

# The ARK EFV-Test™: A Rapid, Automated Immunoassay for Therapeutic Drug Monitoring of Efavirenz

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## Abstract

**Background:** Therapeutic drug monitoring (TDM) in HIV disease may increase antiretroviral (ARV) efficacy by reducing toxicity, monitoring adherence, preventing drug resistance, and managing drug-drug interactions. Measuring ARVs with current techniques (e.g. HPLC or LC/MS/MS) is costly, time consuming, and requires specialized equipment and skilled technicians. A new rapid, automated enzyme immunoassay has been developed for determining plasma efavirenz (EFV) concentrations. Results using the new method were compared to those from a standard HPLC method.

**Methods:** ARK's EFV-Test™ is based on competitive binding to antibody between a drug in the sample and a drug-labeled enzyme. Drug concentration is measured spectrophotometrically in terms of enzyme activity using a Roche COBAS MIRA® bench top analyzer. Each test uses 4 µl of sample. The calibration standards ranged from 0.5 - 12 µg/mL. Patient samples and proficiency testing (PT) samples were run and compared to HPLC results.

**Results:** Validation data for controls (0.25 to 10 µg/mL) show inter-assay precision of <10% CV (n = 40). Accuracy was -4.0% deviation at 0.25 µg/mL and within 14% for remaining controls (n = 40). Assay sensitivity was 0.25 µg/mL. No significant interference was noted from 10 other ARV drugs. A comparison of patient samples analyzed by HPLC and ARK's EFV-Test™ yielded strong correlation:  $y = 0.937x + 0.126$ ,  $r = 0.99$ ,  $n = 44$ . Proficiency testing samples showed excellent agreement with target values.

**Conclusions:** ARK's EFV-Test™ for measuring efavirenz in plasma was validated. The test is an automated enzyme immunoassay (EIA) that requires minimal expertise, small sample volume, no sample pre-treatment, and provides the first result within 7.5 minutes. All reagents are supplied ready-to-use. This test shows good correlation with HPLC and is well-suited for routine TDM use. This test may also provide a cost-effective way to determine EFV concentrations in areas with high HIV prevalence and limited testing resources.

## COBAS MIRA Parameters

**Table 1.** Parameters established for the ARK EFV-Test™ on the COBAS MIRA System:

	Assay Parameter
Sample Volume (µL)	4
Reagent 1 (Antibody) Volume (µL)	150
Reagent 2 (Enzyme) Volume (µL)	75
Assay Temperature (°C)	37
Wavelength (nm)	340
Throughput (tests/hour)	72

## HPLC Procedure

Plasma efavirenz (EFV) levels were determined by a validated reverse-phase high-performance liquid chromatography (RF-HPLC) using UV detection. Briefly, plasma proteins were removed using acetonitrile. After centrifugation, the supernatant was injected directly onto a C-18 RF column and EFV was separated using a buffer of pH 3.1 which included 49% acetonitrile. UV detection was at 245nm. The preferred sample size for HPLC is 300-500µL.

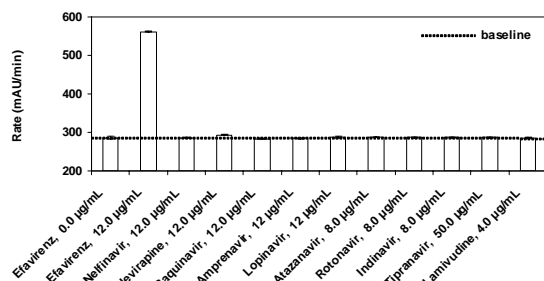
## Inter-Assay Precision and Accuracy Studies

**Table 2.** Five QC samples were tested using the ARK EFV-Test™ for plasma on the COBAS MIRA analyzer. Data are derived from 5 days: 2 runs per day, 4 replicates per run with a total of 40 replicates of each control level.

Conc. (µg/mL)	Assayed (Mean ± SD)	Precision (CV%)	Accuracy (Deviation %)
0.25	0.24 ± 0.02	9.9	-4.0
0.75	0.75 ± 0.03	4.3	0.5
2.0	2.28 ± 0.11	5.0	14.1
4.0	3.88 ± 0.19	4.9	-2.9
8.0	7.42 ± 0.50	6.8	-7.3
10.0	10.1 ± 0.72	7.1	0.8

## Specificity

**Figure 1.** Antiretrovirals whose chemical structure or concurrent therapeutic use would suggest possible cross-reactivity were tested at the levels indicated. None of the compounds tested gave an apparent efavirenz concentration as indicated by rates of change of absorbance within 3% of the baseline value.



## Lower Limit of Quantitation

**Table 3.** Pooled human serum samples were supplemented with known amounts of efavirenz at the concentrations shown below. Each sample was then assayed 20 times. The lowest concentration measured with acceptable accuracy and precision was 0.25 µg/mL.

	Conc. (µg/mL)	Assayed (Mean ± SD)	Precision (CV %)	Accuracy (Deviation %)
Assay result	0.25	0.28 ± 0.02	6.1	11

## Analytical Recovery

**Table 4.** Pooled human serum samples were supplemented with known amounts of efavirenz. Each sample was then assayed 4 times. The amount of efavirenz recovered from nominal ranged from 84% to 112%.

Conc. Tested (µg/mL)	Recovery	
	(Mean ± SD)	(%)
0.25	0.27 ± 0.02	107
0.50	0.56 ± 0.03	112
1.5	1.65 ± 0.05	110
2.5	2.55 ± 0.12	102
4.5	4.02 ± 0.16	89
7.5	6.45 ± 0.17	86
9.0	8.00 ± 0.65	89
10.0	8.42 ± 0.34	84

## AIDS Clinical Trials Group Proficiency Testing

**Table 5.** Proficiency testing (PT) samples were prepared by the AIDS Clinical Trials Group. High, medium, and low concentrations of protease inhibitors and non-nucleoside reverse transcriptase inhibitors were added to drug-free EDTA plasma. The samples were assayed for efavirenz in duplicate by the ARK EFV-Test™ and the mean compared to target values.

I.D.	Target (µg/mL)	ARK Mean (µg/mL)	I.D.	Target (µg/mL)	ARK Mean (µg/mL)
UCSD-RD17-D	5.51	4.84	UCSD-RD16-D	7.65	7.48
UCSD-RD17-E	0.41	0.48	UCSD-RD16-E	0.63	0.73
UCSD-RD17-F	1.54	1.51	UCSD-RD16-F	3.02	3.08
JHU-R17-D	0.24	0.38	JHU-R16-D	4.0	3.9
JHU-R17-E	9.8	8.1	JHU-R16-E	0.41	0.53
JHU-R17-F	4.3	3.8	JHU-R16-F	8.3	7.2

## Endogenous Interference

**Table 6.** Five (5) hypercholesterolemic, 1 hypertriglyceridemic, and 4 hyperbilirubinemic samples obtained from individual patients were supplemented with 6.0 µg/mL of efavirenz and tested in quadruplicate. One sample of normal human serum spiked with 100 mg/mL human gamma globulin was also tested in quadruplicate. The endogenous substances tested did not interfere significantly with the ARK EFV-Test™.

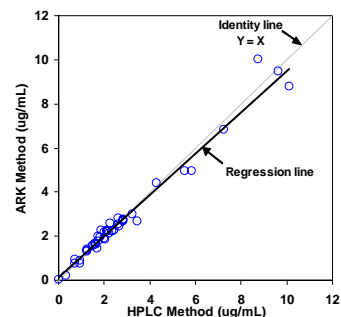
Endogenous Substance	Endogenous Substance Concentration Range	Efavirenz Recovery (% of expected value)
Cholesterol	304 - 346 mg/dL	88 - 94
Triglyceride	316 mg/dL	86
Total Bilirubin	28 - 31 mg/dL	92 - 102
Human Gamma Globulin	100 mg/mL	89

## Comparative Analysis

**Figure 2.** Patient samples dosed with efavirenz were analyzed using the ARK Efavirenz Assay on the COBAS MIRA chemistry analyzer and HPLC, and the results are compared below. Regression was calculated with the Passing-Bablok method.

### ARK EFV-Test™ versus HPLC comparison statistics:

Slope 0.937 (95% CI: 0.866 to 0.997)  
Intercept 0.126 (95% CI: 0.018 to 0.283)  
Pearson's correlation (r) 0.99  
N 44



## Conclusions

The ARK EFV-Test™ is an accurate and precise method to conveniently measure efavirenz in plasma. This assays offer the following advantages to laboratories:

- No sample extraction or pretreatment required
- High specificity and good sensitivity
- Small sample size
- Excellent correlation to an HPLC method for EFV
- Ready-to-use liquid reagents and calibrators
- Rapid turn-around time