Published in final edited form as:

AIDS. 2018 August 24; 32(13): 1891–1898. doi:10.1097/QAD.0000000000001922.

Tenofovir and tenofovir-diphosphate concentrations during pregnancy among HIV-uninfected women using oral pre-exposure prophylaxis

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Abstract

Objectives: Pregnancy is a time of increased HIV acquisition risk and pregnancy reduces concentrations of antiretrovirals used for treatment. We assessed whether pregnancy lowers concentrations of tenofovir (TFV) and tenofovir-diphosphate (TFV-DP) among HIV-uninfected women using oral pre-exposure prophylaxis (PrEP).

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MP, JB, RH, KM, & KT contributed to the analysis plan and MP conducted the analyses. PA, CW, and MM analyzed the specimens. EB, NM, SA, & EK coordinated data and specimen collection. MP & JB drafted and all authors contributed to the manuscript.

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Data Management was provided by DF/Net Research, Inc. (Seattle, WA). PrEP medication was donated by Gilead Sciences.

Methods: We analyzed data from an open-label PrEP study, comparing concentrations of TFV in plasma and TFV-DP in dried blood spots (DBS) among 37 pregnant women and 97 non-pregnant women. Analyses controlled for adherence from daily electronic monitoring.

Results: The average plasma concentration of TFV among pregnant women was 34.7 ng/mL with 22.2 average recorded doses over the prior month versus 86.5 ng/mL with 23.1 doses among non-pregnant women. After controlling for adherence, TFV concentrations were 58% lower among pregnant women, a statistically significant difference of -50.4 ng/mL (95%CI -68.3 to -32.5). The average TFV-DP concentration was 450.3 fmol/punch among pregnant women and 636.7 fmol/punch among non-pregnant women. This difference was not statistically significant after adjusting for adherence; however, among those with quantifiable TFV-DP, concentrations were 27% lower during pregnancy (-202 fmol/punch [95%CI -384 to -19]). Among participants with samples before and during pregnancy, there were significant decreases during pregnancy, controlling for adherence: -28.1 ng/mL TFV (95%CI -52.3 to -4.0) and -289.2 fmol/punch TFV-DP (95%CI -439.0 to -139.3).

Conclusions: Consistent with studies among HIV-infected women on ART, we found TFV and TFV-DP concentrations were lower during pregnancy. There is no established TFV concentration threshold to achieve HIV prevention. Additional pharmacokinetic studies and studies of PrEP efficacy in pregnancy are needed.

Keywords

pregnancy; HIV; pre-exposure prophylaxis; tenofovir

Introduction

Pregnancy is a period of increased risk for HIV acquisition [1–5] which may last for a substantial portion of women's reproductive years in settings with a high burden of HIV. Oral tenofovir-based PrEP is safe for pregnant women and their infants, and breastfed infants are exposed to very low tenofovir levels in breastmilk [6–8], leading the World Health Organization to provide guidance recommending that PrEP can be safely used during pregnancy and breastfeeding for women at high HIV risk [9]. As PrEP becomes widely used in heterosexual populations, an increasing number of women who are pregnant or trying to become pregnant may use PrEP for HIV prevention [10,11].

However, data on the effectiveness of PrEP for HIV prevention during pregnancy are relatively limited. In the clinical trials of PrEP that established its safety and efficacy, women stopped study drug once pregnancy was detected. Pregnancy can affect drug pharmacokinetics in several ways [12]. Most relevant to oral PrEP, pregnancy increases blood volume, leading to a higher volume of distribution, and increases kidney flow rate, leading to higher clearance, with the greatest changes in the 3rd trimester [13,14]. Increases in volume of distribution can result in lower peak drug concentrations and longer half-lives, while increases in clearance can result in lower steady-state drug concentrations and shorter half-lives [15].

In this analysis, we used stored plasma and dried blood spots (DBS) from an open-label study that included women using PrEP during pregnancy to examine potential differences in blood concentrations of tenofovir when compared to non-pregnant women, as well as to compare pre-pregnant and pregnant periods within women. As pregnancy may result in women changing their use of PrEP [16], we controlled for adherence as measured by electronic monitoring data.

Methods

Women in this analysis were members of mutually-disclosed HIV serodiscordant couples who participated in an open-label PrEP demonstration project in Uganda and Kenya (the Partners Demonstration Project), as previously described [17]. Briefly, participants were seen quarterly for up to 24 months. Initially, the study protocol required that women stop using PrEP once pregnant. However, during the study the protocol was updated at each site and women could continue PrEP (coformulated emtricitabine/tenofovir disoproxil fumarate 200mg/300 mg) if they chose to during pregnancy; if they continued PrEP, follow-up became monthly during pregnancy. PrEP adherence was assessed by electronic monitoring (MEMS caps, WestRock, Switzerland), which recorded daily pill bottle openings; for this analysis, openings were limited to one per day. Blood samples were also taken at quarterly study visits and at monthly visits when pregnant; samples were stored as plasma at -80°C and, partway through the study, as dried blood spots (DBS) stored at -20°C . Age, BMI, and creatinine clearance were assessed at baseline. All participants provided written informed consent in their preferred language.

For this analysis, non-pregnant plasma samples came from a selection of HIV-uninfected women (who never became pregnant or were sampled before their first pregnancy during follow-up): either a random sample tested serially at each study visit or women enrolled in a pharmacokinetic substudy tested at a single study visit. Plasma samples were tested from all pregnant HIV-uninfected women who took part in monthly follow-up, as well as any women in the substudy who became pregnant. In addition, we selected one DBS sample from each trimester of pregnancy, as well as a pre-pregnancy sample when available; one non-pregnant sample was also chosen from a random sample of women. Throughout, samples from women who had <1 recorded PrEP dose in the month prior were excluded as non-users. When multiple samples were available for the same time period (non-pregnant or trimester), we selected the one with the most recorded doses.

Plasma was tested for tenofovir (TFV) and DBS were tested for tenofovir-diphosphate (TFV-DP), the active metabolite, using ultra-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) [17,18]. Values below the lower limit of 0.31 ng/mL for plasma TFV and 31.25 fmol/punch for TFV-DP were set to half the lower limit (0.15 ng/mL and 15.6 fmol/punch respectively). With a half-life of 14 hours in plasma, TFV is a marker of recent use [19], while TFV-DP has a half-life of 17 days in red blood cells and DBS, and provides a marker of cumulative adherence over the prior month [20].

We compared the mean concentrations of each analyte between pregnant and non-pregnant women using linear models with concentration as the outcome and pregnancy status as the

primary predictor; women were not included as both pregnant and non-pregnant in these models (only pregnant samples were included if both were available). As pregnant women could contribute multiple samples while pregnant, generalized estimating equations with an unstructured correlation matrix were used to account for repeated observations. The analysis was then repeated comparing each trimester to non-pregnant women. We next compared the mean concentration of each analyte from pre-pregnancy to pregnancy among the subset of women who had samples from both time periods; this comparison of pregnancy within woman is conditional on a random intercept for each woman and mixed effects models were used. We conducted two sensitivity analyses: restricting to those samples with tenofovir above the lower limit of quantitation and restricting to samples with 100% adherence over the prior month, according to MEMS. For all analyses (except the 100% adherence sensitivity analysis), models were adjusted for adherence (number of days with a MEMS opening over the prior month (for TFV-DP), or prior 2 days (for TFV)). Baseline age, BMI, creatinine clearance, and time in study were tested and only included if they changed the estimated pregnancy effect; we did not adjust for BMI during pregnancy, as changes in weight may be part of the causal pathway. All analyses were conducted in SAS 9.4.

Results

Participant characteristics.

Overall, thirty-seven (37) women contributed either plasma or DBS concentrations while pregnant and 97 women contributed only non-pregnant samples. All women were 48 years or younger, with a mean age of 29 at baseline. Overall, 97% were married to their study partner and had an average of 2.4 children (Table 1).

Plasma TFV concentrations.

There were 389 plasma samples (163 from pregnant and 226 from non-pregnant visits) from 116 women (33 pregnant and 83 non-pregnant) available for the comparison between pregnant and non-pregnant women. The average TFV concentration among pregnant women was 34.7 ng/mL, with an average of 22.2 recorded PrEP doses over the prior month; the average TFV concentration among non-pregnant women was 86.5 ng/mL, with an average 23.1 recorded PrEP doses (Table 2). After adjusting for adherence, average TFV concentrations were significantly lower among pregnant women, -50.4 ng/mL (95%CI -68.3, -32.5) (Table 4); this finding translates into a mean concentration of TFV in pregnant women 58% lower compared to non-pregnant women. TFV concentrations were also significantly lower in each trimester ($p < 0.001$) relative to non-pregnant women: -40.0 ng/mL for the 1st trimester; -49.4 ng/mL for the 2nd trimester; and -59.2 ng/mL for the 3rd trimester.

In a sensitivity analysis of the 322 samples from 103 women with quantifiable TFV, the results were similar and remained statistically significant, with an adjusted difference of -50.8 ng/mL for pregnant compared to non-pregnant women. Restricting to the 105 samples from 44 women with 100% MEMS adherence over the prior month, the results remained consistent: -51.8 ng/mL for pregnancy overall, -53.9 ng/mL for the 1st trimester, -48.8

ng/mL for the 2nd trimester, and -52.3 ng/mL for the 3rd trimester compared to non-pregnant women (Figure 1).

DBS TFV-DP concentrations.

In the corresponding analysis using DBS, there were 70 samples contributed by 31 women while pregnant and 32 samples contributed by 32 non-pregnant women. The average TFV-DP concentration was 450.3 fmol/punch among pregnant women and 636.7 fmol/punch among non-pregnant women; the average number of doses recorded by MEMS was 21.4 for pregnant women and 20.7 for non-pregnant women (Table 2). In the fully adjusted model (including adherence, age, and BMI), there were no statistically significant differences between pregnant and non-pregnant women, although the point estimate was lower for pregnant periods: -136.6 fmol/punch (95% CI -318, 44.8); in addition, there was a significant difference between the 2nd trimester and non-pregnant periods in adjusted analysis (Table 3). In the sensitivity analysis restricted to those with quantifiable TFV-DP, the differences between pregnant (-201.9 fmol/punch), 2nd trimester (-278.4 fmol/punch), and 3rd trimester (-259.6 fmol/punch) samples were all statistically significantly lower than non-pregnant samples. There were only 30 samples with 100% adherence over the prior month; differences were larger compared to those with quantifiable TFV-DP, but only remained significant in the 3rd trimester (Table 3, Figure 1).

Pre- and during pregnancy comparisons.

When comparing levels of TFV among the 9 women with plasma samples before and during pregnancy (Table 4), there was a statistically significant decrease during pregnancy of -28.1 ng/mL after controlling for adherence (Supplemental Table 1); by trimester, only the 3rd trimester was statistically different from pre-pregnancy. Among 12 women with DBS samples prior to and during pregnancy (Table 4), there was also a statistically significant decrease during pregnancy, by -289.2 fmol/punch overall and with significant differences in each trimester as well (Supplemental Table 1). These results indicated that concentrations of TFV were 45% lower and concentrations of TFV-DP were 49% lower during pregnancy compared to pre-pregnancy, controlling for adherence. In both analyses, results were similar when restricted to women with any quantifiable tenofovir; samples sizes were too small to conduct a sensitivity analysis restricted to 100% adherence over the prior month.

Discussion

Among HIV-uninfected women using oral PrEP, we found that plasma TFV and intracellular TFV-DP in DBS were 45%–58% lower during pregnancy compared to non-pregnant periods after adjusting for adherence as measured by MEMS openings. Differences in concentrations were generally larger in the second and third trimester than in early pregnancy. These results suggest that pregnancy alters the pharmacokinetics of oral PrEP among HIV-uninfected women.

Prior studies of tenofovir used as antiretroviral treatment in pregnant women living with HIV have shown similar results to those we found for HIV-uninfected women using PrEP, though most have focused on US and European populations. In particular, three studies

reported higher TFV clearance during pregnancy (compared to either non-pregnant or post-partum periods) [21–23]. In addition, two of the studies found lower TFV concentrations in the 3rd trimester compared to post-partum (23% lower C₂₄ and 20% lower AUC) [22,23]. In addition, two studies of TFV-based regimens in HIV-infected pregnant women included viral suppression endpoints and found 83% and 94% of women on TFV were virally suppressed in the 3rd trimester, compared to 82% and 85%, respectively in the post-partum period [22,23]. One study had a target AUC based on the 10th percentile of non-pregnant levels and found that 73% of 3rd trimester samples met the cut-off [22]; the other study reported no cases of vertical transmission among the 34 women studied, indicating sufficient suppression [23]. Some studies have used higher doses (600mg TDF/400mg FTC) at delivery for prevention of mother to child transmission and found it was possible to achieve drug exposures similar to non-pregnant populations [24–27]. Altogether, these findings of similar viral suppression rates in spite of modestly lower tenofovir levels during pregnancy has led to recommendations in the US that TDF/FTC dosing in HIV infected women does not need to be altered during pregnancy [28].

For PrEP, the clinical significance of the differences in blood concentrations of tenofovir that we observed is not clear. Clinical trials of PrEP efficacy stopped women from taking PrEP once pregnancy was detected and the number of pregnant users in reported implementation trials to date is too small to conduct efficacy analyses. There is no established protective concentration of tenofovir for women and protective levels needed for PrEP may differ from suppressive levels when used as ART [19]. Furthermore, it is unclear how blood concentrations of tenofovir correlate with tissue concentrations at the site of infection or in regional lymph nodes, where the protective action presumably occurs [29]. Prior studies, including a directly-observed dosing study, have used 35 ng/mL (or sometimes 40 ng/mL) of serum or plasma TFV as the lower cut-off to indicate consistent, daily PrEP use [29,30]. In our sample, most participants with 100% adherence by MEMS were above this cut-off, regardless of trimester, although the proportion falling below this threshold increases in pregnancy (Figure 1); the appropriate threshold for TFV-DP is not yet clear.

Pharmacokinetic modeling (in men and women) has suggested that 230 fmol/punch of TFV-DP is equivalent to one dose of PrEP per week [31] and our results suggested that pregnancy has an effect on TFV-DP concentrations of a similar magnitude; in other words, a pregnant woman taking seven doses of PrEP per week would have TFV-DP concentrations similar to a non-pregnant woman taking six doses per week. These results, as well as other work indicating women may need 6 doses per week to achieve protection [32], suggest that adherence could be even more crucial for women during pregnancy.

Our study has some limitations. This was not a pharmacokinetic study and the exact timing of doses is unknown, making direct comparisons with previous ART studies difficult. MEMS data can be subject to both over and underreporting and there may be residual adherence effects that were not controlled for. However, MEMS data have been shown to correlate well with plasma TFV in prior work in this population [17,33]. The concentrations among women are lower than expected from direct observation studies. For instance, the mean TFV-DP in non-pregnant women was 636.7 fmol/punch for 20.7 doses/month, or approximately 67% adherence to daily dosing. This value was lower than the mean steady-

state TFV-DP observed for 67% adherence in a directly observed dosing study conducted in the U.S., 997 fmol/punch [20], suggesting controlled studies are needed to define TFV-DP in African participants.

Strengths of this study include comparing the effect of pregnancy on plasma tenofovir and intracellular levels of tenofovir diphosphate in DBS between pregnant and non-pregnant women, as well as within the same woman over the course of her pregnancy. We accounted for changes in PrEP adherence during pregnancy not only by adjusting for adherence, using electronic monitoring, but with sensitivity analyses of those with complete adherence over the prior month and of those with biomarker evidence of PrEP use.

In conclusion, concentrations of tenofovir and its active metabolite tenofovir-diphosphate appear to be lower during pregnancy in the setting of PrEP; however, most women with high adherence still achieved 35 ng/mL of plasma TFV during pregnancy, which correlated with daily dosing in prior observational studies in this population. Epidemiologic studies within ongoing PrEP implementation projects are needed to determine if the effectiveness of PrEP is altered in pregnancy. Additional pharmacokinetic studies[34] are also warranted to understand the protective threshold of tenofovir among women. This work also highlights the importance of including pregnant women in research related to the development of novel prevention products [35].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank the couples who participated in this study and Director of the Kenya Medical Research Institute (KEMRI).

Funding:

The Partners Demonstration Project was funded by the National Institute of Mental Health of the US National Institutes of Health (R01 MH095507), the Bill & Melinda Gates Foundation (OPP1056051), and the US Agency for International Development (AID-OAA-A-12-00023). This analysis was supported by the National Center for Advancing Translational Sciences of the National Institutes of Health (TL1 TR002318). Research reported in this publication was supported by the UW/Fred Hutch Center for AIDS Research, funded by NIAID, NCI, NIMH, NIDA, NICHD, NHLBI, NIA, NIGMS, NIDDK of the National Institutes of Health under award number P30 A1027757 and the HIV Prevention Trials Network (UM1 AI068613). The contents are the responsibility of the authors and do not necessarily reflect the views of USAID, NIH, or the United States Government.

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Figure 1. Analyte Concentrations Among Women With 100% Adherence by MEMS.

Samples with 100% adherence over the prior month, by MEMS cap. Lines represent 25th, 50th and 75th percentiles and diamonds indicate means. A. TFV concentrations among the 55 non-pregnant, 18 1st trimester, 17 2nd trimester, and 15 3rd trimester samples. The reference line of 35 ng/mL represents the 10th percentile of steady-state TFV concentrations [29]. B. TFV-DP concentrations among 8 non-pregnant, 9 1st trimester, 6 2nd trimester, and 7 3rd trimester samples.

Table 1.

Participant Characteristics at Enrollment

	Non-Pregnant	Pregnant
N women	97	37
Mean age (SD)	30.6 (7.4)	25.1 (4.8)
<25 years old	26.8% (26)	46.0% (17)
Married to partner	99.0% (96)	91.9% (34)
Mean parity (SD)	2.8 (2.0)	1.4 (1.0)
Mean BMI (SD)	24.5 (4.3)	24.6 (4.7)
Mean creatinine clearance (SD)	101.8 (18.4)	111.7 (27.5)
% with Plasma Sample	85.6% (83)	89.2% (33)
Median # Plasma Samples (IQR)	1 (1,4)	4 (3,7)
% with DBS Sample	33.0% (32)	86.1% (31)
Median # DBS Samples (IQR)	1 (1,1)	2 (2,3)

Table 2.

PrEP Use & Analyte Concentrations among Pregnant vs Non-Pregnant Women

TFV ng/mL					
	Non-Pregnant (n=83 women, 226 samples)	Pregnant (n=33 women, 163 samples)	1 st Trimester (n=23 women, 42 samples)	2 nd Trimester (n=23 women, 59 samples)	3 rd Trimester (n= 23 women, 62 samples)
Mean doses (SD) *	23.1 (9.4)	22.2 (10.1)	23.6 (9.4)	22.0 (10.2)	21.5 (10.4)
Recent dose **	93.4% (211)	89.0% (145)	90.5% (38)	88.1% (52)	88.7% (55)
Mean TFV ng/mL (SD)	86.5 (90.6)	34.7 (44.5)	45.5 (65.8)	36.6 (36.8)	25.5 (30.0)
% TFV below limit of quantitation (n)	11.5% (26)	25.0% (41)	17.7% (7)	23.7% (14)	32.3% (20)

TFV-DP fmol/punch					
	Non-Pregnant (n=32 women, 32 samples)	Pregnant (n=31 women, 70 samples)	1 st Trimester (n= 25 women, 25 samples)	2 nd Trimester (n=24 women, 24 samples)	3 rd Trimester (n=21 women, 21 samples)
Mean doses (SD) *	20.7 (10.6)	21.4 (10.5)	22.5 (9.4)	19.3 (11.7)	22.4 (10.4)
Mean TFV-DP fmol/punch (SD)	636.7 (523.0)	450.3 (388.1)	561.6 (423.1)	368.1 (340.7)	411.9 (382.2)
% TFV-DP below limit of quantitation (n)	15.6% (5)	20.0% (14)	12.0% (3)	25.0% (6)	23.8% (5)

* Over past month according to MEMS caps

** Over past 2 days according to MEMS cap

Table 3.

Differences in Tenofovir Concentrations between Pregnant and Non-Pregnant Women

TFV ng/mL				
	Pregnant	1 st Trimester	2 nd Trimester	3 rd Trimester
Unadjusted Difference (95%CI)	-52.3 (-70.4, -34.2) (p<0.001)	-41.2 (-68.8, -13.7) (p=0.004)	-51.4 (-72.4, -30.3) (p<0.001)	-61.5 (-80.6, -42.3) (p<0.001)
Adjusted Difference [*] (95%CI)	-50.4 (-68.3, -32.5) (p<0.001)	-40.0 (-66.8, -13.3) (p=0.004)	-49.4 (-69.5, -29.2) (p<0.001)	-59.2 (-77.7, -40.7) (p<0.001)
Adjusted Difference [*] (95%CI), Among Detectable TFV	-50.8 (-69.7, -31.9) (p<0.001)	-41.8 (-71.2, -12.5) (p=0.007)	-49.0 (-70.5, -27.5) (p<0.001)	-60.6 (-80.3, -40.9) (p<0.001)
Unadjusted Difference [*] (95%CI), Among 100% Adherent	-51.8 (-85.3, -18.4) (p=0.003)	-53.9 (-91.7, -16.2) (p=0.009)	-48.8 (-88.7, -9.0) (p=0.02)	-52.3 (-90.5, -14.2) (p=0.01)

TFV-DP fmol/punch				
	Pregnant	1 st Trimester	2 nd Trimester	3 rd Trimester
Unadjusted Difference (95%CI)	-196.1 (-420.2, 28.0) (p=0.08)	-83.4 (-321.6, 154.8) (p=0.48)	-262.8 (-486.1, -39.5) (p=0.02)	-264.0 (-498.0, -29.9) (p=0.02)
Adjusted Difference ^{**} (95%CI)	-136.6 (-318.0, 44.8) (p=0.13)	-52.0 (-249.6, 145.7) (p=0.59)	-187.1 (-367.9, -6.3) (p=0.04)	-178.7 (-373.2, 15.7) (p=0.07)
Adjusted Difference ^{**} (95%CI), Among Detectable TFV-DP	-201.9 (-384.4, -19.4) (p=0.03)	-104.3 (-296.8, 88.3) (p=0.27)	-278.4 (-464.0, -92.8)(p=0.004)	-259.6 (-463.4, -55.8)(p=0.01)
Adjusted Difference ^{***} (95%CI), Among 100% Adherent	-383.3 (-807.7, 41.4) (p=0.07)	-138.8 (-638.9, 361.3) (p=0.52)	-490.1 (-982.2, 2.0) (p=0.05)	-503.8 (-999.7, -7.9) (p=0.04)

GEE models with unstructured correlation matrix and non-pregnant as reference.

* Adjusted for MEMS cap over prior 2 days.

** Adjusted for MEMS cap over prior month, age and BMI at baseline

*** Adjusted for age and BMI at baseline

Table 4.

PrEP Use & Analyte Concentrations Before and During Pregnancy

TFV ng/mL					
	Pre-Pregnancy (n=9 women, 7 samples)	Pregnancy (n=49 samples)	1 st Trimester (n=7 women, 15 samples)	2 nd Trimester (n=7 women, 20 samples)	3 rd Trimester (n=5 women, 14 samples)
Mean doses (SD) *	15.7 (11.5)	23.3 (10.3)	23.9 (9.8)	22.5 (11.3)	23.8 (10.1)
Mean TFV ng/mL (SD)	63.0 (65.0)	43.2 (50.8)	53.5 (55.3)	42.4 (52.1)	33.4 (45.3)
% TFV below limit of quantitation (n)	23.5% (4)	30.6% (4)	13.3% (2)	40% (8)	35.7 % (5)
Recent dose **	82.4% (14)	87.8% (43)	93.3% (14)	85% (17)	85.7% (12)

TFV-DP fmol/punch					
	Pre-Pregnancy (n=12 women, 12 samples)	Pregnancy (n=12 women, 27 samples)	1 st Trimester (n=11 women, 11 samples)	2 nd Trimester (n=10 women, 10 samples)	3 rd Trimester (n=6 women, 6 samples)
Mean doses (SD) *	18.8 (12.1)	15.6 (10.1)	17.3 (10.8)	13.3 (10.0)	16.2 (10.3)
Mean TFV-DP fmol/punch (SD)	591.7 (464.9)	232.1 (233.9)	257.7(270.5)	231.2 (232.1)	186.6 (193.3)
% TFV-DP below limit of quantitation (n)	8.3% (1)	25.9% (7)	18.2% (2)	30.0% (3)	33.3% (2)

* Over past month according to MEMS caps

** Over past 2 days according to MEMS caps