

## NOVEL ATAZANAVIR IMMUNOASSAY

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### Background

Therapeutic drug monitoring (TDM) in HIV disease may increase antiretroviral (ARV) efficacy by reducing toxicity, preventing drug resistance and managing drug-drug interactions. Measuring ARVs with current techniques (eg. 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 atazanavir (ATV) concentrations. Studies were performed to evaluate the performance of the ARK atazanavir assay.

### Methods

The ARK ATV-Test is based on competitive binding to antibody between drug in the sample and drug-labeled enzyme (Figure 1). Drug concentration is measured spectrophotometrically (Roche MIRA® bench top analyzer) in terms of enzyme activity. Although each test uses 5 µL of sample, at least 60 µL sample must be added per sample cup due to dead volume. The calibration standards ranged from 0.25 – 8.0 µg/mL. Assay precision of controls (0.375, 0.75, and 3.0 µg/mL), analytical recovery, sensitivity (0.1 µg/mL), endogenous interference (cholesterol 300 – 400 mg/dL, triglycerides 200 -350 mg/dL, and bilirubin total > 25 mg/dL) and crossreactivity of other ARVs were tested. ARK-ATV standards and calibrators were validated on the LC-MS/MS at the concentration ranges of 0.375-8.0 µg/mL (see poster #17).

### Results

Inter-assay precision of ATV control levels (CV) is <8% (n = 20) from the COBAS instrument. Analytical recovery was within 15% at all levels tested. Sensitivity was demonstrated at 0.1 µg/mL. No interference was noted from other ARV drugs, cholesterol, triglycerides or bilirubin samples. The R<sup>2</sup> is 0.995 for both ARK and LC-MS/MS assay methods for ATV (Figure 2). Mean accuracy for ATV from both instruments was measured at ≥90%, when using ARK-derived ATV standards (Table 1). Mean accuracy for ATV was measured at ≥80%, when using USC-derived ATV standards (Table 2).

Figure 1. ARK's Homogeneous, Competitive Immunoassay Technology

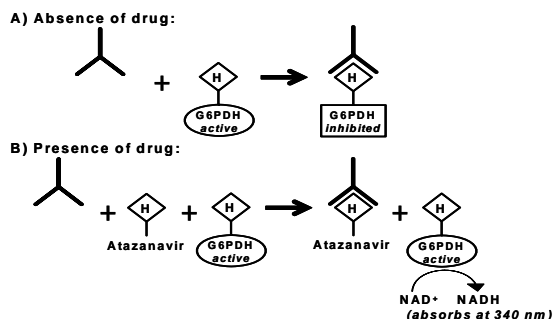


Figure 2. Correlation of ARK Standards with LC-MS/MS

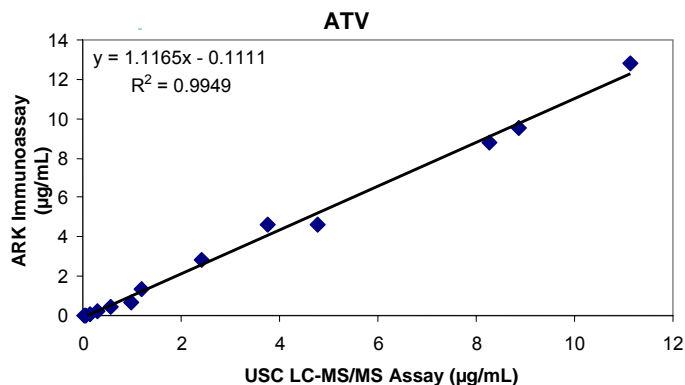


Table 1. ARK Immunoassay: Comparison of Precision & Accuracy

ARK Assay			LC-MS/MS Assay		
Conc (µg/ml)	Precision (%CV)	Mean Accuracy (%)	Conc (µg/ml)	Precision (%CV)	Mean Accuracy (%)
0.25	0	92.00	0.25	0.27	103.00
0.375	3.37	112.00	0.375	0.4	93.33
0.50	1.4	101.00	0.500	0.73	97.50
0.75	1.68	112.00	0.75	0.97	107.40
1.00	0.68	104.50	1.00	0.42	100.40
2.00	0	100.50	2.00	0.03	101.38
3.00	1.31	108.33	3.00	0	105.97
4.00	1.55	91.50	4.00	0.2	96.71
8.00	0	100	8.00	0.69	94.54

Table 2. USC LC-MS/MS Assay: Comparison of Precision & Accuracy

ARK Assay			LC-MS/MS Assay		
Conc (µg/ml)	Precision (%CV)	Mean Accuracy (%)	Conc (µg/ml)	Precision (%CV)	Mean Accuracy (%)
0.25	3.81	118.80	0.25	2.43	104.60
0.375	1.05	107.70	0.375	3.59	99.87
0.50	5.88	103.50	0.50	4.38	100.10
0.75	1.38	102.20	0.75	5.06	104.30
1.00	1.05	94.20	1.00	5.37	104.10
2.00	1.77	87.85	2.00	5.86	102.50
3.00	1.64	94.73	3.00	6.02	103.80
4.00	1.65	84.89	4.00	6.08	100.50
8.00	0.89	78.56	8.00	6.19	94.66

### Conclusions

ARK ATV-Test for measuring atazanavir in plasma was evaluated and validated via LC-MS/MS. The test is an automated EIA that requires minimum expertise, small sample volume, no sample pre-treatment and provides the first result within 7.5 minutes. All reagents are supplied ready-to-use. The ARK ATV-Test has great potential for making antiretroviral drug assays more readily available to facilitate therapeutic drug monitoring in pediatric and adult clinical trials and practice.