Poster 1298, Session P-G1

March 5, 2019

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

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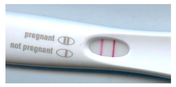
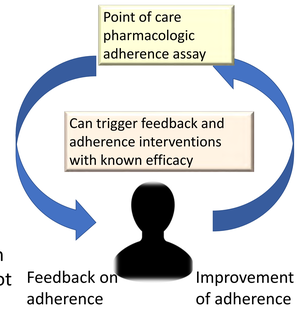
BACKGROUND

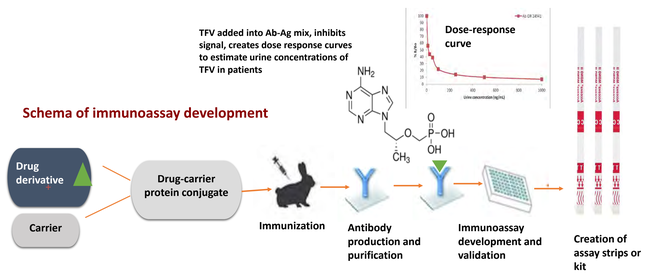
- Pharmacologic measures of adherence critical to interpretation of PrEP clinical trials (e.g. self-reported adherence in VOICE 91% but only 29% with tenofovir (TFV) in plasma)
- ARV concentrations in plasma, PBMCs, dried blood spots (DBS), hair all measured by liquid chromatography/tandem mass spectrometry (LC-MS/MS) – expensive, trained personnel
- Antibody-based tests allow for point-of-care (POC) testing at home or clinic, like urine pregnancy tests
- POC tests allow for real-time monitoring which can trigger adherence interventions on the spot (moreover, feedback can motivate adherence)





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 inhibited by adding TFV to mix
- Tested in proof of concept study[1] – ELISA immunoassay high sensitivity, specificity, and correlation/agreement with LC-MS/MS



- TARGET study administered TDF 300mg/FTC 200mg directly-observed 7 (high adherence), 4 (moderate adherence) and 2 doses per week (low adherence) to 30 volunteers (10 per group, randomized) in Thailand[2]
- Collected 637 urine samples over 6 weeks of administration and during wash-out (mean 21 samples per participant)
- Measured urine TFV levels by the immunoassay using ELISA and by a validated LC-MS/MS-based method in the UCSF Hair Analytical Laboratory (HAL)
- Calculated sensitivity and specificity of the immunoassay compared to the gold standard, along with Spearman's correlation coefficients and agreement
- To predict probabilities of being below different cut-offs of urine TFV levels for the POC assay, a mixed-effects interval regression model was used with log urine-immunoassay concentration as the dependent variable and days since the last dose as the independent variable

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).

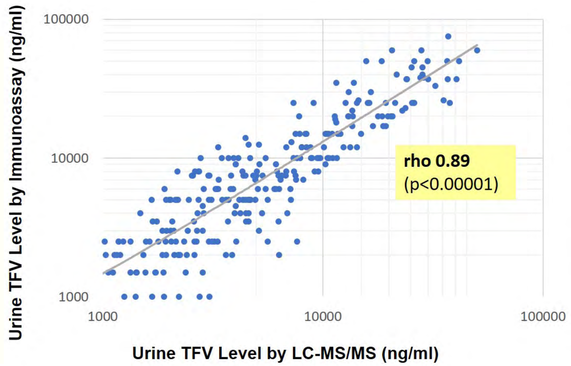
Table 1: Probability of urine TFV level by immunoassay being below different cut-offs based on hours since last witnessed dose in TARGET DOT study

Hours since dose	Probability below 1000 ng/ml	Probability below 1500 ng/ml	Probability below 2000 ng/ml	Probability below 2500 ng/ml	Probability below 3000 ng/mL	Probability below 3500 ng/ml
12	0%	**1%**	1%	2%	4%	5%
16	0%	**1%**	2%	4%	5%	7%
20	0%	**2%**	3%	5%	8%	10%
24	1%	**2%**	5%	7%	10%	13%
30	2%	**4%**	8%	11%	16%	20%
36	3%	**7%**	12%	17%	22%	27%
42	5%	**11%**	18%	24%	31%	37%
48	8%	**16%**	25%	33%	40%	46%
54	13%	**24%**	34%	43%	50%	56%
60	19%	**32%**	43%	53%	60%	66%
66	26%	**42%**	54%	63%	70%	75%
72	35%	**52%**	63%	72%	78%	82%
78	45%	**62%**	72%	80%	85%	88%
84	55%	**71%**	80%	86%	90%	92%
90	65%	**79%**	87%	91%	94%	95%
96	74%	**86%**	91%	94%	96%	97%
102	81%	**91%**	95%	97%	98%	99%
108	87%	**94%**	97%	98%	99%	99%
114	92%	**97%**	98%	99%	99%	100%
120	95%	**98%**	99%	100%	100%	100%

- 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

ELISA	LC-MS/MS Positive	LC-MS/MS Negative
Positive	**274** *TP*	**4** *FP*
Negative	**19** *FN*	**340** *TN*

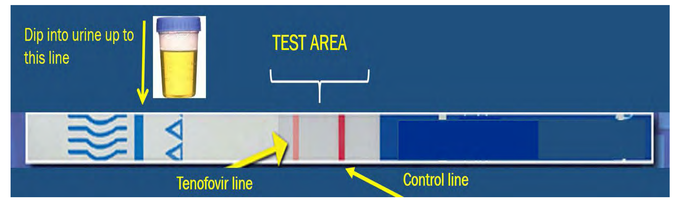
LC-MS/MS gold standard. TP = True positive; FP = false positive; FN= false negative; TN = true negative



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)

Prototype of rapid strip test



REFERENCES and ACKNOWLEDGEMENTS

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

- Funding: NIAID/NIH R01AI143340 (P.I. Gandhi); R21AI127200 and R01AI136648 (P.I. Drain)
- We acknowledge and thank the participants of the TARGET study and their families
- We thank the other laboratory members of the UCSF Hair Analytical Laboratory (Karen Kuncze, Nhi Phung, Alexander Louie, Kevin Zhang, Regina Tallerico; Deserie Arredondo; Leslie Z. Benet PhD)
- We sincerely thank Hao Zhang PhD, program officer, NIAID

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