Performance of the VITROS® Immunodiagnostic Products HIV Combo Assay#

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Method

Antibody detection in the VITROS® Immunodiagnostic Products HIV Combo Assay# (VITROS® HIV Combo Assay) is achieved using recombinant transmembrane envelope proteins for HIV-1 group M and O and HIV-2. Antigen detection is accomplished using monoclonal antibodies against HIV p24. Biotinylated antigens or antibody are pre-bound to MicroWells coated with streptavidin. Sample is added to the well in the first stage of the reaction and HIV analyte is captured by the biotinylated proteins. After wash, HRP conjugated envelope proteins and anti-p24 antibodies are added. Following final wash, bound HRP conjugates are detected using VITROS® signal reagent.

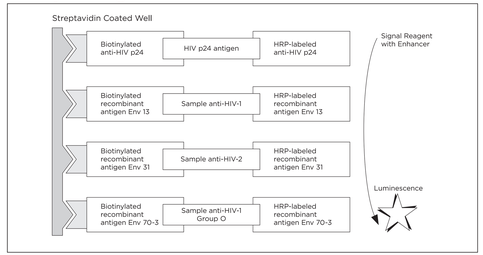
Seroconversion Sensitivity

Thirty-four commercially available HIV-1 seroconversion panels were tested using the VITROS® HIV Combo Assay# and a Commercially available 4th generation HIV assay.

Panel ID	VITROS® HIV Combo Assay		Commercially Available HIV Ag/Ab Combo Assay		Difference in Days to First Reactive Result
	Number of Reactive Panel Members	Days to First Reactive Result	Number of Reactive Panel Members	Days to First Reactive Result	
HIV12007	6	117	6	117	0
HIV12008	6	23	5	28	5
HIV9012	4	14	3	16	2
HIV9013	1	25	1	25	0
HIV9014	6	0	6	0	0
HIV9015	2	30	2	30	0
HIV9018	4	31	3	34	3
HIV9020	3	89	3	89	0
HIV9028	2	53	2	53	0
HIV9032	8	22	7	24	2
HIV9075	3	22	3	22	0
HIV9077	16	42	16	42	0
HIV9089	3	16	3	16	0
PRB930	4	0	4	0	0
PRB934	3	0	3	0	0
PRB950	3	18	2	21	3
PRB951	4	8	4	8	0
PRB954	2	17	2	17	0
PRB955	4	3	4	3	0
PRB963	2	17	2	17	0
PRB966	3	44	3	44	0
PRB943	5	7	5	7	0
PRB956	2	47	2	47	0
PRB957	2	23	3	16	-7
PRB960	2	28	2	28	0
PRB962	2	14	2	14	0
PRB964	1	22	1	22	0
PRB961	2	21	2	21	0
HIV6243	4	24	4	24	0
HIV6244	2	27	2	27	0
HIV6247	4	21	4	21	0
HIV6248	2	18	2	18	0
HIV9021	4	46	4	46	0
HIV9079	17	40	17	40	0
Total	138	929	134	937	8

The VITROS® HIV Combo Assay# shows higher HIV-1 seroconversion sensitivity than a commercially available 4th generation assay.

Assay Design



Detection of HIV-1 Antibody Subtypes

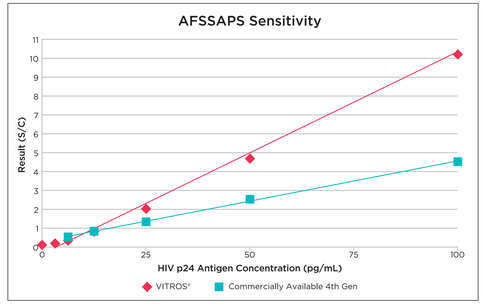
A total of 83 HIV-1 group M subtype samples were tested on the VITROS® HIV Combo Assay#.

Antibody Subtype	Number Tested	VITROS® HIV Combo Number Reactive
A	5	5
A1	1	1
A2	2	2
B	2	2
C	7	7
D	4	4
F	2	2
F1	2	2
F2	5	5
G	7	7
H	3	3
H/A1	1	1
H/U	1	1
J	4	4
K	3	3
CRF01	2	2
CRF01/AE	5	5
CRF01/CRF15	1	1
CRF02/AG	11	11
CRF02/G	2	2
CRF06	1	1
CRF06/CPX	2	2
CRF09/K	1	1
CRF09/CPX	3	3
CRF11/CPX	2	2
CRF18/CPX	4	4
Total	**83**	**83**

The VITROS® HIV Combo Assay# detected all 83 samples.

HIV p24 Antigen Sensitivity

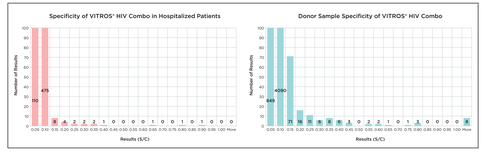
One HIV p24 standard was diluted and tested on both the VITROS® HIV Combo Assay# and on a commercially available HIV Ag/Ab Combo assay. The AFSSAPS standard (Bio-Rad 72217) was used.



- VITROS® Sensitivity = **13.1pg/mL**
- Commercially Available 4th Gen = **17.2pg/mL**

Clinical Specificity

Specificity of the VITROS® HIV Combo Assay# was assessed using 5077 blood donor samples and 608 samples from hospitalized patients.



Statistics for 608 Hospitalized Samples	
Mean S/C	0.07
Number Initial Reactive Samples	1*
Number Repeat Reactive Samples	1*
% Specificity	100%
95% Exact Confidence Interval	99.39 to 100%

Statistics for 5077 Blood Donor Samples	
Mean S/C	0.07
Number Initial Reactive Samples	16
Number Repeat Reactive Samples	8
% Specificity	99.84%
95% Exact Confidence Interval	99.69 to 99.93%

*Reactive sample was confirmed as HIV positive.

Precision

Total within lab precision for the VITROS® HIV Combo Assay# was evaluated over 22 days consistent with CLSI EP05-A3 using one lot of reagent tested on a VITROS® 3600 Immunodiagnostic System.

Analyte	Mean (S/C)	Repeatability	Within-Calibration	Total Within Lab*
Negative	0.06	0.010**	0.013**	0.013**
Negative	0.07	0.008**	0.011**	0.012**
Negative Control	0.07	0.009**	0.014**	0.014**
Anti-HIV-1	0.56	4.4%	9.3%	9.3%
Anti-HIV-1	0.98	3.2%	7.8%	7.6%
Anti-HIV-1	2.17	2.7%	5.7%	5.4%
Anti-HIV-1 Reactive Control	1.90	3.8%	6.1%	6.0%
Anti-HIV-2	0.72	5.1%	10.1%	10.0%
Anti-HIV-2	1.04	3.7%	7.3%	7.0%
Anti-HIV-2	2.44	3.1%	5.4%	5.1%
Anti-HIV-2 Reactive Control	4.20	3.0%	4.6%	4.5%
Anti-HIV-1 Group O	0.86	5.2%	8.7%	8.7%
Anti-HIV-1 Group O	1.10	4.2%	7.3%	7.3%
Anti-HIV-1 Group O	2.38	4.0%	5.6%	5.6%
Anti-HIV-1 Group O Reactive Control	3.30	3.3%	5.3%	5.0%
HIV p24 Ag	0.78	2.3%	7.4%	7.5%
HIV p24 Ag	1.40	2.0%	5.3%	5.4%
HIV p24 Ag	3.33	1.5%	3.3%	3.4%
HIV p24 Ag Reactive Control	1.92	1.7%	4.7%	4.7%

*All precision estimates calculated using single calibration data except for Total Within Lab (4 calibration events)
**Precision for Negative samples are presented as standard deviation

Detection of HIV-1 Antigen Genotypes

A total of 52 specimens were evaluated on a VITROS® 3600 Immunodiagnostic System. Three commercially available HIV-1 Group M specimens were run without dilution. The remaining 49 specimens were received as cell culture supernatants and diluted in HIV negative human plasma to 200,000 RNA copies/mL.

Source (# of Specimens)	Subtype	Number of Specimens Tested	VITROS® HIV Combo	Commercially Available 4th Generation HIV Ag/ Ab Assay
			Number of Reactive Interpretations (%)	Number of Reactive Interpretations (%)
Group M Specimens (3)				
Uganda	A	1	1 (100)	1 (100)
Uganda	D	1	1 (100)	1 (100)
Romania	F	1	1 (100)	1 (100)
Viral Isolates (49)				
Ghana (1), Kyrgyzstan(1), Uganda (2)	A	4	4 (100)	4 (100)
Brazil (1), Thailand (1), USA (6)	B	8	8 (100)	7 (87.5)
Djibouti (1), Ethiopia (1), Senegal (1), Somalia (1), Uganda (1), Zambia (1), Unknown (1)	C	7	5 (71.4)	5 (71.4)
Senegal (1), Uganda (2)	D	3	3 (100)	3 (100)
Brazil (3), Romania (2)	F	5	5 (100)	5 (100)
Kenya (1), Democratic Republic of vthe Congo (1)	G	2	2 (100)	1 (50)
Democratic Republic of the Congo (1)	H	1	1 (100)	1 (100)
Indonesia (2), Thailand (8)	CRF01_AE	10	10 (100)	10 (100)
Djibouti (2), Liberia (1)	CRF02_AG	3	3 (100)	3 (100)
Cameroon (2), USA (1), Spain (1)	Group O	4	4 (100)	4 (100)
Unknown	IIIB	1	1 (100)	0 (0.0)
Unknown	HIV-2	1	1 (100)	1 (100)
	Total	**52**	**50 (96.2)**	**47 (90.4)**

The VITROS® HIV Combo Assay# detects all HIV-1 Genotypes tested.

Conclusions

Ortho is developing a 4th generation HIV assay that has demonstrated the following:

- Observed within lab precision that ranged from 3.4% to 10.0%
- Seroconversion sensitivity consistent with a commercially available 4th generation assay
- HIV p24 antigen sensitivity of 13.1 pg/mL when determined using the AFSSAPS standard
- Detection of all antigen genotypes (96.2% of samples at 200,000 RNA copies/mL) and 100% of antibody subtype samples tested
- 100% specificity with clinical samples and 99.84% specificity with donor samples
- Developed to be used on random access VITROS® Systems

#Under Development

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