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 (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 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 (Table 1) were validated on the LC-MS/MS assay methods for ATV (Figure 2). Mean accuracy for ATV from both instruments was measured at ≥80%, when using USC-derived ATV standards (Table 1). Mean accuracy for ATV was measured at ≥80%, when using USC-derived ATV standards (Table 2). Sensitivity was demonstrated at 0.1µg/mL. No interference was noted from other ARV drugs, cholesterol, triglycerides or bilirubin samples. The R² 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-ATV standards (Table 1). Mean accuracy for ATV was measured at ≥80%, when using USC-derived ATV standards (Table 2).

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 0.1µg/mL. No interference was noted from other ARV drugs, cholesterol, triglycerides or bilirubin samples. The R² 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-ATV standards (Table 1). Mean accuracy for ATV was measured at ≥80%, when using USC-derived ATV standards (Table 2).

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.