ARK Diagnostics, Inc.

For Export Only – Not For Sale in USA

ARK™ *Efavirenz Assay*

This ARK Diagnostics, Inc. package insert for the ARK Efavirenz Assay must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

CUSTOMER SERVICE

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KEY TO SYMBOLS USED

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE	CE Mark
IVD	In Vitro Diagnostic Medical Device		Temperature limitation
	Consult Instructions for Use	R1 R2	Reagent 1/ Reagent 2
Rx Only	For Prescription Use Only		

1 NAME

ARK™ *Efavirenz Assay*

2 INTENDED USE

The ARK™ Efavirenz Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of efavirenz in human serum or plasma on automated clinical chemistry analyzers. The serum or plasma concentration of efavirenz, used in combination with other clinical information, may aid in the management of patients treated with efavirenz. Measurements of efavirenz are intended to help ensure appropriate therapy or to monitor adherence.

3 SUMMARY AND EXPLANATION OF THE TEST

Efavirenz (SUSTIVA®, Bristol-Myers Squibb Company) is a non-nucleoside reverse transcriptase inhibitor indicated in combination with other antiretroviral agents for the treatment of human immunodeficiency virus type 1 infection in adults and in pediatric patients at least 3 months old and weighing at least 3.5 kg.[1]

4 PRINCIPLES OF THE PROCEDURE

ARK Efavirenz Assay is a homogeneous enzyme immunoassay based on competition between drug in the specimen and efavirenz labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for binding to the antibody reagent. As the latter binds antibody, enzyme activity decreases. In the presence of drug from the specimen, enzyme activity increases and is directly proportional to the drug concentration. Active enzyme converts the coenzyme nicotinamide adenine dinucleotide (NAD) to NADH that is measured spectrophotometrically as a rate of change in absorbance. Endogenous serum G6PDH does not interfere with the results because the coenzyme NAD functions only with the bacterial enzyme used in the assay.

5 REAGENTS

REF	Product Description	Quantity/Volume
5017-0001-00	**ARK Efavirenz Assay** **Reagent R1 – Antibody/Substrate** rabbit polyclonal antibodies to efavirenz, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 28 mL
	Reagent R2 – Enzyme Efavirenz labeled with bacterial G6PDH, buffer, bovine serum albumin, sodium azide, and stabilizers	1 X 14 mL

Reagent Handling and Storage

ARK Efavirenz Assay reagents are provided liquid, ready to use and may be used directly from the refrigerator. When not in use, reagents must be stored at 2–8°C (36–46°F), upright and with screw caps tightly closed. If stored as directed, reagents are stable until the expiration date printed on the label. Do not freeze reagents. Avoid prolonged exposure to temperatures above 32°C (90°F). **Improper storage of reagents can affect assay performance.**

ARK Efavirenz products contain ≤0.09% sodium azide. As a precaution, affected plumbing including instrumentation should be flushed adequately with water to mitigate the potential accumulation of explosive metal azides. No special handling is required regarding other assay components.

6 WARNINGS AND PRECAUTIONS

- For In Vitro Diagnostic Use. For prescription use only.
- Reagents R1 and R2 are provided as a matched set and should not be interchanged with reagents from different lot numbers.
- Reagents contain ≤0.09% sodium azide.

7 SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- Serum or plasma is required. For consistency, using the same specimen matrix for individual patients is a good practice. A steady state, trough (pre-dose) sample is generally accepted as most consistent for therapeutic drug monitoring of efavirenz. Time of blood draw since last dose should be noted.
- Whole blood cannot be used. The following anticoagulants were shown not to interfere with this assay.
  - Sodium heparin
  - Lithium heparin
  - Potassium EDTA
- Blood collection must be performed with collection tubes compatible for use with therapeutic drug monitoring (TDM).

- Do not induce foaming and avoid repeated freezing and thawing to preserve the integrity of the specimen from the time it is collected until the time it is assayed.
- Fibrin, red blood cells, and other particulate matter may cause an erroneous result. Ensure adequate centrifugation.
- **Handle all patient specimens as if they were potentially infectious.**

8 PROCEDURE

Materials Provided

ARK Efavirenz Assay – REF 5017-0001-00

Materials Required – Provided Separately

ARK Efavirenz Calibrator – REF 5017-0002-00
Quality Controls – ARK Efavirenz Control – REF 5017-0003-00

Instruments

Reagents R1 and R2 may need to be transferred to analyzer-specific reagent containers prior to use. Avoid cross-contamination of R1 and R2 .

Assay Sequence

To run or calibrate the assay, see the instrument-specific operator's manual and instrument-specific application sheet.

Calibration

Perform a calibration using the ARK Efavirenz Calibrators A, B, C, D, E, and F. Calibration is required with each new reagent kit lot number. Verify the calibration curve with quality controls according to the established laboratory quality assurance plan.

When to Re-Calibrate

- Whenever a new lot number of reagents is used
- Whenever indicated by quality control results
- Whenever required by standard laboratory protocols

Quality Control (QC)

Follow QC procedures for the ARK Efavirenz Assay. All quality control requirements and testing should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Manual Dilution Protocol

The measurement range of the ARK Efavirenz Assay is 0.2 – 10.0 µg/mL. Specimens containing efavirenz in higher concentrations (>10.0 µg/mL) are assayed by dilution of the specimen into the measurement range. Dilute the specimen with zero calibrator (CAL A). A four-fold dilution factor is suggested. Multiply the assayed result by the dilution factor.

$$\text{Manual Dilution Factor} = \frac{(\text{Volume of Specimen} + \text{Volume of CAL A})}{\text{Volume of Specimen}}$$

9 RESULTS

Report result units as µg/mL or µmol/L. To convert results from µg/mL efavirenz to µmol/L efavirenz, multiply µg/mL by 3.168. The efavirenz value from this assay should be used in conjunction with other clinical information. Refer to the instrument specific operator's manual for any result error codes.

10 LIMITATIONS OF PROCEDURE

This assay is designed for use with serum or plasma only; refer to the sections Specimen Collection and Preparation for Analysis. It is generally good practice to use the same method (as well as matrix) consistently for individual patient care due to the potential for method-to-method variabilities. See the section **Expected Values** below.

11 EXPECTED VALUES

A therapeutic range for efavirenz has not been well established. The reference range of 1.0 µg/mL to 4.0 µg/mL has been proposed.[2,3] Efavirenz plasma concentrations below 1.0 µg/mL have been associated with increased virological failure and an increased risk in development of drug resistance, while adverse effects have been observed at efavirenz plasma concentrations above 4.0 µg/mL.[4-6]

CYP2B6 polymorphism has been shown to be a key factor in inter-individual variability in efavirenz plasma concentrations.[7-9] However, gender, race, body mass index, and time after last drug intake are considered important factors in determining inter-individual variability in efavirenz plasma concentrations.[4,10] Multiple samples over time may be needed to determine steady-state concentrations for individual patients.

HIV treatment guidelines in the United States and Europe recommend using therapeutic drug monitoring (TDM) to help optimize antiretroviral (ARV) treatment in certain individuals, such as those with renal or hepatic impairment, with suspected drug-drug or drug-food interactions, with suspected non-adherence, with potential drug concentration-related toxicities, and pregnant women and children.[11-17]

Efavirenz drug concentrations should not be the only means of therapeutic drug management. The assay should be used in conjunction with information available from clinical evaluations and other diagnostic procedures. Clinicians should carefully monitor patients during therapy and dosage adjustments. Consideration should be given to the requirements for pediatric use, since metabolism in children may be different than for adults.

12 SPECIFIC PERFORMANCE CHARACTERISTICS

Each laboratory is responsible for verification of performance using instrument parameters established for their analyzer. The following performance characteristics were obtained on the Beckman Coulter AU480® System unless otherwise noted.

Sensitivity

Limit of Quantitation (LOQ)

The following characteristics were determined according to CLSI EP17-A2 for the ARK Efavirenz Assay. Analyzer-specific performance may vary.

Criterion	Efavirenz Concentration (µg/mL)
Limit of Blank (LoB); N = 60 µB + 1.645 SD , where SD = 0.0063	0.02
Limit of Detection (LoD); N = 60 LoB + 1.652 SD, where SD = 0.0142	0.04
Limit of Quantitation (LoQ); N = 40 LoQ – 2 SD > LoD With acceptable recovery and linearity	0.2

Each laboratory is responsible for determining reporting criteria for Efavirenz concentrations. The following suggestion from CLSI EP17-A2 may be appropriate:

Result ≤ LoB	report "not detected; concentration < LoD"
LoB < Result < LoQ	report "analyte detected; concentration < LoQ"
Result ≥ LoQ	report the result as measured

Measurement Range

The measurement range of the ARK Efavirenz Assay is 0.2 – 10.0 µg/mL. Specimens containing efavirenz in higher concentrations (>10.0 µg/mL) are assayed by dilution of the specimen into the measurement range. Refer to **Section 8 Procedure - Manual Dilution Protocol**.

Recovery

Analytical recovery was assessed by adding concentrated efavirenz drug into human serum negative for efavirenz. A stock concentrate of efavirenz in methanol was added volumetrically to human serum negative for efavirenz, representing drug concentrations across the assay range. Six replicates of each sample were assayed. The results were averaged and compared to the target concentration and percent recovery calculated.

$$\%\ \text{Recovery} = \frac{100 \times \text{Mean recovered concentration}}{\text{Theoretical concentration}}$$

Theoretical Concentration (µg/mL)	Mean Recovered Concentration (µg/mL)	Percent Recovery
0.50	0.53	105.0
1.50	1.61	107.3
3.00	2.81	93.8
5.00	4.57	91.3
7.00	6.48	92.5
9.00	8.87	98.5

Mean percent recovery: 98.1

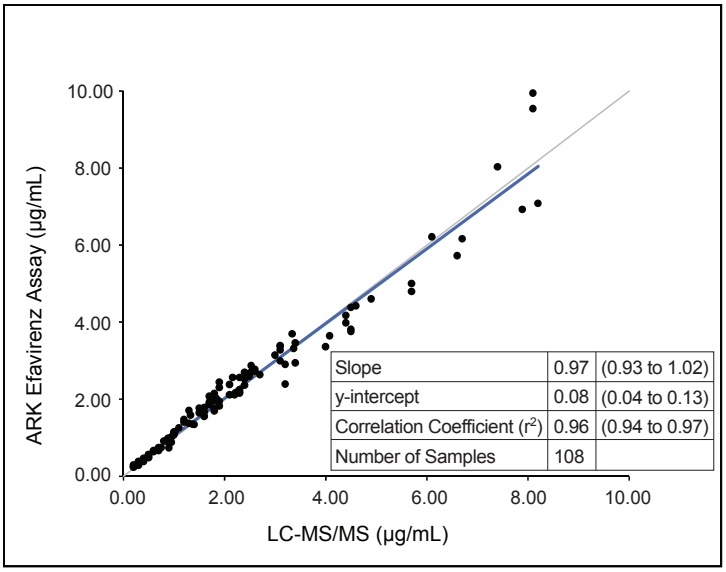
Linearity

Linearity studies were performed as suggested in CLSI/NCCLS Protocol EP6-A. A 15.0 µg/mL efavirenz serum sample was prepared and dilutions were made proportionally with human serum negative for efavirenz. Linearity at specific dilutions was considered acceptable if the percent difference was ±10% between the predicted 1st and 2nd order regressed values, or ≤ 0.2 µg/mL at concentrations ≤ 1.0 µg/mL. A linear relationship was demonstrated between 0.0 and 10.0 µg/mL.

Nominal (µg/mL)	Measured Results (µg/mL)	1st Order Predicted Results	2nd Order Predicted Results	Difference
0.00	0.01	-0.03	0.13	0.16 µg/mL
0.50	0.52	0.46	0.55	0.09 µg/mL
1.00	1.03	0.95	0.98	0.03 µg/mL
2.00	2.02	1.93	1.85	-4.3%
4.00	3.78	3.90	3.70	-5.1%
6.00	5.54	5.86	5.67	-3.1%
8.00	7.68	7.82	7.78	-0.5%
10.00	10.11	9.78	10.01	2.4%

Method Comparison

Method comparison studies were performed using CLSI Protocol EP9-A3 as a guideline. Testing was performed on the Abbott Architect automated clinical chemistry analyzer. Results from the ARK Efavirenz Assay were compared with results from LC-MS/MS for sera obtained from 108 patients with efavirenz concentrations ranging from 0.20 µg/mL to 8.20 µg/mL. Passing-Bablok[18] regression statistics are shown below (with 95% confidence limits).



Precision

Precision was determined as described in CLSI Protocol EP5-A3. Tri-level controls and three human serum pooled specimens containing efavirenz were used in the study. Each level was assayed in quadruplicate twice a day for 20 days. Each of the runs per day was separated by at least two hours. The within run, between day, total SD, and percent CVs were calculated. Results are shown below. Acceptance criteria: ≤10% total CV.

Sample	N	Mean	Within Run		Between Day		Total	
		(µg/mL)	SD	CV (%)	SD	CV (%)	SD	CV (%)
ARK Efavirenz Control								
LOW	160	1.09	0.046	4.2	0.020	1.8	0.051	4.6
MID	160	3.74	0.182	4.9	0.150	4.0	0.230	6.2
HIGH	160	7.71	0.450	5.8	0.231	3.0	0.506	6.6
Human Serum								
LOW	160	1.09	0.041	3.8	0.023	2.1	0.053	4.9
MID	160	3.95	0.202	5.1	0.145	3.7	0.250	6.3
HIGH	160	7.98	0.441	5.5	0.217	2.7	0.502	6.3

Interfering Substances

Interference studies were conducted using CLSI Protocol EP7-A2 as a guideline. Clinically high concentrations of the following potentially interfering substances in serum with known levels of efavirenz (1.0 and 4.0 µg/mL) were evaluated. Each sample was assayed using the ARK Efavirenz Assay, along with a serum control of efavirenz. Measurement of efavirenz resulted in ≤10% error in the presence of interfering substances at the levels tested.

		Percentage Recovery	
Interfering Substance	Interferent Concentration	1.0 µg/mL Efavirenz	4.0 µg/mL Efavirenz
Albumin	12 g/dL	99.7	93.5
Bilirubin - conjugated	70 mg/dL	104.8	94.9
Bilirubin - unconjugated	70 mg/dL	105.6	98.8
Cholesterol	400 mg/dL	97.1	92.4
Gamma-Globulin	12 g/dL	102.4	106.0
Hemoglobin	1000 mg/dL	103.8	105.2
Rheumatoid Factor	1000 IU/mL	100.2	102.6
Triglycerides	1000 mg/dL	99.4	92.6
Uric Acid	30 mg/dL	100.2	98.1

Specificity

Metabolism

Efavirenz displays highly variable non-linear pharmacokinetics, which are primarily due to polymorphic CYP2B6 metabolism. After oral administration, efavirenz is extensively metabolized to inactive metabolites including 7-hydroxyefavirenz, 8-hydroxyefavirenz and 8,14-dihydroxyefavirenz.[19] Efavirenz and its major metabolite, 8-hydroxyefavirenz, are present quantitatively in human plasma[20,21], while hydroxylated metabolites are easily excreted into urine. The concentration of 8-hydroxyefavirenz metabolite usually does not exceed concentration of the parent drug. Pharmacokinetics of efavirenz may be further influenced by other drug metabolizing enzymes and age-based differences in drug metabolism.

Metabolite

The crossreactivity of 8-hydroxyefavirenz metabolite (10.0 µg/mL) in the ARK Efavirenz Assay was not clinically significant (0.2% crossreactivity) when tested in the presence of efavirenz (2.0 µg/mL) in human serum.

Crossreactivity

The compounds listed below did not interfere with the ARK Efavirenz Assay when tested in presence of efavirenz (2.0 µg/mL). Levels tested were at or above maximum physiological or pharmacological concentrations. Efavirenz concentrations of samples containing interferent were compared to the efavirenz level in a normal serum control. Measurement of efavirenz resulted in ≤10% error in the presence of drug compounds at the levels tested.

Compound	Concentration (µg/mL)	Compound	Concentration (µg/mL)
Abacavir	30.0	Lopinavir	30.0
Amprenavir	30.0	Maraviroc	30.0
Atazanavir	30.0	Nelfinavir	30.0
Atovaquone	30.0	Nevirapine	30.0
Cycloguanil	5.0	Pyrazinamide	100.0
Didanosine	30.0	Rifampicin	50.0
Emtricitabine	30.0	Ritonavir	30.0
Ethambutol	20.0	Saquinavir	30.0
Indinavir	30.0	Stavudine	30.0
Isoniazid	20.0	Tenofovir	30.0
Lamivudine	30.0	Tipranavir	30.0

13 REFERENCES

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14 TRADEMARKS

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