

# Therapeutic Drug Monitoring Immunoassay for Efavirenz

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## Abstract

A homogeneous enzyme immunoassay for Efavirenz (EFV) was developed. Adherence to effective antiretroviral (ARV) therapy is important for achieving and maintaining viral suppression. Verification of plasma drug level ensures that intended targets are met. Therapeutic drug monitoring (TDM) used during initiation of therapy has been proposed to benefit viral suppression that might be suboptimal in patients who fail to achieve expected drug levels under standard dosing. Efavirenz in plasma (or serum) and drug-labeled-enzyme compete for binding to antibody. Enzymatic activity increases proportionally with drug concentration. Calibration standards ranged from 0.00 – 12.00 µg/mL. Performance was established on the Roche COBAS MIRA<sup>®</sup> and a preliminary application was developed for the Roche Hitachi 917<sup>®</sup> analyzer. Precision, limit of quantification, analytical recovery, specificity, interference by endogenous substances, linearity and comparative analysis versus HPLC were assessed. Data for a representative lot on the MIRA are given. Total (within-laboratory precision) coefficient of variation was 8.3% (1.00 µg/mL), 8.3% (4.00 µg/mL) and 9.2% (8.00 µg/mL) for tri-level control solutions. Within-run precision was 5.3%, 5.4%, and 6.0% respectively. The limit of quantification was 0.20 µg/mL and efavirenz measurements were accurate within 14% between 0.50 and 9.00 µg/mL for spiked levels tested. The assay was linear from 0.45 to 10.80 µg/mL and did not crossreact with seventeen other ARV drugs that could be co-administered with Efavirenz. Interference from endogenous substances was tested by spiking 2.00 µg/mL efavirenz into normal serum (control), patient samples with elevated cholesterol, triglycerides, bilirubin (total), and normal serum to which was added hemoglobin, human IgG, or albumin. Quantitative recovery (% versus control) ranged 91.1 – 93.2% in cholesterol (267.9 – 301.0 mg/dL), 82.0 – 95.9% in triglycerides (919.5 – 1060.0 mg/dL), 82.5 – 88.1% in bilirubin (23.6 – 24.4 mg/dL), 101.8% in hemoglobin (1000 mg/dL), 85.3% in human IgG (approx. 11 g/dL; added 9 g/dL) and 82.6% in human albumin (approx. 9 g/dL; added 5 g/dL). The assay may use serum or plasma anticoagulated with either heparin or EDTA. Comparative analysis (Passing-Bablok) of ARK (y) versus HPLC (x) yielded good correlation where  $y = 0.87x + 0.25$  (r=0.99; n=43). Improvement to precision and interference was observed on the Hitachi 917 analyzer. Sufficient precision and accuracy was obtained for general clinical laboratory performance to verify that dosage achieves a target serum/plasma concentration of efavirenz.

## Introduction

Efavirenz (SUSTIVA<sup>®</sup>, Bristol-Meyers Squibb) is an antiretroviral non-nucleoside reverse transcriptase inhibitor used in the treatment of Human Immunodeficiency Virus (HIV Type 1). The suggested minimum target trough concentration for naïve patients with wild-type HIV-1 is 1.00 µg/mL. Trough levels above 4.00 µg/mL are associated with increased risk of neurotoxicity.

**Scenarios for Use of TDM.** There are multiple scenarios in which both data and expert opinion indicate that information on the concentration of an antiretroviral agent may be useful in patient management ([www.aidsinfo.nih.gov/guidelines](http://www.aidsinfo.nih.gov/guidelines); [www.bhiva.org](http://www.bhiva.org); [www.HIVpharmacology.com](http://www.HIVpharmacology.com)). Consultation with an expert clinical pharmacologist may be advisable. These scenarios include:

- **clinically significant drug-drug or drug-food interactions** that may result in reduced efficacy or increased dose-related toxicities;
- **changes in pathophysiological states** that may impair gastrointestinal, hepatic, or renal function, thereby potentially altering drug absorption, distribution, metabolism, or elimination;
- **in persons such as pregnant women** who may be at risk for virologic failure as a result of their pharmacokinetic characteristics that result in plasma concentrations lower than those achieved in the typical patient;
- **in treatment-experienced persons** who may have viral isolates with reduced susceptibility to antiretroviral agents;
- **use of alternative dosing regimens** in which safety and efficacy have not been established in clinical trials;
- **concentration-dependent toxicities**; and
- **lack of expected virologic response** in a treatment-naïve person.

## HPLC Procedure

Plasma efavirenz (EFV) levels were determined by a validated reverse-phase high-performance liquid chromatography (RF-HPLC) using UV detection (University of California, San Francisco). EFV and its internal standard were isolated from plasma (200 µL) by a simple acetonitrile precipitation of proteins followed by centrifugation. The supernatant was then transferred into glass culture tubes, evaporated to dryness under nitrogen, and reconstituted with mobile phase for injection onto a C-18 RF column. EFV was separated using an isocratic mobile phase of sodium phosphate and acetonitrile. The system used a photodiode array detector set at 247 nm for detection.

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## Instrument Parameters

Table 1. Parameters have been established for the ARK Efavirenz Assay on the Roche COBAS MIRA and Hitachi 917 Systems.

	COBAS MIRA	Hitachi 917
Sample Volume (µL)	4	3
Reagent 1 Volume (µL)	150	150
Reagent 2 Volume (µL)	75	75
Assay Temperature (°C)	37	37
Wavelength (nm)	340	340
Throughput (tests/hour)	72	800

## Precision

Table 2. Three Quality Control (QC) samples were tested using the ARK Efavirenz Assay on the COBAS MIRA and Hitachi 917 analyzer. The data were derived from 20 days: 2 runs per day, 4 replicates per run with a total of 160 replicates of each control level for the COBAS MIRA and 5 days: 2 runs per day, 4 replicates per run with a total of 40 replicates of each control level for the Hitachi 917. Percentage recovery was based on nominal QC values.

	COBAS MIRA	Hitachi 917	
<b>QC Low (1.0 µg/mL)</b>	Assayed µg/mL (Mean ± SD)	1.01 ± 0.08	0.92 ± 0.07
	Precision (CV%)	8.3	6.5
	% Recovery	101	92
<b>QC Mid (4.0 µg/mL)</b>	Assayed µg/mL (Mean ± SD)	4.27 ± 0.35	3.82 ± 0.16
	Precision (CV%)	8.3	4.1
	% Recovery	107	96
<b>QC High (8.0 µg/mL)</b>	Assayed µg/mL (Mean ± SD)	7.87 ± 0.72	7.73 ± 0.50
	Precision (CV%)	9.2	5.7
	% Recovery	98	97

## Lower Limit of Quantitation

Table 3. Limit of quantitation was evaluated according to CLSI/NCCLS EP17-A. Pooled human serum was supplemented with known amounts of Efavirenz and assayed 40 times. The lowest concentration with acceptable accuracy (% Recovery) and precision was 0.20 µg/mL.

Conc. 0.20 µg/mL	COBAS MIRA	Hitachi 917
Assayed (Mean ± SD)	0.21 ± 0.05	0.20 ± 0.02
Precision (CV %)	22.9	9.9
% Recovery	105	100

## Analytical Recovery

Table 4. Pooled human serum samples were supplemented with known amounts of efavirenz. Each sample was then assayed 5 times. The amount of efavirenz recovered from nominal ranged from 92.0% to 114.0% for the COBAS MIRA and 90.0% to 105.2% for the Hitachi 917.

Conc. Tested (µg/mL)	COBAS MIRA Recovery		Hitachi 917 Recovery	
	(Mean ± SD)	(%)	(Mean ± SD)	(%)
0.5	0.48 ± 0.03	95.6	0.45 ± 0.03	90.0
1.0	0.92 ± 0.06	92.0	0.93 ± 0.03	93.4
2.0	2.20 ± 0.10	110.0	2.10 ± 0.02	105.2
4.0	4.05 ± 0.11	101.2	4.17 ± 0.15	104.3
5.0	5.70 ± 0.43	114.0	4.98 ± 0.19	99.6
7.0	7.32 ± 0.04	104.5	6.98 ± 0.27	99.7
9.0	9.44 ± 0.43	104.9	8.86 ± 0.26	98.5

## Specificity

Table 5. Other antiretroviral drugs were tested for possible cross-reactivity at 30 µg/mL in the presence of 2.00 µg/mL of Efavirenz in pooled negative human serum. Solvent controls were also prepared and tested. None of the compounds tested crossreacted, since the recovery of efavirenz was within the error of the method.

Compound	Efavirenz Recovery (%)	
	COBAS MIRA	Hitachi 917
Abacavir	103.6	97.7
Amprenavir	99.5	103.1
Atazanavir	99.6	100.3
Didanosine	100.7	99.7
Emtricitabine	96.7	100.0
Indinavir	95.4	100.0
Lamivudine	101.7	99.3
Loginavir	100.2	100.8
Nelfinavir	99.8	100.6
Nevirapine	97.9	98.8
Ritonavir	100.1	101.0
Saquinavir	95.9	98.8
Stavudine	91.1	101.5
Tenofovir	95.7	100.4
Tipranavir	94.5	98.5
Zalcitabine	95.2	98.1
Zidovudine	99.4	97.1

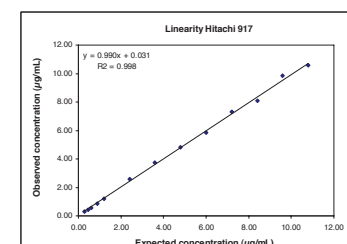
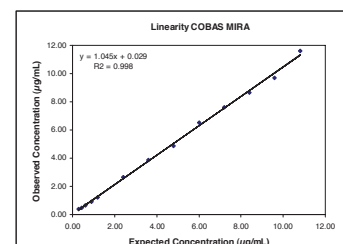
## Endogenous Interference

Table 6. High levels of endogenous substances were evaluated, and the recovery observed was within 20% allowable error of the target level of Efavirenz. Two elevated cholesterol, five elevated triglyceride, and two hyperbilirubinemic plasma samples obtained from individual clinical patients were tested. Negative human serum was supplemented with gammaglobulin, hemoglobin, or human albumin. All samples were supplemented with 2.00 µg/mL of Efavirenz.

Endogenous Substance	COBAS MIRA Recovery (%)	Hitachi 917 Recovery (%)
Cholesterol (267.9 - 301.0 mg/dL)	91.1-93.2	92.7-98.8
Triglyceride (919.5 - 1060.0 mg/dL)	82.0-95.9	87.3-96.8
Total Bilirubin (23.6 - 24.4 mg/dL)	82.5-88.1	95.6-98.7
Hemoglobin (1000 mg/dL)	101.8	103.7
Human Albumin (9 g/dL)	82.6	96.1
Human Gamma Globulin (11 g/dL)	85.3	100.9

## Linearity

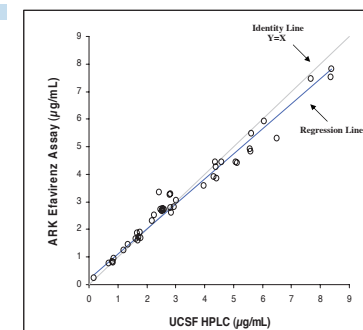
Figures 1 and 2. Linearity was determined according to CLSI EP6-A. The assay was considered linear when the percentage difference between the predicted 1st order and 2nd order polynomial was within 10%. Negative pooled human serum was supplemented with Efavirenz to give 12.00 µg/mL and then diluted proportionally. The assay was linear from 0.30 to 10.80 µg/mL on the MIRA and on the Hitachi 917. Regression plots of observed versus expected concentrations are shown.



## Comparative Analysis

Figure 3. Clinical specimens from patients treated with Efavirenz were analyzed retrospectively using the ARK Efavirenz Assay on the COBAS MIRA chemistry analyzer and HPLC. Comparison by Passing-Bablok regression of the results is shown in the figure below.

ARK Efavirenz Assay vs HPLC	
Slope	0.87
Intercept (µg/mL)	0.25
Correlation	0.99
N	43



## Conclusions

The ARK Efavirenz Assay is an accurate and precise method to conveniently measure Efavirenz in serum or plasma on automated clinical chemistry analyzers. The assay offers the following advantages to laboratories:

- Small sample size – Serum or Plasma (heparin or EDTA)
- No sample extraction or pretreatment
- Excellent specificity, analytical sensitivity and linearity
- Acceptable correlation to HPLC and accuracy
- Ready-to-use liquid reagents and calibrators
- Rapid turn-around time

\*For Investigational Use Only. The Performance Characteristics of This Product Have Not Been Established\*