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Clinical Validation of a Novel Diagnostic HIV-2 Total Nucleic Acid Qualitative Assay

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BACKGROUND:

The 2014 CDC 4th generation HIV screening algorithm includes an orthogonal immunoassay to confirm and discriminate HIV-1 and HIV-2 antibodies. Additional HIV nucleic acid testing (NAT) is recommended to resolve indeterminate or undifferentiable HIV seroreactivity. HIV-2 NAT requires a second-line assay that detects HIV-2 total nucleic acid (TNA) in patients' blood cells, as one third of untreated HIV-2 infected patients have undetectable plasma HIV-2 RNA.

AIM:

To establish and validate a qualitative HIV-2 TNA assay for peripheral blood mononuclear cells (PBMC) using the Abbott m2000 platform

MATERIALS & METHODS:

Clinical samples:

- PBMC and plasma from 30 Senegalese HIV-2 seropositive and 8 HIV-1/-2 dual-seropositive participants, either ART-experienced or ART-naïve
- PBMC and plasma from 25 HIV-1-infected and 25 HIV-negative individuals provided by UW CFAR

Assay controls and standards:

- Plasmid pROD9 (containing the full genome of the HIV-2 group A ROD strain) linearized by XhoI, then measured by NanoDrop 1000 spectrophotometer to determine the copy number
- Serial dilutions of pROD9 spiked to one million PBMC

HIV-2 TNA assay:

- Digestion of PBMC with proteinase K in lysis buffer
- Abbott customer-specific DNA/RNA extraction protocol
- HIV-2 amplification and detection using the Abbott qualitative LDA program
- Internal and endogenous controls included in the assay: in-house RNA transcripts added in the extraction lysis buffer and amplification of human house-keeping gene, respectively

HIV tests:

- Abbott HIV-1/-2 Ag/Ab Combo assay, Bio-Rad Multispot Rapid test
- UW HIV-2 quantitative RNA assay, Abbott RealTime HIV-1 assay, Modified Abbott HIV-1 TNA assay

RESULTS:

The limit of detection of the assay: 26 copies/million cells (95% CI, 18-55 copies/million cells)

pROD copies /million cells	Number of replica tested	Number detected	Percent detected
46	16	16	100
23	16	15	93.4
12	16	10	62.5
6	16	9	56.3
3	16	3	18.8

Specificity

All PBMCs from 30 Senegalese HIV-2 seropositive participants were detected by the HIV-2 TNA assay (see below), while all 50 PBMCs from HIV-1-infected or -negative individuals tested negative (not shown).

			Plasma Sample		PBMC Sample	
		HIV Ag/Ab Combo	Multispot^	HIV-2 RNA	HIV-2 TNA	HIV-1 TNA
Specimen ID	HIV-2 Group	Result (S/CO+)	Interpretation	Result (c/mL)	Result	Result
H2A 32	A	5.89	HIV NEG	TND*	Detected	Not detected
H2A 76	A	22.07	HIV-2 (wk)	TND	Detected	Not detected
H2A 24	A	54.78	HIV-2-after 1:10	32	Detected	Not detected
H2A 67	NT$	66.66	HIV-2 (wk)	TND	Detected	Not detected
H2A 72	B	99.79	HIV-2	TND	Detected	Not detected
H2A 25	A	122.55	HIV-2	<10,detected	Detected	NT$
H2A 66	NT$	123.75	HIV-2	TND	Detected	Not detected
H2A 64	A	139.49	HIV-2-after 1:10	TND	Detected	Not detected
H2A 26	A	142.28	HIV-2	TND	Detected	NT$
H2A 53	A	149.89	HIV-2-after 1:100	TND	Detected	Not detected
H2A 84	NT$	157.64	HIV-2	TND	Detected	Not detected
H2A 14	A	160.84	HIV-2-after 1:100	n/a	Detected	Not detected
H2A 78	NT$	165.91	HIV-2	TND	Detected	Not detected
H2A 3	A	168.28	HIV-2-after 1:100	115	Detected	Not detected
H2A 30	A	179.93	HIV-2-after 1:100	TND	Detected	Not detected
H2A 10	A	186.91	HIV-2	TND	Detected	Not detected
H2A 34	A	189.62	HIV-2	<10,detected	Detected	Not detected
H2A 38	NT$	192.39	HIV-2-after 1:100	<10,detected	Detected	Not detected
H2A 74	A	192.51	HIV-2	TND	Detected	Not detected
H2A 62	A	196.98	HIV-2	TND	Detected	Not detected
H2A 57	A	205.59	HIV-2	TND	Detected	Not detected
H2A 77	A	206.43	HIV-2-after 1:10	TND	Detected	Not detected
H2A 22	A	207.58	HIV-2-after 1:10	TND	Detected	Not detected
H2A 55	A	219.28	HIV-2	TND	Detected	Not detected
H2A 6	A	222.62	HIV-2	TND	Detected	Not detected
H2A 33	A	223.00	HIV-2-after 1:100	TND	Detected	Not detected
H2A 20	A	225.10	HIV-2	16	Detected	Not detected
H2A 82	NT$	234.44	HIV-2	TND	Detected	Not detected
H2A 9	A	234.58	HIV-2	TND	Detected	Not detected
H2A 31	A	293.67	HIV-2	TND	Detected	Not detected

^Multispot results interpreted without dilution are stated while determined to be HIV-2 seropositive after 1:10 or 1:100 dilutions as stated.
$NT, Not tested; *S/CO, Signal to cut-off ratio; *TND, Target not detected

Diagnostic Performance: HIV-2 infection status was evaluated by comparing the outcome of PBMC tested by the HIV-2 TNA assay with the serological results obtained from the Abbott HIV-1/-2 Combo and Multispot immunoassays using matching plasma samples. **The agreement between the HIV-2 TNA assay and the combined results of the immunoassays was 98.75% (79 of 80), as summarized below.**

PBMC tested by HIV-2 TNA assay	The matching plasma tested by HIV Ag/Ab Combo and Multispot assays	
	HIV-2 Ab reactive	HIV-2 non-reactive
Detected	29	1*
Not Detected	0	50

*HIV reactive by Abbott HIV Ag/Ab Combo assay and HIV nonreactive by Multispot. Participant's baseline HIV testing: HIV reactive by Alere Determine and HIV-2 reactive by Immunocomb II

Results of Multispot-undifferentiated* from 8 HIV-1/HIV-2 dually seropositive participants' paired PBMC and plasma samples

	Plasma sample		PBMC sample	
Specimen ID	HIV-1 RNA copies/mL	HIV-2 RNA copies/mL	HIV-1 TNA	HIV-2 TNA
HD01	Not detected	Not detected	Detected	Detected
HD02	85120	2592	Detected	Detected
HD06	510596	311	Detected	Detected
HD09	Not detected	Not detected	Detected	Detected
HD12	Not detected	Not detected	Detected	Detected
H2A036	Not detected	136	Detected	Detected
HD13	Detected,<40	28726	Not detected	Detected
HD10	13730	Not detected	Detected	Not detected

*HIV Ab was not differentiated for the sample at a 1:100 dilution.

CONCLUSIONS:

- The HIV-2 TNA assay detected HIV-2 DNA/RNA in 100% of PBMC tested including from patients with undetectable HIV-2 plasma viral loads.
- We demonstrated the utility of the HIV-2 TNA assay in patients' yielding indeterminate (reactive to HIV Ag/Ab Combo and non-reactive to Multispot) or undifferentiated HIV-1/-2 Ab results when tested using the 4th generation HIV screening algorithm.

The details of this study are published by the Journal of Clinical Virology in January 2017 (PMID: 27951466)
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