

Rapid, automated immunoassay for atazanavir in plasma and cerebrospinal fluid (CSF) using the ARK ATV-Test™

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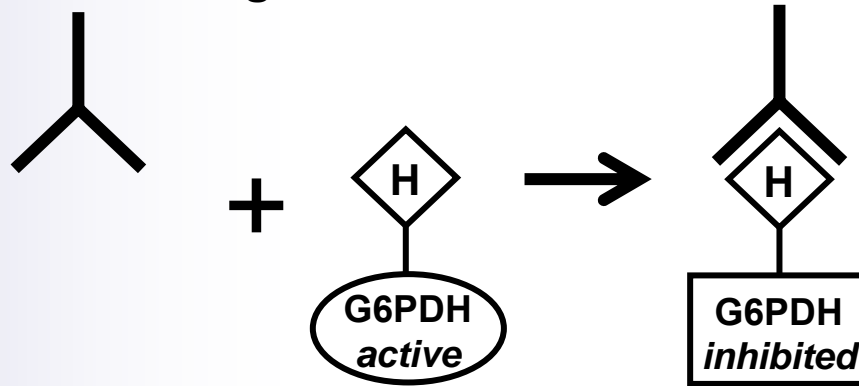
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Significance of TDM in plasma and CSF

- **Plasma**
 - US DHHS guidelines describe (and BHIVA guidelines recommend) specific indications in which TDM may provide useful information.
 - C_{\min} (trough level) may be used with phenotypic tests to determine IQ and with genotypic tests to determine GIQ.
 - General goals of TDM:
 - Improve efficacy
 - Prevent toxicity
- **CSF**
 - TDM in CSF is a current area of research.
 - CSF levels of antiretrovirals have been shown to be lower than plasma levels.
 - Maintaining adequate drug exposure may help suppress virus in this reservoir site and prevent neurocognitive impairment.
- ARK's immunoassays are enabling tools for further studies.

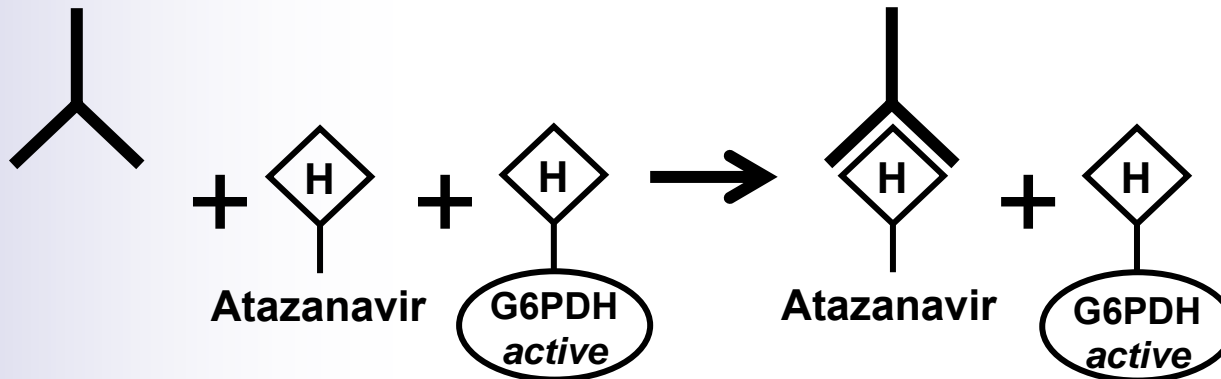
ARK's homogeneous, competitive immunoassay technology

A) Absence of drug:



Low rate

B) Presence of drug:



High rate

NAD⁺ → NADH
(absorbs at 340 nm)

Features of the ARK ATV-Test™

	Plasma assay	CSF assay
Sample size	5 µL	36 µL
Calibration range	0 – 8 µg/mL	0 – 50 ng/mL
Sensitivity (LLOQ)	50 ng/mL	5 ng/mL
Instrumentation	Roche COBAS MIRA and similar	Roche COBAS MIRA and similar
Time to <u>first</u> result*	7.5 minutes	7.5 minutes

- *Assumes calibrators and controls have already been run.*
- *Running calibrators and controls adds approximately 15 minutes, so the time to first result is then 23 minutes.*
- *Subsequent results are available every 30 seconds after the first result.*

Ready-to-use kit



2 reagents

7 calibrators

**3 quality
controls**

Accuracy and Precision

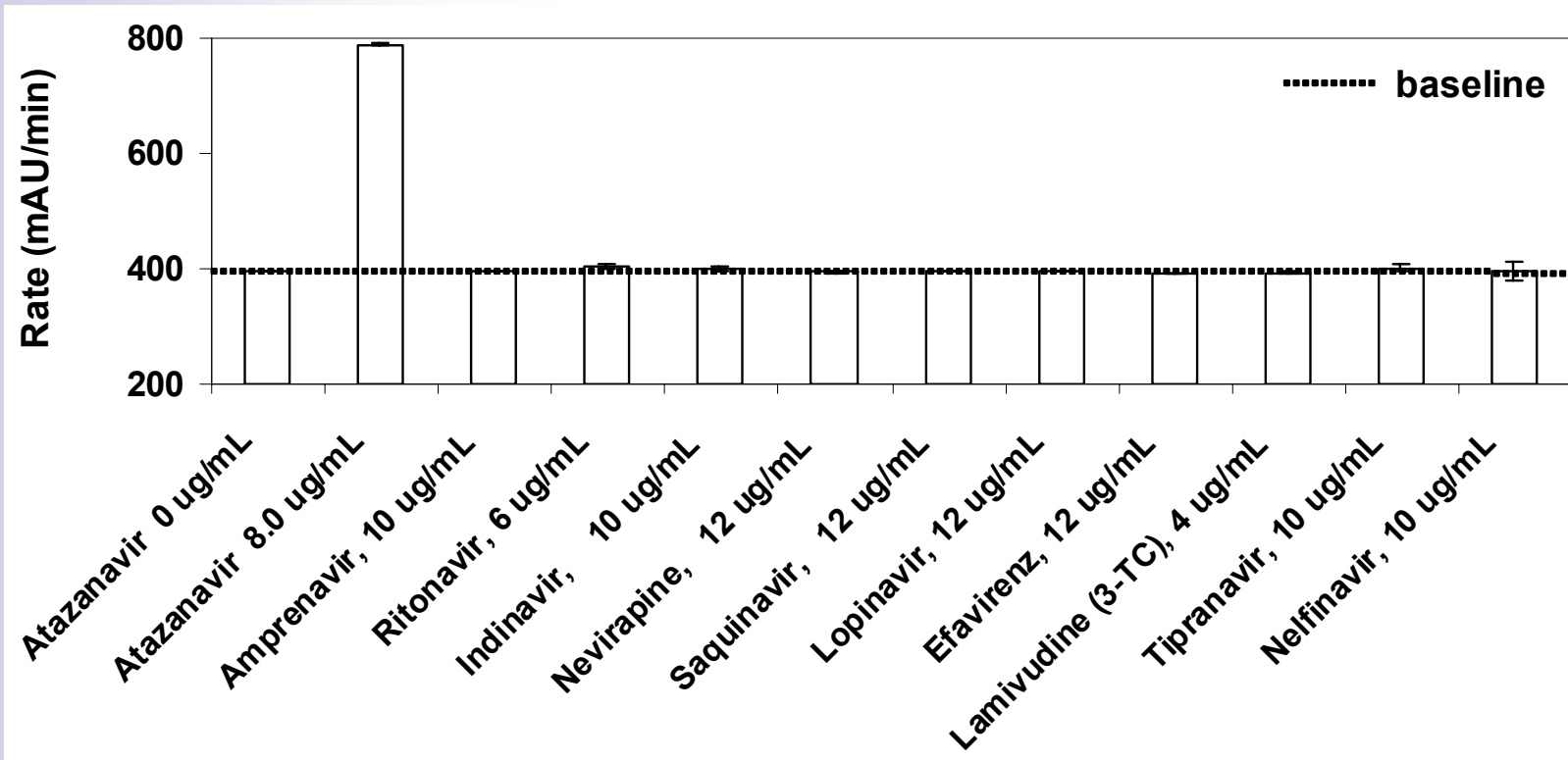
- **Plasma assay:**
 - ATV from 0.1 to 6 $\mu\text{g/mL}$
 - N = 32 to 40 over 4-5 days
- **Precision (%CV) = $\leq 11\%$**
- **Accuracy (%dev*) = $\pm 6\%$**
- **CSF assay:**
 - ATV from 5 to 60 ng/mL
 - N = 21 over 3 days
- **Precision (%CV) = $\leq 19\%$**
- **Accuracy (%dev*) = $\pm 15\%$**

** Accuracy is measured as deviation (as a %) from the expected value.*

Sensitivity (LLOQ)

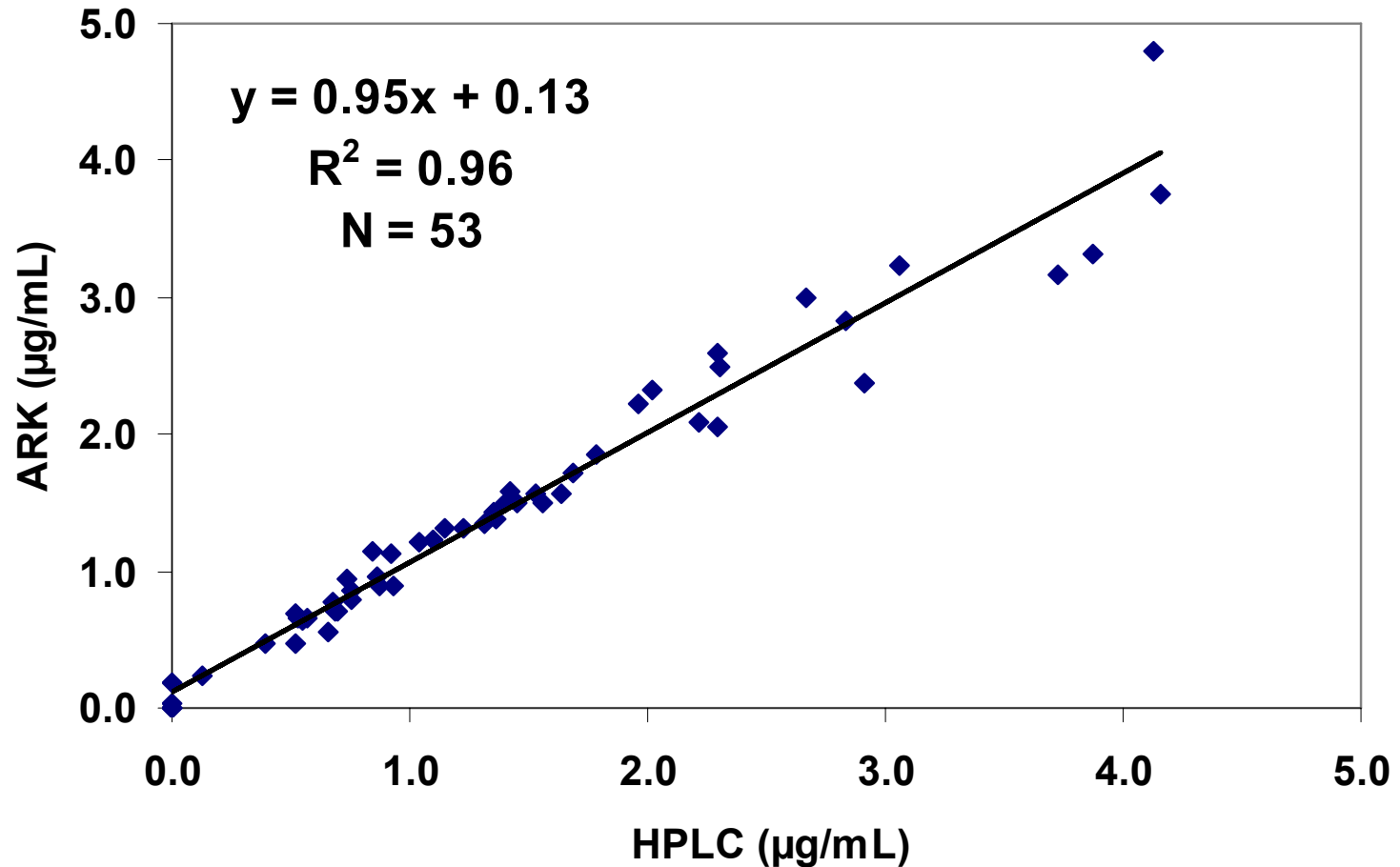
- Plasma assay
 - 50 ng/mL ATV
 - N = 20 over 1 day
 - CV = 15%
 - Deviation = 10%
- CSF assay
 - 5 ng/mL ATV
 - N = 21 over 5 days
 - CV = 19%
 - Deviation = -14%

Specificity and robustness to interference of the plasma assay



- In the presence of 10 antiretrovirals at high levels, the rate is within 2% of baseline.
- In the presence of normal human serum and abnormal serum with pathological levels of bilirubin, cholesterol, and triglycerides, recovery of 2 μ g/mL ATV is within 16% of the expected value.

Correlation to HPLC using patient samples



Application of CSF assay

- 76 CSF samples from 57 patients were measured with the ARK ATV-Test™
- Results:
 - Median ATV = 10.3 ng/mL
 - Range = BQL to 38.4 ng/mL
 - CSF ATV concentrations were ~1% that of plasma
- Conclusions:
 - ATV is low in CSF considering the IC_{50} (plasma protein binding-corrected value for wild-type HIV) is 11 ng/mL
 - 55% of samples were below this IC_{50} value.
 - These concentrations may not protect against HIV replication and HIV neurocognitive impairment.
- Reference:
 - Best, B. et al. (2006) Low Atazanavir Concentrations in Cerebrospinal Fluid. Poster #576 at CROI 2006.

Conclusions

- **ARK's ATV-Test™ is**
 - **Rapid**
 - **Simple**
 - **Accurate**
 - **Precise**
 - **Sensitive**
 - **Specific**
 - **Robust to interference**
 - **Shows excellent correlation to HPLC**
 - **Shows excellent agreement with proficiency samples**
 - **Is useful for measuring plasma and CSF samples**
- **Special thanks to our colleagues at UCSD and USC**
- **Please visit us online at www.ark-tdm.com**