SUPPLEMENTAL TABLE 1. CONRAD A15-140 Schedule of Assessments

Visit #	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**
	Enrollment	**Baseline**	**Product Assignment**	**Post Product Use**
Phase of menstrual cycle		**Luteal**	**Follicular**	**Luteal**
Informed consent, Medical history, Vital Signs, Physical Exam	✓			
Pregnancy test, urine	✓	(✓)	✓	(✓)
Pap Smear, STI Testing, Chemistry Panel, Complete Blood Count	✓	(✓)	(✓)	(✓)
Product assignment and dispensation product			✓	
Plasma, cervical and vaginal biopsies and swabs (PK)				✓
Cervical and vaginal biopsies and swabs (PD)		✓		✓

(✓) = as indicated

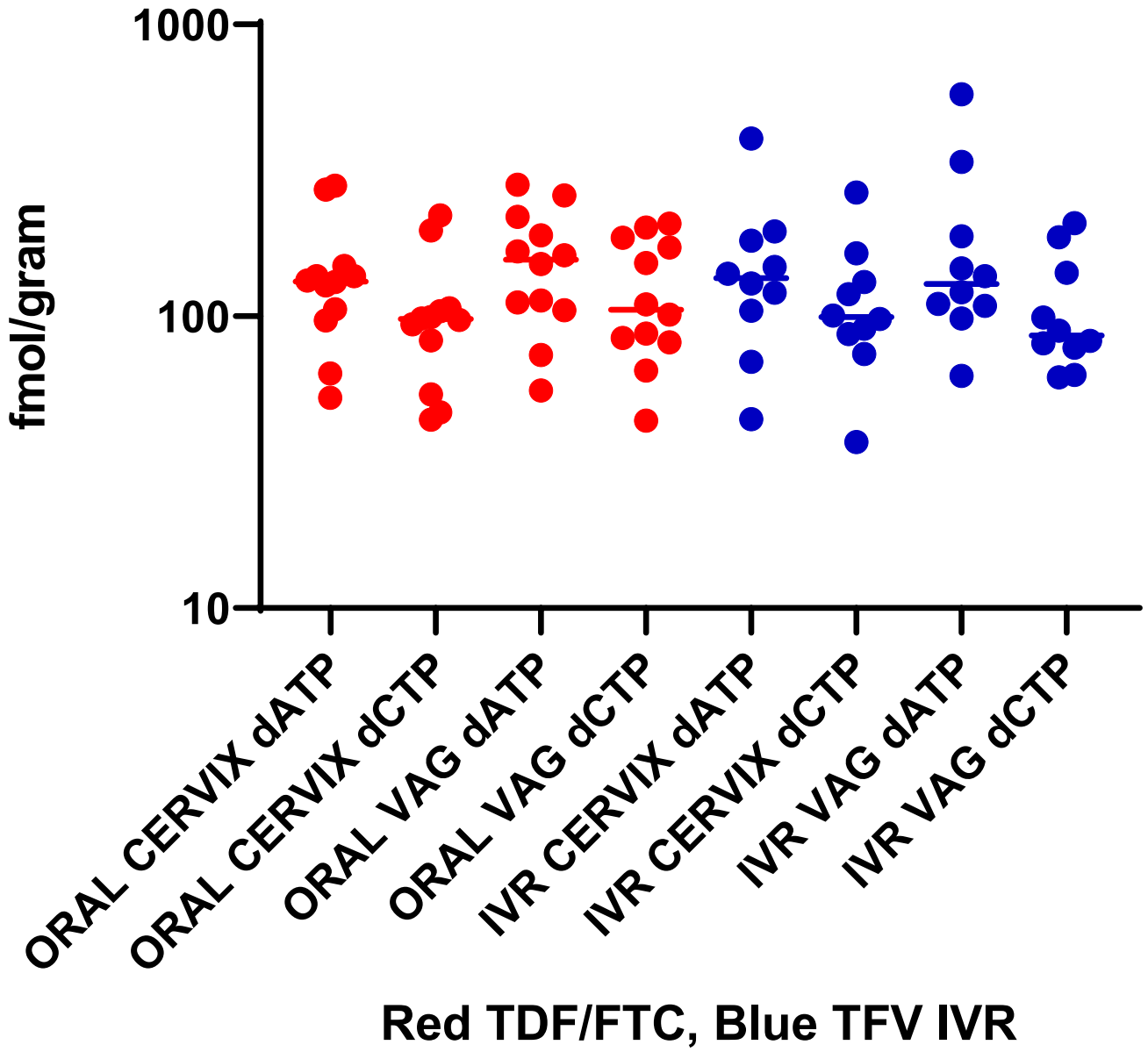
Supplemental Methods

Assessment of Plasma, Ectocervical and Vaginal Fluid and Tissue PK Parameters and Endogenous Nucleotides: All analytes were quantified by liquid chromatography, tandem mass spectrometry (LC-MS/MS) assays with precision and accuracy of 15% (plasma and tissue assays) or 20% (CVF assay). CVF on Dacron swabs was diluted with 1.5 ml of 0.9% sodium chloride and TFV and FTC were extracted from diluted CVF and human plasma by protein precipitation with the following isotopically-labeled internal standards (IS): $^{13}C_5$-TFV and $^{13}C^{15}N$-FTC, respectively. Analytes were separated by reverse phase chromatography with a Waters T3 (100 X 2.1mm 5µm particle size) column then detected on an AB Sciex API-5000 triple quadrupole mass spectrometer. The dynamic range was 1 – 4000 ng/mL for plasma and 0.2 – 100 ng/mL of extract for CVF.

Frozen tissue biopsies were homogenized in 1ml of 70:30 acetonitrile:1mM ammonium phosphate (pH 7.4) and all analytes were extracted by protein precipitation with the following isotopically-labeled IS: $^{13}C_5$-TFV (for TFV); $^{13}C^{15}N$-FTC (for FTC); $^{13}C_5$-TFV-DP (for TFV-DP); $^{13}C^{15}N$-FTC –TP (for FTC-TP); $^{13}C_{10}$,$^{15}N_5$-dATP (for dATP); and $^{13}C_9$,$^{15}N_3$-dCTP (for dCTP). Tissue homogenate extract (300 µL) was evaporated to dryness and reconstituted with 1mM ammonium phosphate (pH 7.4) for TFV, FTC, TFV-DP and FTC-TP quantification, and 200 µl of the extract was evaporated to dryness then reconstituted with purified water for dATP and dCTP quantification. Analytes were separated by reverse phase chromatography with a Waters T3 (100 X 2.1mm 5µm particle size) column (TFV/FTC) or by anion exchange chromatography with a Thermo Scientific BioBasic AX (50 X 2.1mm 5µm particle size) column (TFV-DP, FTC-TP, dATP, dCTP), then detected on an AB Sciex API-5000 triple quadrupole mass spectrometer. The dynamic range was 0.3 – 300 ng/mL homogenate for TFV, TFV-DP, FTC, and FTC-TP and 0.1 - 20 ng/mL homogenate for dATP and dCTP.

PK concentrations of parent drug (FTC and TFV) in plasma, CVF and ectocervical and vaginal tissues and active metabolites (FTC-TP and TFV-DP) and competing nucleotides (dATP and dCTP) in tissue were analyzed and summarized by treatment group and sample collection using descriptive statistics, which included the coefficient of variation, geometric mean, and geometric coefficient of variation. The geometric mean and geometric coefficient of variation were computed by calculating the mean and coefficient of variation of the log-transformed concentration values and then converting to the original scale by calculating the anti-log. Concentrations that were detectable but below the lower limit of quantification (LLOQ) were set to 0.5*LLOQ; concentrations below the limit of detection were set to zero.

Supplemental Figure 1: dATP dCTP in Cervical and Vaginal Tissues

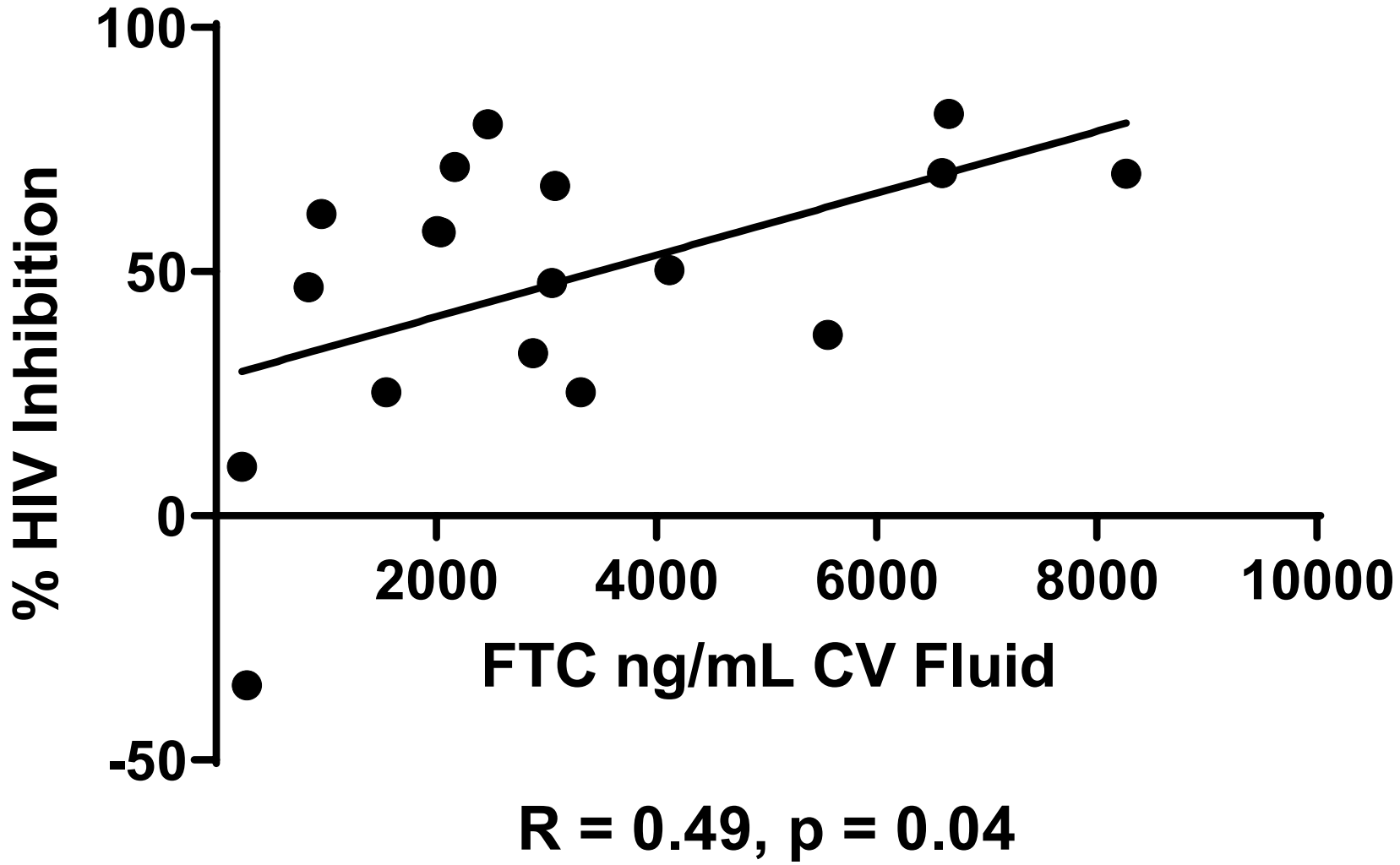


Red TDF/FTC, Blue TFV IVR

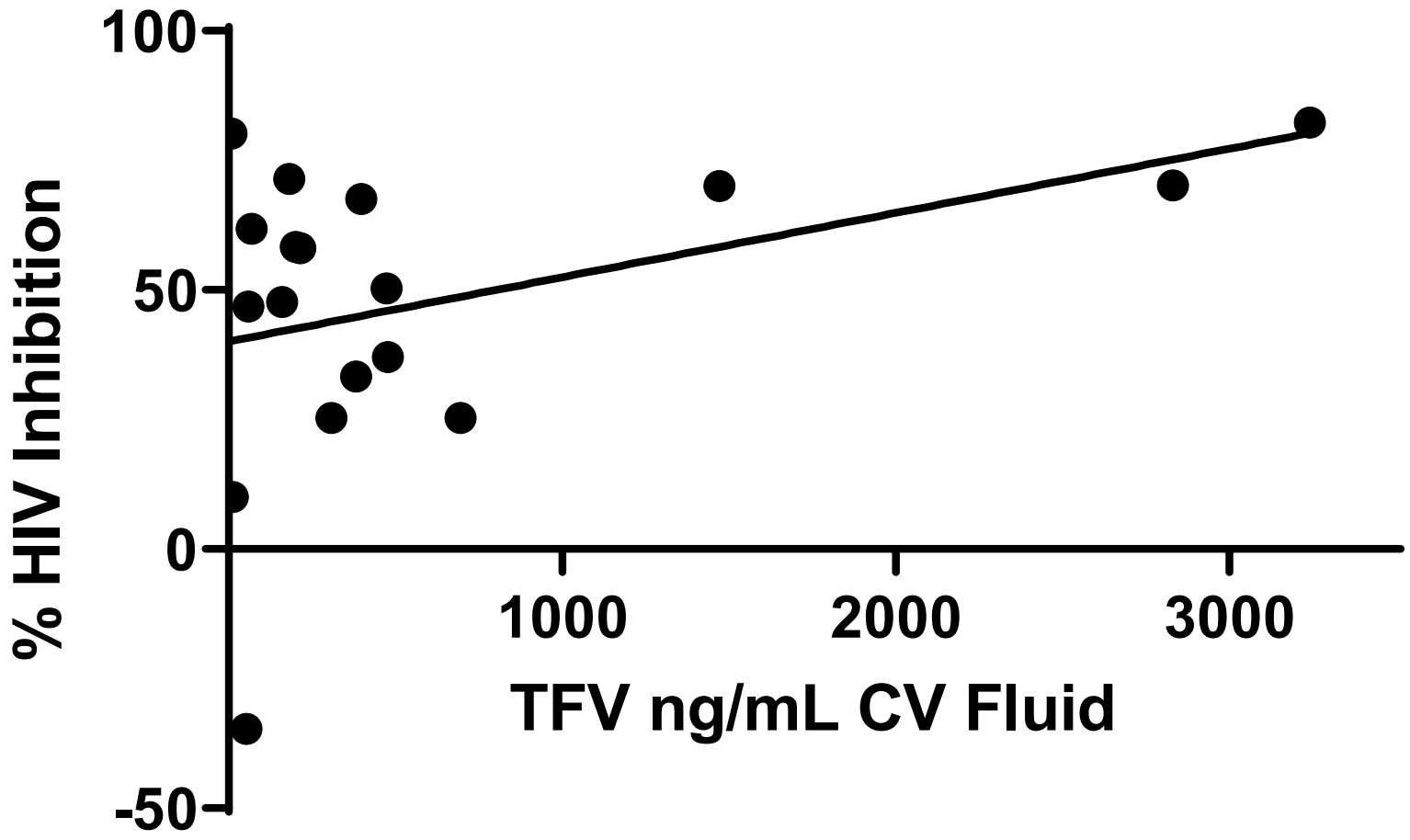
Supplemental Table 2: *In vitro* Inhibition of HIV-1_{BaL} by Vaginal and Ectocervical Fluids

Visit	Vaginal Fluid HIV Inhibition (%)				Ectocervical Fluid HIV Inhibition (%)				P value
	N	Mean	SD	Median	N	Mean	SD	Median	
TDF/FTC Users									
Pre-Treatment Baseline Visit 2	11	-3.4	34.5	-3.2	10	8.7	28.0	7.8	0.75
Post Treatment Visit 4	11	47.2	23.6	54.3	10	31.9	49.6	48.5	0.33
TFV IVR Users									
Pre-Treatment Baseline Visit 2	8	27.0	20.6	22.3	8	16.9	44.5	34.0	0.74
Post Treatment Visit 4	8	96.6	1.9	96.6	8	77.6	28.5	91.7	0.29

SUPP. FIG. 2a. TDF/FTC Users: FTC PK PD Correlation

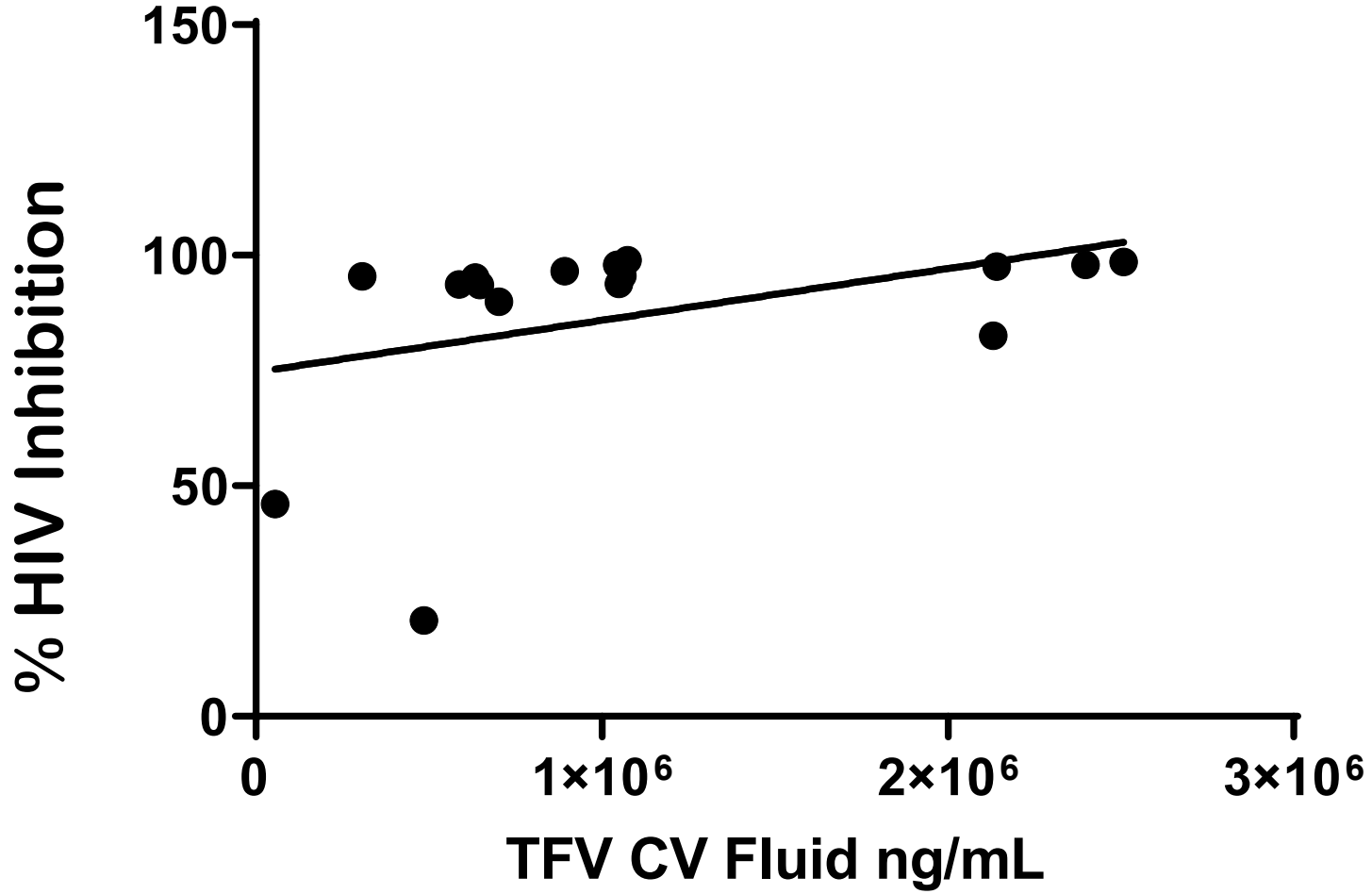


SUPP. FIG. 2b. TDF/FTC Users: TFV PK PD Correlation



R = 0.26, p = 0.29

SUPP. FIG. 2c. TFV IVR Users: TFV PK PD Correlation



R = 0.65 p <0.001