Validation of a urine TFV immunoassay for real-time PrEP and ART adherence testing

Monica Gandhi1, Peter Bacchetti2, Hideaki Okochi3, Matthew Spinelli1, Rachel Kubak4, Orphan Siriprakaisil5, Virat Klinbuayaem3, Pra-ornsuda Sukrakanchana4

Yardpiron Tawon1, Jared M. Baeten7, Warren C. Rodrigues5, Guohong Wang5, Michael Vincent5, Tim R. Cressey6, Paul K. Drain1

CONCLUSIONS

• We developed one of the first TFV-specific immunoassays for point-of-care testing in urine: has high specificity, high sensitivity, high correlation/agreement, and precision compared to LC-MS/MS

• Estimated the appropriate cut-off (1500 ng/mL) for a yes/no POC assay using urine samples collected in a directly-observed therapy study performed in Thailand

• Now being packaged into a lateral flow immunoassay as a rapid strip test for POC testing by Alere™ using this cut-off

• Time to results 5 minutes; low cost (<$2/assay)

• Limitations: short-term measure, first packaging will be yes/no but more-expensive test with reader will have gradations of adherence (high, medium, low)

METHODS

• Tenofovir derivatives (haptens) synthesized and conjugated

• Rabbits immunized with immunogens, bled monthly to evaluate for Abs that bind to enzyme-labeled TFV derivatives using ELISA & where Ag:Ab signal by adding TFV mix

• Tested in proof of concept study1 – ELISA immunoassay high sensitivity, specificity, and correlation/agreement with LC-MS/MS

• An immunoassay cut-off of 1500ng/mL accurately classified 98% of patients who took a dose 24 hours ago as adherent and was chosen as the cut-off for the lateral flow immunoassay (LFA) – the POC assay

• Specificity and sensitivity of the immunoassay compared to LC-MS/MS at the 1500ng/mL cut-off were 99% and 94%, respectively

• Correlation between TFV levels generated by the two assays was 0.92 (p <0.00001) for all 637 samples; correlation among levels in samples with detectable drug in both assays (n=274) was 0.89 (p<0.00001)

• Bland-Altman analysis of the average relative difference between log-transformed values in samples positive by both assays suggests that 95% of immunoassay values would fall within 70% below and 98% above the LC-MS/MS value

RESULTS

• Among participants in all three adherence groups, median TFV levels in urine by the immunoassay were 12,000 ng/mL (IQR 7500-25,000) one day after dosing; 5000 ng/mL (IQR 2500-8000) two days after dosing; 1500 ng/mL (IQR 500-2750) three days after dosing and below the immunoassay’s LLOQ thereafter (≥4 days).

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REFERENCE AND ACKNOWLEDGEMENTS

1. Gandhi M et al. EClinicalMedicine 2018
2. Cressey T et al. BMC Infectious Diseases 2017

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Improvement of adherence

Can trigger feedback and adherence interventions with known efficacy

ARVs administered to mice, rabbits, chickens, dogs, monkeys, cats, and rats in nonclinical studies and clinical trials of TFV in humans

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