

## The ARK APV-Test™:

### A Rapid, Automated Immunoassay for Amprenavir in Human Plasma

Michael K. Helms<sup>1</sup>, Byung S. Moon<sup>1</sup>, Johnny J. Valdez<sup>1</sup>, Brookie Best<sup>2</sup>, Diane T. Holland<sup>2</sup>,  
Rowena J. Espina-Quinto<sup>2</sup>, Rebecca Jauregui<sup>2</sup>, and James D. Connor<sup>2</sup>

<sup>1</sup>ARK Diagnostics, Inc., Sunnyvale, CA, USA; <sup>2</sup>University of California, San Diego, La Jolla, CA, USA

#### Abstract

**Background:** Current antiretroviral (ARV) assays for pharmacokinetics, adherence, and therapeutic drug monitoring are costly, time-consuming, and require specialized equipment and technologists. A rapid, automated enzyme immunoassay system has been developed for several drugs and we have applied the ARK APV Test™ for amprenavir (APV) to human plasma samples. Here we report the performance results from the immunoassay and a comparison to a validated HPLC method.

**Methods:** The ARK APV Test™ is based on competitive binding to an antibody between drug in the sample and drug-labeled enzyme. All reagents are supplied ready-to-use, the test is automated, and is read with the Roche COBAS MIRA® bench-top analyzer. A 50 µL sample is required and each test uses 4 µL in duplicate. Calibration standards ranged from 0.5 to 12.0 µg/mL, with a sensitivity of 0.25 µg/mL. Proficiency testing (PT) samples were run in parallel by both the ARK APV Test™ and HPLC and the results were compared.

**Results:** The inter-assay precision was ≤ 9.3% and accuracy was within 4.5% deviation (n = 40 over 5 days). There was no detectable interference from other ARV drugs, endogenous interferents, anticoagulants, or blank plasma. All spiked samples tested were within 8% of the target value. All PT samples tested (n = 8) were within 15% of the target value. PT samples (n = 8) analyzed by HPLC and the ARK APV Test™ yielded the following results based on a Passing-Bablok regression:  $y = 1.146x - 0.0186$ , with  $r = 1.00$ .

**Conclusions:** The ARK APV Test™ showed good correlation with a validated HPLC method; it provides the first result within 7.5 minutes; it does not require sample pre-treatment; and it is moderately complex. The volume of routine reporting of ARV concentrations for patients on standard therapy can be dramatically increased (and the cost reduced) by using this new immunoassay, which would benefit hospitals and field work. Next, the ARK APV Test™ will be compared to HPLC with a large number of patient samples.

#### COBAS MIRA Parameters

**Table 1.** Parameters established for the ARK APV-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 amprenavir (APV) 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 APV 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.

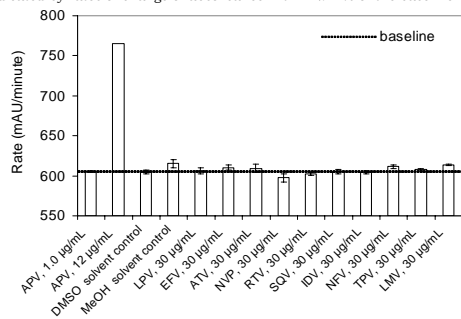
#### Inter-Assay Precision and Accuracy Studies

**Table 2.** Five QC samples were tested using the ARK APV-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	0.26 ± 0.02	9.3	4.5
0.75	0.78 ± 0.03	4.1	3.7
3.00	3.06 ± 0.11	3.4	1.8
6.00	5.98 ± 0.24	4.0	-0.4
8.00	7.88 ± 0.52	6.6	-1.5

#### 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 amprenavir concentration as indicated by rates of change of absorbance within 2.11% of the baseline value.



#### Lower Limit of Quantitation

**Table 3.** Pooled human serum samples were supplemented with known amounts of amprenavir 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.

Assay result	Conc. (µg/mL)	Assayed (Mean ± SD)	Precision (CV %)	Accuracy (Deviation %)
	0.25	0.27 ± 0.02	8.5	6.8

**Note:** This device is currently in development and has not yet been approved by the US FDA.

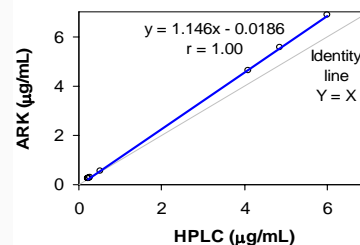
#### Analytical Recovery

**Table 4.** Pooled normal human serum samples were supplemented with known amounts of amprenavir. Each sample was then assayed 10 times. The amount of amprenavir recovered from nominal ranged from 92% to 108%.

Conc. Tested (µg/mL)	Recovery	
	(Mean ± SD)	(%)
0.50	0.46 ± 0.03	92
1.00	0.97 ± 0.04	97
2.50	2.70 ± 0.17	108
5.00	4.86 ± 0.31	97
8.00	7.79 ± 0.79	97

#### AIDS Clinical Trials Group Proficiency Testing

**Figure 2.** 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 amprenavir in duplicate by the ARK APV-Test™ and the mean compared to HPLC results. A Passing-Bablok linear regression was performed.



#### Interference

**Table 6.** Endogenous interference was assessed with 5 hypercholesterolemic, 5 hypertriglyceridemic, and 5 hyperbilirubinemic plasma samples obtained from individual patients and tested in duplicate. The endogenous substances tested did not result in an apparent amprenavir concentration.

Endogenous Substance	Endogenous Substance Concentration Range	Apparent APV Concentration (µg/mL)
Cholesterol (5)	304 - 327 mg/dL	0.000
Triglyceride (5)	277 - 312 mg/dL	0.000
Total Bilirubin (5)	26.4 - 32.4 mg/dL	0.015
"Normal" Serum (3)	Normal	0.000

**Table 7.** Anticoagulant interference was assessed with plasma samples collected in vacutainer tubes containing Na EDTA, K<sub>2</sub> EDTA, and Na heparin obtained from individual patients. One sample of normal human serum without anticoagulants was also tested as a control. The samples were supplemented with 2 µg/mL amprenavir and assayed at least 8 times.

Anticoagulant (n)	Recovery (%)
Na EDTA (24)	96.5
K <sub>2</sub> EDTA (24)	94.8
Na heparin (24)	95.7
Serum control (no anticoagulant) (8)	100

#### Long-Term, Real-Time Stability at 2-8°C

**Table 8.** Reagents were stored at 2-8°C and tested up to 245 days. At each time point, 2 calibration curves and 3 controls (n = 20) were assayed.

Time (days)	Day 0	Day 53	Day 245
<b>Total curve size:</b>			
Total curve size (AmA)	310	314	304
<b>Control quantitation:</b>			
0.75 µg/mL (% recovery)	0.73 ± 0.07 (97.3%)	0.84 ± 0.06 (112%)	0.83 ± 0.04 (101%)
3.0 µg/mL (% recovery)	3.1 ± 0.1 (103%)	3.0 ± 0.3 (102%)	3.1 ± 0.1 (104%)
6.0 µg/mL (% recovery)	6.1 ± 0.4 (101%)	6.0 ± 0.5 (101%)	6.1 ± 0.3 (101%)

#### Conclusions

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

- No sample extraction or pretreatment required
- High specificity and good sensitivity
- Small sample size
- Ready-to-use liquid reagents and calibrators
- Rapid turn-around time (7.5 minutes for the first result)