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Blood Systems Research Institute

Comparison of Detection Limits of 4th and 5th Generation Combination HIV Antigen/Antibody, p24 Antigen and Viral Load Assays on Diverse HIV Isolates

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BACKGROUND

Detection of acute HIV infection is critical for HIV public health and diagnostics. Clinical 4th and 5th-generation antigen-antibody (Ag/Ab) combination (combo) and p24 Ag immunoassays have enhanced detection of acute infection compared to Ab alone assays, but require ongoing evaluation with currently circulating diverse subtypes. Blinded panels of serial dilutions of genetically and geographically diverse HIV clinical isolates were used to assess sensitivity (p24 Ag relative to viral loads) of HIV clinical diagnostic, blood screening and next generation p24 Ag assays.

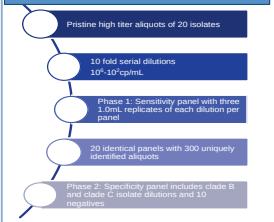
METHODS

Panel Composition and Characteristics

Sample ID	Subtype	Country of Origin	Donation Year	Infection Stage	VL Copies/mL	p24 pg/mL	VL:p24 cp/pg
DEMA106ES002	A1	Spain	2006	UNK	1.07E+06	47.13	22705
DEURF10US008	A1B (URF)	USA	2010	I/II	2.45E+06	53.86	45487
DEURF09GQ001	ADG	Guinea	2009	UNK	2.72E+06	35.95	75664
DEMB10ES003	B	USA	2010	I/II	1.26E+06	54.48	23129
DEMB09US002	B	China	2010	Chronic	1.70E+06	53.71	31650
DEMB10US004	B	Venezuela	2010	Acute	1.10E+06	25.16	43719
DEURF07BR002	BC (URF)	Brazil	2007	V	1.90E+06	43.86	43323
DEMBF09ES003	BF	Spain	2009	Acute	1.53E+06	35.13	43550
DEMC07AO001	C	Angola	2007	UNK	2.14E+06	71.90	29765
DEMC07BR003	C	Brazil	2007	VI	1.20E+06	21.08	56925
DE00109CN003	CRF01_AE	China	2009	IV	2.04E+06	60.13	33929
DE00208CM004	CRF02_AG	Cameroon	2008	VI	1.58E+06	40.54	38974
DE01405BR001	CRF14_BG	Brazil	2005	UNK	6.18E+05	17.41	35489
DE04708ES003	CRF47_BF	Spain	2008	Acute	6.42E+05	30.22	21245
DEMD10CM009	D	Cameroon	2010	VI	7.95E+05	39.51	20121
DEMD07UG002	D	Uganda	2007	I	1.70E+06	35.60	47756
DEMF110ES001	F1	Spain	2010	Recent	1.22E+06	21.96	55556
DEMF210CM001	F2	Cameroon	2010	VI	1.62E+06	62.66	25853
DEMG09ES002	G	Spain	2009	Acute	1.10E+06	25.68	42838
DEOXXUS001	O	USA	unknown	UNK	8.36E+05	41.71	20042

Isolate Name	N
A1	1
B	4
C	2
D	2
F	2
G	1
O	1
CRF01 AE	1
CRF02 AG	1
CRF14 BG	1
CRF47 BF	1
URF BC	1
URF ADG	1
URF A1B	1
Total Isolates	20
5 Dilutions 10^6 to 10^2	x5
3 Replicates	x3
Total Panel Members	300

Study Scheme



Participating Labs and Assays

Site	Assay(s) Run
Abbott	ARCHITECT HIV Ag/Ab Combo
Ortho Clinical Diagnostics	VITROS Anti-HIV 1+2 Assay
Bio-Rad	Bio-Rad GS HIV Combo Ag/Ab EIA
	Bio-Rad Genscreen HIV-1 Ag Assay
Henry M Jackson Foundation	Bio-Rad GS HIV Combo Ag/Ab EIA
	Bio-Rad Genscreen HIV-1 Ag Assay
	Alere Determine HIV-1/2 Ag/Ab Combo
	SD BIOLINE HIV Ag/Ab Combo (RUO)
University of Washington	ARCHITECT HIV Ag/Ab Combo
	Abbott Real-Time HIV Viral Load Assay
Public Health England	Bio-Rad Bioplex 2200; 5th Gen Assay
EQAPOL iVQAC	Perkin Elmer p24
MSD	Ultrasensitive P24
Quanterix	Ultrasensitive Simoa
FDA	Alere Determine HIV-1/2 Ag/Ab Combo
Hologic	Aptima HIV-1 Quant Dx assay

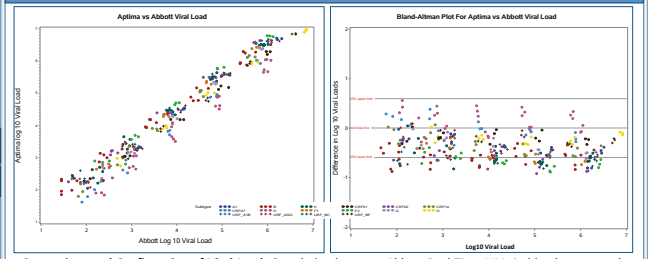
Proportion Positive Results and Viral Loads at Cutoff by Assay

Assay Category	Assay	Lab(s)	Proportion Positive on Performance Panel (N=300)	Estimated VL at manufacturer's cutoff (95% cutoff)	Specificity Panel (N=20)
4th Gen HIV Ag/Ab	Bio-Rad GS HIV Combo Ag/Ab EIA	BR	0.4	4.71 (4.61, 4.80)	0
		HJF	0.33	4.88 (4.79, 4.97)	ND
	Abbott Architect HIV Ag/Ab Combo	ABT	0.37	4.70 (4.62, 4.79)	0
		UW	0.37	4.60 (4.52, 4.69)	ND
	VITROS HIV Combo Assay	OCD	0.32	5.06 (4.96, 5.15)	0
5th Gen HIV Ag/Ab	Bio-Rad Bioplex AG	PHE	0.31	4.91 (4.77, 5.06)	ND
HIV p24	Bio-Rad Genscreen HIV-1 Ag Assay	BR	0.4	4.41 (4.32, 4.50)	0
		HJF	0.4	4.60 (4.50, 4.69)	ND
Ultrasensitive HIV p24	Quanterix Ultrasensitive Simoa	Qt	0.99	2.34 (1.45, 4.15)*	0
	Meso Scale Discovery p24 Assay	MSD	0.69	2.68 (1.45, 3.77)*	0
POC Rapid Assays	Alere Determine HIV-1/2 Ag/Ab Combo Assay	FDA	0	N/A	ND
		HJF¶	0	N/A	ND
	SD BIOLINE HIV Ag/Ab Combo	HJF¶	0	N/A	ND

*median (min, max)

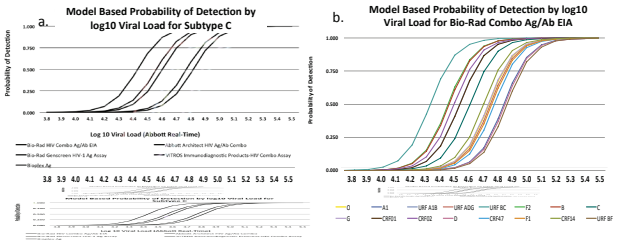
RESULTS

1. Comparison and Confirmation of Viral Load



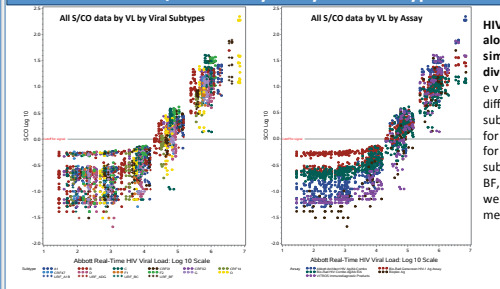
Comparison and Confirmation of Viral Load. Correlation between Abbott Real Time HIV viral load assay results (performed by UW) and Hologic Aptima HIV-1 Quant Dx assay results (a.) The difference in the Aptima and Abbott viral loads (b.) Each dot represents a sample with a total N of 300. The color denotes the HIV subtype of the sample. The Pearson's r = 0.98.

2. Model Based Proportion Detected by Subtype and Assay



Model Based Proportion Detected by Assay for Subtype 'C' and by Viral Subtype Detected by Bio-Rad HIV Combo Ag/Ab EIA assay. The predicted proportion of samples detectable by each of the four FDA approved Ag/Ab combo and one CE-marked p24 Ag alone assays utilizing HIV subtype C to illustrate the relative rank order performance with reactive S/CO by VL (a). The predicted proportion of viral subtypes detectable by the Bio-Rad HIV Combo Ag/Ab EIA assay which was used as representative of the relative rank order performance with reactive S/CO by VL (b).

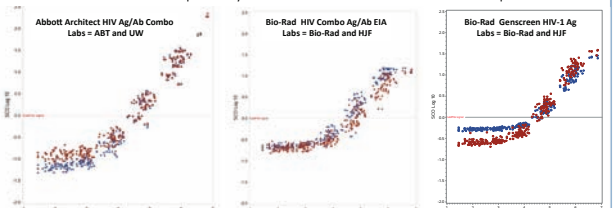
3. All S/CO data by VL by Viral Subtypes and Assay



HIV Ag/Ab combo and Ag alone assays demonstrated similar performance on diverse subtypes. There is evidence for small differences in detection by subtype on Abbott Architect for subtype CRF02, Vitros for subtype C, and MSD for subtypes CRF01 and URF-BF, but these differences were likely not clinically meaningful.

4. Comparing Assays Run in Both Manufacturer and Clinical Labs

Blinded Panels tested in parallel by the manufacturer and an end user lab on three platforms:

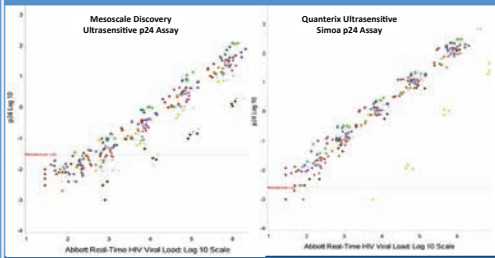


	Proportion Positive	VL at cutoff (cp/ml)
ABT	0.37	4.70 (4.62, 4.79)
UW	0.37	4.60 (4.52, 4.69)

	Proportion Positive	VL at cutoff (cp/ml)
BR	0.4	4.71 (4.61, 4.80)
HJF	0.33	4.88 (4.79, 4.97)

	Proportion Positive	VL at cutoff (cp/ml)
BR	0.4	4.71 (4.32, 4.50)
HJF	0.4	4.60 (4.50, 4.69)

5. Ultrasensitive Next-gen p24 Assays



Ultrasensitive p24 Ag signal intensity compared to Viral Load. Newer generation pre-clinical p24 Ag detection platforms achieved enhanced sensitivity relative to currently approved 4th gen Ag/Ab combo and stand-alone p24 Ag assays.

SUMMARY

- Excellent correlation was observed across the range of viral loads (VL) evaluated for all subtypes for the Abbott and Hologic VL assays
- Similar sensitivity was demonstrated across most clades and recombinant forms for commercially available p24 Ag/Ab and stand-alone p24 Ag assays
- Similar performance of Abbott Architect and Bio-Rad Genscreen assays was observed in manufacturer and end-user labs, although the Bio-Rad Combo performed by the manufacturer showed slightly higher proportion positives and lower VL at cutoff
- Newer generation pre-clinical p24 Ag detection platforms achieved enhanced sensitivity relative to currently approved 4th gen Ag/Ab combo and stand-alone p24 Ag assays
- Poor performance was observed for p24 Ag detection by rapid POC assays available in the U.S.

CONCLUSIONS

Development and comparative testing of diverse blinded panels is essential to assess the relative performance of commercially available and pre-clinical assays. We confirmed similar performance across subtypes between currently FDA-approved 4th gen and stand-alone p24 Ag assays, demonstrated enhanced sensitivity of next generation p24 digital detection platforms, and observed poor performance of POC assays.

ACKNOWLEDGEMENTS

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