical Study, HIV Prevention Trials Network 073

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ABSTRACT Daily oral tenofovir disoproxil fumarate (TDF)-emtricitabine (FTC) is a safe and effective intervention for HIV preexposure prophylaxis (PrEP). We evaluated the performance of a qualitative assay that detects 20 antiretroviral (ARV) drugs (multidrug assay) in assessing recent PrEP exposure (detection limit, 2 to 20 ng/ml). Samples were obtained from 216 Black men who have sex with men (208 HIV-uninfected men and 8 seroconverters) who were enrolled in a study in the United States evaluating the acceptability of TDF-FTC PrEP (165 of the uninfected men and 5 of the seroconverters accepted PrEP). Samples from 163 of the 165 HIV-uninfected men who accepted PrEP and samples from all 8 seroconverters were also tested for tenofovir (TFV) and FTC using a quantitative assay (detection limit for both drugs, 0.31 ng/ml). HIV drug resistance was assessed in seroconverter samples. The multidrug assay detected TFV and/or FTC in 3 (1.4%) of the 208 uninfected men at enrollment, 84 (40.4%) of the 208 uninfected men at the last study visit, and 1 (12.5%) of the 8 seroconverters. No other ARV drugs were detected. The quantitative assay confirmed all positive results from the multidrug assay and detected TFV and/or FTC in 9 additional samples (TFV range, 0.65 to 16.5 ng/ml; FTC range, 0.33 to 14.6 ng/ml). Resistance mutations were detected in 4 of the 8 seroconverter samples. The multidrug assay had 100% sensitivity and specificity for detecting TFV and FTC at drug concentrations consistent with daily PrEP use. The quantitative assay detected TFV and FTC at lower levels, which also might have provided protection against HIV infection.

KEYWORDS antiretroviral drug, HIV, HPTN 073, preexposure prophylaxis, Truvada, adherence

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Daily preexposure prophylaxis (PrEP) with a combination of tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) is a safe and effective intervention for preventing HIV infection (1, 2). The efficacy of this regimen has been demonstrated in different risk groups, including men who have sex with men (MSM), transgender and cisgender women, serodiscordant couples, and persons who inject drugs (1, 3–7). In 2012, the U.S. Food and Drug Administration (FDA) approved daily oral use of TDF-FTC

for PrEP (8). TDF-FTC PrEP is now recommended by the U.S. Centers for Disease Control and Prevention and the World Health Organization for prevention of HIV infection (9, 10). A nondaily regimen that includes TDF-FTC dosing before and after sex events has also been shown to be effective for preventing HIV infection in MSM (11). The efficacy of TDF-FTC PrEP regimens is strongly associated with adherence (1, 2).

Antiretroviral (ARV) drug testing provides an objective biomedical measure of ARV drug use (12). ARV drug testing for evaluation of adherence to TDF-FTC regimens is often performed using quantitative assays based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) (13–17). Steady-state concentrations of tenofovir (TFV) in plasma are achieved after a few days of daily oral dosing. In a placebo-controlled PrEP efficacy trial, plasma TFV concentrations of >40 ng/ml were shown to provide 91% risk reduction for individuals receiving daily TDF-FTC (16). HIV Prevention Trials Network [HPTN] 066 was a 5-week, directly observed TDF-FTC dosing study (13); the study included a daily dosing regimen and a 4-dose/week regimen. The ~90% sensitivity thresholds for serum TFV and FTC concentrations in HPTN 066 were 35.5 and 49.1 ng/ml, respectively, for daily dosing and 4.2 and 4.6 ng/ml, respectively, for the 4-dose/week regimen (13). We previously developed a qualitative method based on high-resolution mass spectrometry (HRMS) (18) that is less costly than LC-MS/MS methods. That assay detected 15 or 16 ARV drugs in three drug classes (19–21). The multidrug assay was recently modified to detect 20 ARV drugs in five drug classes (22).

In this report, we validated the new version of the multidrug assay for ARV drug detection and determined its performance characteristics. We then evaluated the performance of the assay for monitoring PrEP exposure in a cohort of Black MSM enrolled in a clinical study evaluating PrEP uptake and adherence at three sites in the United States (Washington, DC; Los Angeles, California; and Chapel Hill, North Carolina), the HPTN 073 study (2013 to 2015) (23). Because the multidrug assay detects most ARV drugs currently in use, we were also able to assess the use of other ARV drugs among HIV-uninfected men and seroconverters in the HPTN 073 cohort.

RESULTS

Validation of the multidrug assay. Validation studies were performed to determine the performance characteristics of the multidrug assay. As a first step, we tested the ability of the assay to detect ARV drugs at the limit of detection (LOD) of the assay by injecting five replicates of an LOD control twice a day for 10 days. More than 95% of the samples spiked with compounds at the LOD were identified over the course of the experimental period. Carryover studies indicated that no carryover was present for any of the ARV drugs in blank injections following an injection of the same drug at a concentration of 2,000 ng/ml. Some carryover was detected at concentrations of 5,000 and 10,000 ng/ml. Therefore, a 2,000 ng/ml control was added to each plate. If any test sample had a signal above this control level for any ARV drug in the assay and the subsequent sample was positive for the same ARV drug, then the second sample was flagged for reinjection. The multidrug assay was further validated for detection of ARV drugs by performing an external method comparison study with 25 blinded samples (provided by the Johns Hopkins Clinical Pharmacology Analytical Laboratory) that had been analyzed previously using the gold standard, a quantitative LC-MS/MS assay. These samples contained 16 of the 20 drugs included in the current version of the multidrug assay (tipranavir, nelfinavir, darunavir, and zidovudine were not present in the blinded sample set). The multidrug assay correctly identified 98% of ARV drugs present in the blinded sample set (see Table S1 in the supplemental material). The sensitivity and specificity of the multidrug assay in this analysis were 98.7% and 100%, respectively. To supplement this analysis, an internal blinded spiking experiment was performed using 20 samples that contained the four ARV drugs not present in the previous validation sample set. These four drugs were present in the validation samples at various concentrations, and the validation samples were also spiked with TFV and FTC prior to testing. These samples were prepared in a different laboratory by a technologist who was not involved in sample analysis. In this set of samples, the

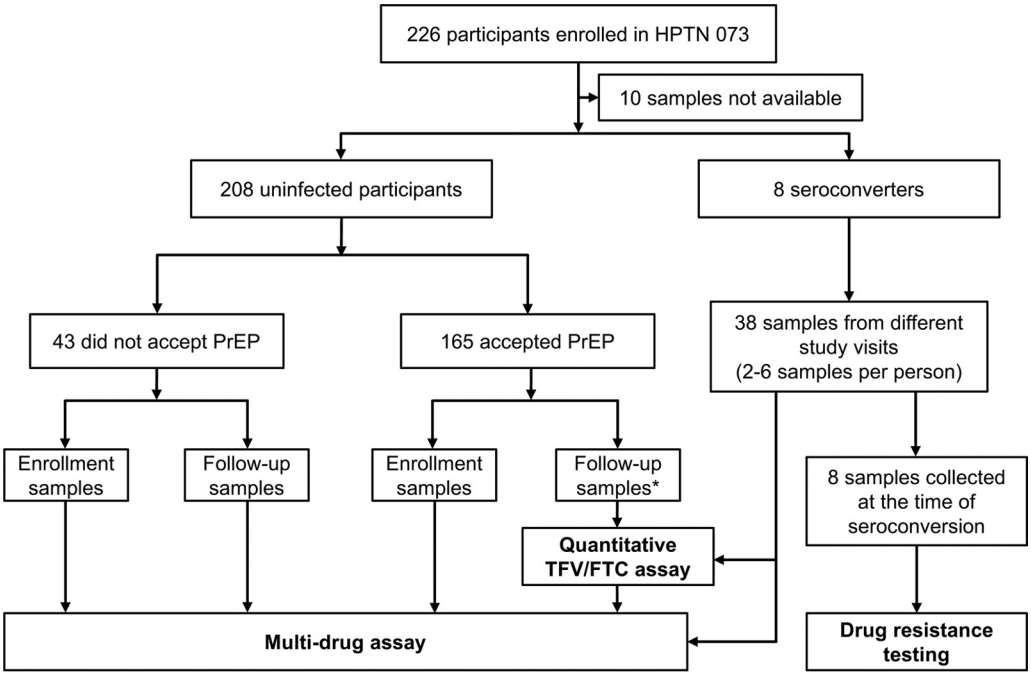

FIG 1 Flow chart of laboratory testing. All samples from 208 uninfected participants and 8 seroconverters were tested for the presence of ARV drugs using the qualitative multidrug assay; this assay detects 20 ARV drugs based on HRMS. The lower limit of detection was 2 to 20 ng/ml. A subset of follow-up samples from uninfected participants who accepted PrEP were tested using a quantitative assay for TFV and FTC that was based on LC-MS/MS. The lower limit of quantification was 0.31 ng/ml for both drugs. HIV drug resistance was assessed using the ViroSeq HIV-1 genotyping system and next-generation sequencing. The asterisk in the figure indicates that all 165 follow-up samples from uninfected PrEP acceptors were tested with the multidrug assay but two of the samples were not tested with the quantitative TFV-FTC assay due to insufficient sample volume.

multidrug assay detected all of the spiked compounds (100% agreement). Finally, 40 additional samples from ARV-naïve individuals were analyzed; no ARV drugs were detected in those samples.

Analysis of ARV drug use in HIV-uninfected men enrolled in HPTN 073. We further evaluated the performance of the multidrug assay by testing plasma samples collected in the HPTN 073 study (Fig. 1). Paired plasma samples collected from HIV-uninfected men at study enrollment and at the end-of-study visit were available for 208/226 (92%) of the men enrolled in the study. The 208 men included 43 men who did not accept PrEP and 165 men who accepted PrEP; 29 (17.6%) of the 165 men who accepted PrEP permanently discontinued PrEP during the study and 136 did not. The median time between study enrollment and collection of the end-of-study sample for the 208 men was 52 weeks (range, 4 to 89 weeks). The only ARV drugs detected using the multidrug assay were TFV and FTC. The multidrug assay detected TFV and/or FTC in 3 (1.4%) of the 208 enrollment samples and 84 (40.4%) of the 208 end-of-study samples (Table 1). All 3 of the enrollment samples and 81 (96.4%) of the 84 end-of-study samples in which drugs were detected were from men who accepted PrEP; 3 (3.7%) of the 81 men who accepted PrEP and had drugs detected at their last study visit reported that they had permanently discontinued PrEP before their last visit. TFV and FTC were not detected in any of the enrollment samples from the 43 men who did not accept PrEP, but they were detected in 3 (7.0%) of the end-of-study samples from those men.

Comparison of TFV and FTC detection using the multidrug assay and a quantitative TFV-FTC assay. The performance of the multidrug assay in detecting TFV and FTC was further evaluated by comparing results from the multidrug assay with results obtained for these two drugs using a more sensitive quantitative TFV-FTC assay. The latter assay was used in the HPTN 066 study, which determined the concentrations of TFV and FTC achieved during 5 weeks of directly observed TDF-FTC dosing with a daily

TABLE 1 Antiretroviral drugs detected using the multidrug assay

ARV drugs detected	No. of samples[a]				
		Enrollment visit (n = 208)		Last visit (n = 208)	
	Total (n = 416)	Did not accept PrEP	Accepted PrEP	Did not accept PrEP	Accepted PrEP
FTC	3	0	0	1	2
TFV	1	0	1	0	0
TFV and FTC	83	0	2	2	79
None	329	43	162	40	84

[a]Plasma samples were collected at the enrollment visit and the end-of-study visit from 208 participants in HPTN 073, including 165 who accepted PrEP and 43 who did not accept PrEP. Twenty-nine of the 165 men who accepted PrEP permanently discontinued PrEP before their last study visit. Samples were tested for the presence of 20 ARV drugs using the multidrug assay, and TFV and FTC were the only drugs detected in the samples.

dosing regimen and a 4-dose/week regimen (13). That benchmark study found that the 90% sensitivity thresholds for TFV and FTC were 35.5 ng/ml and 49.1 ng/ml, respectively, for the daily dosing regimen and 4.2 and 4.6 ng/ml, respectively, for the 4-dose/week regimen (13). Results from the quantitative TFV-FTC assay were available for 163 of 165 HIV-uninfected men who accepted PrEP (end-of-study visits only; two participants did not have samples available for testing). The quantitative TFV-FTC assay detected TFV in 85 (52.1%) of the 163 samples (median concentration, 87.5 ng/ml [range, 0.7 to 424.0 ng/ml]) (Table 2) and detected FTC in 87 (53.4%) of the 163 samples (median concentration, 342.0 ng/ml [range, 0.3 to 3,230.0 ng/ml]) (Table 2). Most men for whom TFV and/or FTC was detected had drug concentrations consistent with daily TDF-FTC use; 70 (42.9%) had TFV concentrations above 35.5 ng/ml and 73 (44.8%) had FTC concentrations above 49.1 ng/ml. The multidrug assay detected TFV and FTC only in samples in which the drugs were detected using the quantitative TFV-FTC assay, and it detected the drugs in all cases in which those drugs were present at concentrations above 20 ng/ml. In nine cases, TFV and/or FTC were detected only using the quantitative TFV-FTC assay; in all nine cases, the drugs were present at concentrations below 20 ng/ml (TFV range, 0.65 to 16.50 ng/ml; FTC range, 0.33 to 14.60 ng/ml) (see Table S2). Using the quantitative TFV-FTC assay as the gold standard, the sensitivity of the multidrug assay for this sample set was 91.8% for TFV and 92.0% for FTC; the accuracy was 95.7% and the specificity was 100% for both drugs.

Analysis of ARV drug use and drug resistance among men in HPTN 073 who acquired HIV infection. As a final step, we analyzed ARV drug use and HIV drug resistance among the eight men who acquired HIV infection during the HPTN 073 study; this included five men who accepted PrEP and three who did not (Fig. 2). The only drugs detected in samples from these men were TFV and FTC. In the three cases in which men did not accept PrEP (cases A to C), TFV and FTC were not detected in any samples using either the multidrug assay or the quantitative TFV-FTC assay (Fig. 2). In three of the five cases in which men accepted PrEP (cases D, E, and F), TFV or FTC was

TABLE 2 Detection of TFV and FTC using the multidrug assay and the quantitative TFV/FTC assay[a]

Multidrug assay result	TFV			FTC		
	Quantitative assay positive	Quantitative assay negative	Total	Quantitative assay positive	Quantitative assay negative	Total
Positive	78	0	78	80	0	80
Negative	7	78	85	7	76	83
Total	85	76	163	87	78	163

[a]Plasma samples from HPTN 073 were tested using the multidrug assay and the quantitative TFV-FTC assay. The number of samples in which TFV or FTC was detected with one or both test methods is shown. Positive indicates that TFV or FTC was detected, and negative indicates that the corresponding drug was not detected. Both drugs were detected in 85 (52.1%) of the 163 samples.

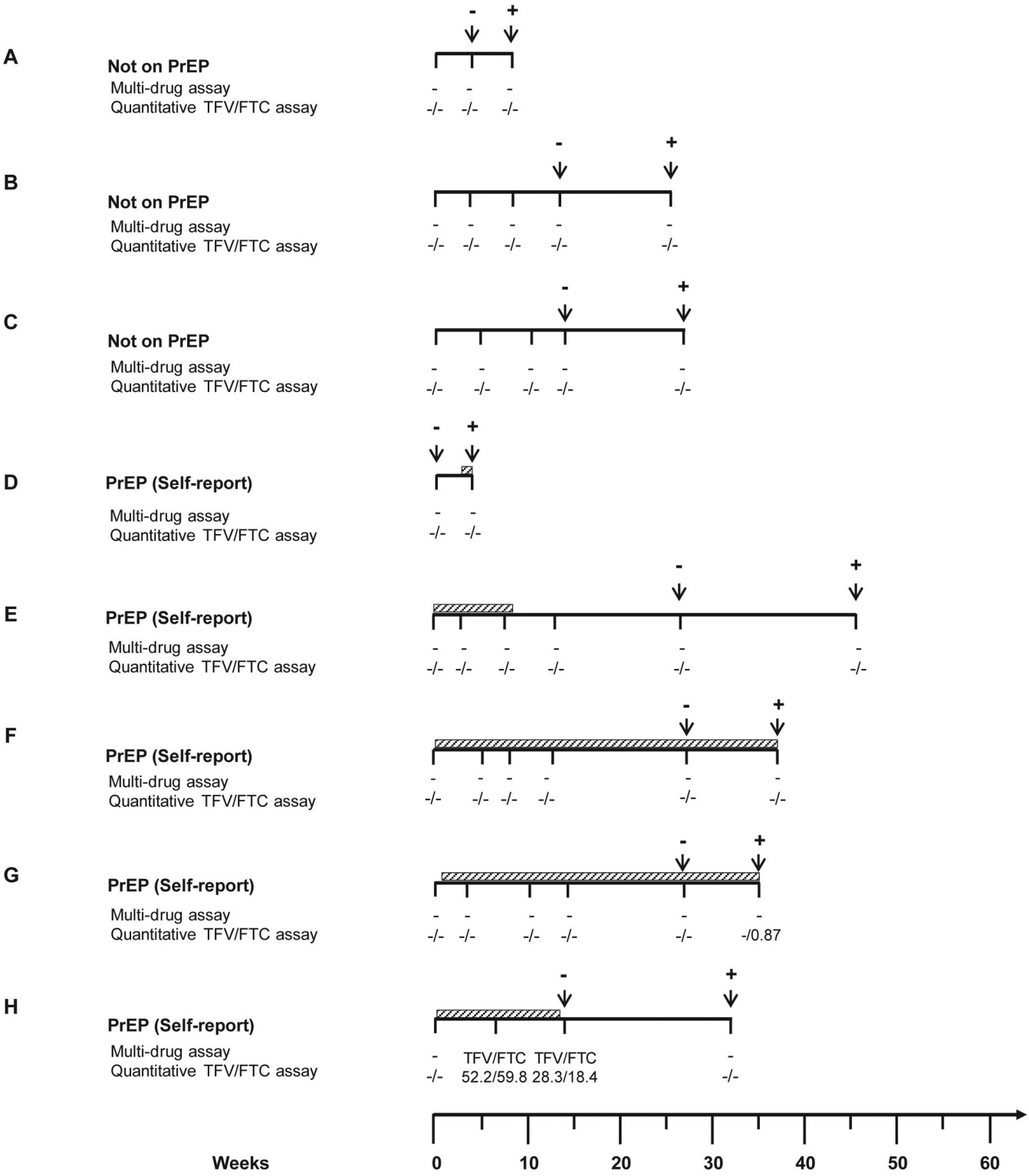

FIG 2 ARV drug use among men who acquired HIV infection (cases A to H). Plasma samples were obtained from eight men who acquired HIV infection during the HPTN 073 study. The arrows with negative signs indicate the last study visit at which the participants tested negative for HIV infection, and the arrows with positive signs indicate the first visit at which the participants tested positive for HIV infection. The solid lines indicate the time period before the diagnosis of HIV infection. The number of weeks after the enrollment visit is shown. Samples were tested using the qualitative multidrug assay (assay cutoff value, 2 to 20 ng/ml) and the quantitative TFV-FTC assay (lower limit of quantification, 0.31 ng/ml for both drugs). Hatched bars indicate the periods during which the participants had access to PrEP in the study. Self-reports included the percentage of TDF-FTC pills taken over the prior month (data are noted only for visits at which the participant reported taking PrEP). Participants who accepted PrEP reported the following levels of PrEP use: case D, 90% at the week 4 visit; case E, 10% at the week 4 visit and 40% at the week 8 visit; case F, 100% at the week 4, 8, 13, and 26 visits and 90% at the week 39 visit; case G, 10% at the week 4 and 13 visits, 40% at the week 8 visit, 90% at the week 26 visit, and 100% at the week 39 visit (note that this visit occurred 35 calendar weeks after enrollment); case H, 20% at the week 4 visit and 60% at the week 13 visit.

TABLE 3 HIV drug resistance mutations detected using the ViroSeq HIV-1 genotyping system and next-generation sequencing

Case	Mutation (frequency of reads [%])[a]						Resistance-associated drugs[b]
	Protease		Reverse transcriptase		Integrase		
	ViroSeq	NGS	ViroSeq	NGS	ViroSeq	NGS	
A	None	None	**K103N**	**K103N** (99.12)	None	None	EFV, NVP
B	None	None	None	None	None	**N155S** (2.3)	EVG, RAL
C	None	None	None	None	None	None	
D	None	None	None	None	T97A,[c] E157Q[c]	T97A[c] (98.6), E157Q[c] (99.11)	EVG, RAL
E	None	**M46I** (2.7)	None	None	None	None	ATV, LPV
F	None	None	None	None	E157Q[c]	E157Q[c] (98.8)	EVG, RAL
G	None	None	**K103N**	**K65R** (2.5), **K103N** (99.0)	None	None	TDF, DDI, ABC, D4T, FTC, 3TC, EFV, NVP
H	None	None	None	None	None	Q146L[d] (5.2)	

[a]HIV drug resistance testing was performed using two methods, ViroSeq HIV-1 Genotyping System, v2.8 (Abbott Molecular, Des Plaines, IL) and NGS using the MiSeq system (Illumina, San Diego, CA). The mutations detected in HIV protease, reverse transcriptase, and integrase are indicated. The frequency of NGS reads with each mutation is shown in parentheses. Major resistance mutations are shown in bold, and drugs with reduced susceptibility are shown. Polymorphic mutations (e.g., L10V and L71T in HIV protease) are not shown.

[b]EFV, efavirenz; NVP, nevirapine; EVG, elvitegravir; RAL, raltegravir; ATV, atazanavir; LPV, lopinavir; TDF, tenofovir disoproxil fumarate; DDI, didanosine; ABC, abacavir; D4T, stavudine; FTC, emtricitabine; 3TC, lamivudine.

[c]Accessory mutation.

[d]Q146P is associated with reduced susceptibility to elvitegravir; Q146L is an unusual mutation.

not detected in any of the samples tested. In one case (case G), a low concentration of FTC (0.87 ng/ml, consistent with ≥1 dose/week) was detected at the HIV seroconversion visit using the quantitative TFV-FTC assay. In the last case (case H), both drugs were detected by both assays at two study visits prior to seroconversion (TFV and FTC concentrations of 52.2 and 59.8 ng/ml, respectively, at the first visit and 28.4 and 18.4 ng/ml at the second visit, consistent with daily dosing and ≥4 doses/week, respectively). Self-reported data on PrEP adherence are provided in the Fig. 2 legend. Some discrepancies were noted between self-reported data and data from ARV drug testing. For example, the participant in case F reported 100% PrEP use at all visits but TFV and FTC were not detected by either assay at any visit. This participant was diagnosed with HIV infection at the study site at the week 52 visit and reported using PrEP until that time; however, retrospective HIV testing at the HPTN Laboratory Center revealed that the participant had acute HIV infection at the prior study visit (at week 39).

HIV drug resistance was assessed using an FDA-cleared HIV genotyping system based on population sequencing (ViroSeq) and next-generation sequencing (NGS) (Table 3). This testing was performed using samples collected at the first HIV-positive visit for all cases except case F; in that case, resistance testing was performed using a sample collected at the visit when HIV infection was diagnosed at the study site (week 52). Mutations associated with TFV and FTC resistance were not detected using the ViroSeq system. The K65R mutation was detected at a low frequency (2.5%) in one case using NGS; in that case (case G), a very low concentration of FTC was detected in plasma at the time of HIV seroconversion, consistent with one dose of TDF-FTC in the prior week (Fig. 2). The nonnucleoside reverse transcriptase inhibitor (NNRTI) resistance mutation K103N was detected in two cases using both methods. The low-frequency protease inhibitor (PI) resistance mutation M46I was detected in one case using NGS only. The polymorphic integrase strand transfer inhibitor (INSTI) resistance mutations T97A and E157Q were detected in two cases using both methods. In one case, N155S (reported as a rare nonpolymorphic mutation selected *in vitro* [24]) was detected using NGS only.

DISCUSSION

Many methods used to monitor adherence to antiretroviral therapy (ART) and PrEP, such as self-reporting, pill counting, and electronic monitoring, have limitations (12, 25, 26). When data are collected by self-report, some individuals may forget when they

took the drugs, and others may choose to provide socially desirable answers (27). In a clinical trial setting, enrollment criteria and other features of the trial design may inadvertently encourage some participants not to disclose knowledge of their HIV status or ARV drug use (19, 28). ARV drug testing provides an objective biomedical measure of ARV drug use. This study evaluated the performance of a high-throughput, qualitative, multidrug assay in evaluating exposure to TDF-FTC PrEP. Results obtained with the multidrug assay were also compared to quantitative adherence assessments.

Comparison of results from the multidrug assay and a quantitative, gold-standard, LC-MS/MS assay for TFV and FTC showed that the multidrug assay detected TFV and FTC in all cases in which the drugs were present at concentrations above the 90% sensitivity thresholds established for daily TDF-FTC use in a directly observed dosing study (13). Because of its lower sensitivity, however, the multidrug assay may not detect drugs with less than daily TDF-FTC dosing. Also, because the lower LOD of the multidrug assay (20 ng/ml for TFV and FTC) is below the 90% sensitivity threshold for daily adherence, it is possible that individuals who are taking TDF-FTC less often than daily (in the 4-dose/week range) could be misclassified as adherent to a daily regimen. The high level of agreement between the multidrug assay and the quantitative LC-MS/MS assay observed in this report reflects the high level of adherence to PrEP in the HPTN 073 cohort. Quantitative data are required to determine the level of adherence to TDF-FTC PrEP, particularly for individuals with less than daily dosing.

In addition to detecting TFV and FTC, the multidrug assay detects 18 other ARV drugs in five drug classes. This allowed us to explore whether the men enrolled in HPTN 073 were using any other ARV drugs. In a previous study, we analyzed ARV drug use among HIV-infected men in the HPTN 061 study (2009 to 2011), which enrolled 1,553 Black MSM in six cities in the United States, including the three sites in HPTN 073 (19, 21). At the time when HPTN 061 was performed, there were no ARV drugs approved for PrEP for sexual HIV transmission. Many of the men in HPTN 061 who reported no current or prior ARV drug use had ARV drugs detected in study samples (21). In most cases, the drugs detected were consistent with ART regimens recommended at the time of the study. Many of the men for whom drugs were detected did not demonstrate viral suppression and many were using unusual combinations of drugs, which likely contributed to the high frequency of drug resistance observed in that cohort (19). Unusual patterns of ARV drug use were also detected among seroconverters in the HPTN 061 cohort (19). In another study (HPTN 064 [2009 to 2010]), we found unusual patterns of ARV drug use among HIV-uninfected women in the United States who were at increased risk of HIV acquisition (20, 29). Those findings prompted analysis of ARV drug use among HIV-uninfected men and seroconverters in HPTN 073, which began in 2013, after FDA approval of TDF-FTC for PrEP. In HPTN 073, the study drugs (TFV and FTC) were the only drugs detected. The availability of an approved and proven intervention for preventing HIV infection might have reduced the motivation of participants in HPTN 073 to use other ARV drugs off-label. However, it is also possible that we did not observe the use of other drugs among the Black MSM in HPTN 073 because the number of participants was relatively small and because many ARV drugs have short half-lives and would not be detected unless they were taken close to the time of sample collection.

Another interesting finding in this report was the detection of TFV and FTC in samples from 3 (1.4%) of 208 men at the time of study enrollment, which indicated that these men did not disclose the use of PrEP to the study staff when they enrolled in the study; disclosure of PrEP use within 60 days of enrollment would have excluded them from participation in the study. This provides further support for the use of an objective biomedical measure to assess ARV exposure in clinical studies. The actual frequency of PrEP exposure at study entry might have been higher, since the multidrug assay has relatively high cutoff values for TFV and FTC detection (20 ng/ml) and does not reliably detect nondaily PrEP use.

In HPTN 061, HIV drug resistance mutations were detected in samples from 9

(23.1%) of 39 newly infected Black MSM (seroconverters and men with recent HIV infection) using the ViroSeq system (integrase genotyping and NGS were not performed in that study) (19). In HPTN 073, resistance mutations were detected for 4 (50.0%) of the 8 seroconverters. Resistance to the study drugs (TDF and FTC) was detected in only one case. K65R, which is associated with resistance to TDF, FTC, and other nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), was detected in one seroconverter sample that also contained the NNRTI resistance mutation K103N. TDF was not detected in any of the samples from this participant, and FTC was detected at a low level in only one sample, indicating infrequent PrEP use. We did not detect resistance to TDF and FTC in samples from the six men for whom no study drugs were detected at any study visit. In one case, study drugs were consistently detected in study samples, indicating high levels of PrEP adherence; no major resistance mutations were detected in the seroconversion sample from that case.

A limitation of both assays used in this study (the multidrug assay and the quantitative TFV-FTC assay) is that the assays are subject to the "white coat" effect (i.e., detection of drugs in individuals who were not taking the drugs regularly but took the drugs within 1 day before sample collection). Assays that detect TFV in hair or TFV diphosphate in peripheral blood mononuclear cell (PBMC) or dried blood spot (DBS) samples are less susceptible to this white coat effect but do not reflect very recent drug dosing. However, the frequency of a white coat effect, as quantified by comparing data from plasma assays with data from other sample types, was consistently very low in several PrEP studies (C. W. Hendrix, unpublished data).

Quantitative drug assessments using LC-MS/MS methods represent the gold standard for monitoring adherence to TDF-FTC PrEP. This report demonstrates that the multidrug assay provides a complementary approach to quantitative assessments of PrEP adherence by providing information about exposure to a daily TDF-FTC regimen. It also provides new and reassuring data suggesting that the use of other ARV drugs among HIV-uninfected and newly infected Black MSM in the United States is infrequent. The multidrug assay is currently being modified to include the newer INSTIs cabotegravir and elvitegravir, which will be useful in future surveys of ARV drug use in different populations and settings (e.g., ART in HIV-infected individuals, daily TDF-FTC PrEP, postexposure prophylaxis [PEP], and recreational ARV drug use). The relatively low cost of the multidrug assay allows analysis of large sample sets (20, 22), which may also be useful for surveillance of ARV drug use and larger research studies.

MATERIALS AND METHODS

Clinical samples. Plasma samples were obtained from men enrolled in HPTN 073 (ClinicalTrials registration no. NCT01808352). Eligibility criteria for HPTN 073 included high risk for HIV acquisition, no prior HIV diagnosis, and no record of ARV drug use for PrEP or PEP in the 60 days prior to anticipated enrollment. Study interventions included offering once-daily oral TDF-FTC (Truvada, a fixed-dose combination tablet containing 300 mg TDF and 200 mg FTC) and client-centered care coordination, which was provided to promote and to support PrEP use. The study enrolled 226 HIV-uninfected Black MSM; 79% of the men accepted PrEP. HIV testing was performed at the study sites. Additional testing was performed retrospectively at the HPTN Laboratory Center (Johns Hopkins University, Baltimore, MD); this included additional HIV testing for quality assurance and confirmation of HIV infection. Study participants were followed for 12 months; 8 men acquired HIV infection during the study (5/178 who accepted PrEP and 3/48 who did not accept PrEP).

ARV drug testing. (i) Multidrug assay. Plasma samples were tested for the presence of 20 ARV drugs using a qualitative assay based on HRMS (18). The 20 drugs included 6 NRTIs (abacavir, FTC, lamivudine, stavudine, TFV, and zidovudine), 3 NNRTIs (efavirenz, nevirapine, and rilpivirine), 9 PIs (amprenavir, atazanavir, darunavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, and tipranavir), a CCR5 receptor antagonist (maraviroc), and an INSTI (raltegravir). Briefly, 100 μl plasma was prepared with 300 μl methanol plus 0.025% formic acid, containing FTC 13C 15N2 (internal standard) at 20 ng/ml, in individual wells of a Phenomenex Impact protein precipitation plate. ARV drugs were detected by high-performance liquid chromatography (HPLC) coupled with high-resolution accurate mass (HRAM) mass spectrometry (MS) (QExactive-Orbitrap; Thermo Scientific, San Jose, CA). The mobile phase system was as follows: solvent A, water plus 10 mM ammonium formate plus 0.05% formic acid; solvent B, methanol plus 0.05% formic acid. After the samples were loaded onto a Thermo Synchronis C_{18} guard column (10 by 4.0 mm; particle size, 5 μm), elution occurred during a 120-s ramp to 100% organic mobile phase. Full-scan data-dependent MS2 analysis was performed with a

resolution of 17,500 at *m/z* 200 for both full MS and MS2 scans. An effective analysis time of 2.0 min/sample was achieved by multiplexing with a 4-channel chromatography system. Controls were included with each batch or plate (2, 20, 200, and 2,000 ng/ml for each drug); if the results for the controls on each plate did not match within a predetermined tolerance, then the plate was reanalyzed. Abacavir, maraviroc, nelfinavir, nevirapine, raltegravir, and rilpivirine were consistently detected at or above 2 ng/ml; the remaining 14 drugs, including TFV and FTC, were consistently detected at or above 20 ng/ml.

(ii) Quantitative TFV-FTC assay. Plasma samples were also analyzed using an assay based on LC-MS/MS that quantified TFV and FTC (14). Briefly, following the addition of isotopically labeled internal standards and sample extraction via protein precipitation, samples were subjected to LC-MS/MS analysis. Chromatographic separation was performed using an Agilent Zorbax Eclipse Plus C_{18} column (2.1 by 50 mm; particle size, 3.5 μm), with detection using an API4000 mass analyzer (SCIEX, Foster City, CA) operated in positive ionization and selective reaction monitoring (SRM) modes. The lower limits of quantification were 0.31 ng/ml for both drugs. The assay was validated in accordance with U.S. FDA guidelines and was externally reviewed by the Clinical Pharmacology Quality Assurance (CPQA) program sponsored by the Division of AIDS (30). Testing was performed for seroconverters at all study visits; testing was limited to end-of-study samples for uninfected men, due to cost considerations.

HIV drug resistance testing. HIV genotyping was performed using the ViroSeq HIV-1 v2.8 genotyping system (Abbott Molecular, Des Plaines, IL). Viral RNA extracted from plasma samples with the ViroSeq HIV-1 genotyping system was used for next-generation sequencing. Next-generation sequencing was performed using methods adapted from a previous study (31). The MiSeq system (Illumina, San Diego, CA) was used to generate paired-end reads (2 by 250 bp). The reads were then trimmed for quality (limit threshold, 0.05) and ambiguity (2-nucleotide maximum) by using CLC Genomics Workbench v9.5 software (Qiagen, Aarhus, Denmark). After the PCR primers were removed, reads were aligned to a reference sequence (HXB2 [GenBank accession no. NC_001802]) with the following alignment settings: mismatch, 2; insertion, 3; deletion, 3; length fraction, 0.7; similarity fraction, 0.8. The low-frequency variant detection tool from the CLC Genomics Workbench software was used to identify resistance mutations (frequency cutoff value: 2%; minimum number of variants: 2,000 reads). The Stanford University HIV drug resistance database (https://hivdb.stanford.edu/hivdb/by-mutations) was used to generate HIV drug resistance reports.

Statistical methods. Sensitivity, specificity, and accuracy were calculated to characterize the performance of the multidrug assay, using the quantitative TFV-FTC assay as the gold standard.

Ethics approval. All study participants provided informed consent for participation in the HPTN 073 study. The study was approved by the participating academic institutions and ethics committees for each study site.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.02743-16.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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