

Evaluation of Three New Combined HIV p24 Antigen and Antibody Assays

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Introduction

Early detection of HIV infection is critical for clinical diagnosis and safety of blood products. Combined HIV p24 antigen and antibody assays licensed in Europe between 1997 and 2003 exhibited a higher detection limit of p24 antigen (21 to 160 pg of HIV antigen per mL) than that of current specific antigen assays (under 20 pg of HIV antigen per mL).

In the present study, three new commercially available combined HIV antigen (Ag) and antibody (Ab) assays were evaluated for (i) sensitivity to detect p24 antigen from diverse virus isolates including HIV-1 group M subtypes and HIV-1 group O, (ii) seroconversion sensitivity using 24 HIV seroconversion panels, (iii) ability to detect antibodies to HIV-1 group M, group O and HIV-2.

Materials and Methods

Three new commercially available HIV combined antigen-antibody (Ag/Ab) assays ARCHITECT HIV Ag/Ab Combo (Abbott Diagnostics, Delkenheim, Germany), Genscreen Ag/Ab HIV Ultra (Bio-Rad, Marnes La Coquette, France) and Modular HIV Combi (Roche Diagnostics GmbH, Mannheim, Germany), were compared to (i) 6 other combined HIV antigen and antibody assays, including AxSYM HIV Ag/Ab Combo (Abbott Diagnostics, Delkenheim, Germany), Enzygnost HIV Integral (Dade Behring, Marburg, Germany), Genscreen Plus HIV Ag/Ab (Bio-Rad, Marnes La Coquette, France), Murex HIV Ag/Ab Combo (Abbott, Dartford Kent, England), Cobas Core HIV Combi (Roche Diagnostics GmbH, Mannheim, Germany) and Vironostika HIV Uniform II Ag/Ab (Organon Teknika, Boxtel, The Netherlands), (ii) 2 third-generation HIV antibody assays Genscreen HIV 1/2, version 2, (Bio-Rad, France), Ortho HIV1/HIV2 Ab Capture ELISA (Ortho Clinical Diagnostics, U.S.A.) and an HIV antigen assay (Coulter HIV-1 p24, Coulter Corporation, Miami, U.S.A.).

The sensitivity was evaluated with several panels:

- Panel 1: 7 HIV-1 p24 Ag reference samples with HIV-1 Ag concentrations from 5 to 500 pg HVI/mL (French Society of Blood Transfusion, SFTS96 HIV-1 p24 Antigen Panel).
- Panel 2: 124 specimens from 31-cell culture derived HIV-1 supernatants (group M and O subtypes) infected with different HIV-1 subtypes including: 1 A; 7 B; 5 C; 3 D; 3 F; 1 G; 1 O; 8 CRF01_AE (circulating recombinant forms) and 2 CRF02_AG. Each panel has 4 different concentrations of HIV Ag pg/mL quantified with Innostest HIV Antigen mAB assay (Innogenetics): 4 – 24, 10 – 75, 22 – 150, 61 – 356 pg HIV/mL.
- Panel 3: Twenty-four HIV-1 seroconversion panels with 168 samples provided by different suppliers, were tested. Ten panels were from Boston Biomedica, Inc (BBI), West Bridgewater, Massachusetts (PRB 923, 932, 941, 944, 951, 952, 953, 954, 957, 958); 10 were from North American Biologicals, Inc (NABI), Boca Raton, Florida (NABI 251, 261, 281, 321, 331, 401, 404, 4888, 37748, 122399); and 4 were from BioClinical Partners (BCP), Franklin, Massachusetts (BCP 6243, 9013, 9016, 9017).
- Panel 4: 553 HIV-1 group M antibody reactive samples, including 113 subtype A (Cameroon, France, Ghana, Uganda, United Kingdom), 75 subtype B (Brazil, Cameroon, France, South Africa, Thailand, United Kingdom), 71 subtype C (South Africa, Uganda, United Kingdom, France), 40 subtype D (Cameroon, France, South Africa, Uganda, United Kingdom), 11 subtype F (Argentina, Brazil, Cameroon, France), 9 subtype G (Cameroon, Ghana), 83 CRF01_AE (Thailand), 92 CRF02_AG (Cameroon, United Kingdom), and 59 mosaic (Argentina, Brazil, Cameroon, Ghana, Uganda, United Kingdom).
 - 6 HIV-1 group O (Cameroon, Equatorial Guinea, Spain, France, U.S.A.)
 - 110 HIV-2 samples (West Africa)

Results

Sensitivity Analysis Using the SFTS96 HIV-1 p24 Antigen Panel

Genscreen Ultra and ARCHITECT Combo exhibited the best analytical sensitivity with respectively 13 and 18 pg/mL HIV Ag and showed performance comparable to the Coulter HIV-1 Ag assay (15.7 pg/mL). The Modular Combi analytical sensitivity was assessed at 126.5 pg/mL HIV Ag (Table 1).

Table 1. HIV Antigen Detection Limit of Different Combined Assays (by using HIV Ag SFTS French panel: LAI subtype B strain)

	Combined Assays	Detection Limit of HIV Ag pg/mL
1998	Enzygnost HIV Integral	160
1998	Vironostika HIV Uniform II Ag/Ab	150
2000	Genscreen HIV Ag/Ab Plus	140
2001	Murex HIV Combo	29
2001	Cobas Core HIV Combi	29
2002	AxSYM HIV Ag/Ab Combo	21
2004	Modular HIV Combi or Elecsys	126.5
2004	ARCHITECT HIV Ag/Ab Combo	18
2005	Genscreen HIV Ag/Ab Ultra	13
p 24 Ag assay		
1994	Coulter HIV p24 Ag	15.7

HIV Ag Sensitivity in Different Genotypes

HIV Ag detection limits of the combined tests vary for HIV isolates (Figure 1). These results reflect the quality of monoclonal antibodies used for the capture of p24 Ag. Regarding the HIV Ag genotype sensitivity, Genscreen Ultra and ARCHITECT Combo were the most sensitive with 6 – 41 pg/mL for all genotypes, except one exception for Genscreen Ultra in one group O sample (180 pg/mL). Modular Combi showed a detection limit of 86 – 280 pg/mL. Vironostika and Enzygnost failed to detect any samples of these 31 panels (Table 2).

Figure 1. Limit of HIV Ag Detection for Different HIV Strains

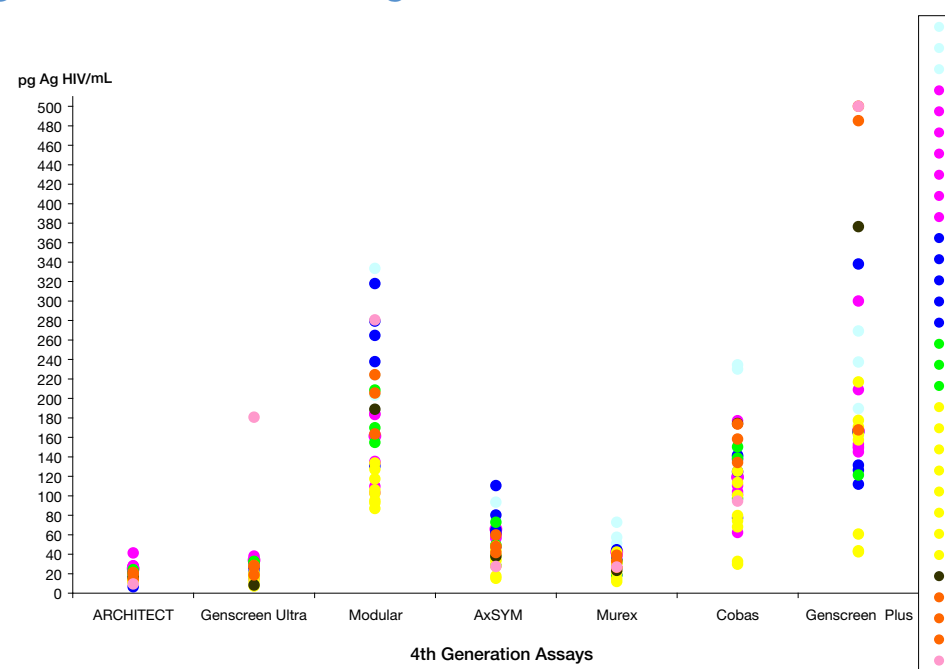


Table 2. HIV Ag Detection Limit of Combined Assays in Different Subtypes (pg HIV/mL)

Sub-type	ARCHITECT Combo	Genscreen Ultra	Modular Combi	AxSYM Combo	Murex Combo	Cobas Combi	Genscreen Plus
A	16.5	28.7	333.4	58.0	50.8	149.4	189.6
AG	22.6	16.1	224.0	93.5	72.7	230.1	269.2
AG	24.9	26.6	203.3	74.7	57.4	234.5	237.3
B	28.0	29.9	135.2	56.2	43.4	124.1	149.1
B	27.9	14.4	133.5	47.7	26.4	101.2	>153
B	27.2	38.0	184.3	60.8	42.8	177.2	>209
B	41.2	28.2	109.8	60.5	38.5	110.4	>300
B	22.6	31.4	103.1	47.9	39.3	104.0	145.0
B	25.1	33.9	161.2	65.7	42.4	119.2	166.3
B	23.8	27.4	183.5	57.6	37.0	62.3	150.5
C	21.0	29.4	318.0	61.8	44.6	137.5	338.0
C	20.5	22.1	279.4	110.4	34.1	141.5	131.4
C	6.6	16.8	130.4	47.8	18.5	77.3	111.9
C	15.8	26.6	264.7	64.8	33.3	140.7	164.6
C	16.7	26.3	237.7	80.1	31.5	125.0	126.4
D	24.1	32.5	154.9	72.7	33.5	138.7	166.9
D	20.8	32.0	208.7	49.4	31.2	97.1	121.3
D	19.1	17.9	169.7	28.3	18.6	150.3	165.0
AE	16.1	17.8	133.7	48.1	30.8	100.4	162.6
AE	14.1	11.5	86.9	17.5	14.9	32.6	42.3
AE	12.4	28.1	95.1	39.3	30.8	79.6	157.4
AE	14.1	18.8	117.4	33.1	21.3	113.5	177.5
AE	11.7	7.3	102.5	15.2	12.0	29.7	43.4
AE	14.4	20.8	106.2	35.0	26.0	68.1	60.7
AE	10.4	16.8	127.1	44.2	42.2	125.3	171.0
AE	11.6	15.3	92.7	28.1	32.3	74.3	217.0
G	16.1	8.3	188.8	37.9	23.4	174.1	376.3
F	21.2	28.3	224.3	59.8	38.9	158.3	485.2
F	14.3	18.7	205.6	48.0	26.4	173.6	167.7
F	19.8	26.5	163.6	41.6	32.9	134.2	>500
O	9.4	180.7	280.7	27.3	26.9	94.4	>500

Vironostika and Enzygnost failed to detect any samples of these 31 panels.

Results (cont.)

Detection of HIV Infection in 24 Seroconversion Panels

The results obtained are presented and expressed as the day of first positive bleed (Table 3). Using the calculation model for time delays between assays established by the Paul Ehrlich Institute (Germany), the mean time delay in days between PCR assay and different tests were respectively ARCHITECT 3.54; Genscreen Ultra 3.68; Coulter HIV-1 Ag 3.96; AxSYM 4.08; Murex 4.79; Cobas Core 5.21; Genscreen Plus 5.37; Modular Combi 5.41; Genscreen v.2 6.92; Ortho 6.96; Enzygnost 7.87 and Vironostika 8.17 days (Table 4).

Table 3. Bleed Day With First Positive Result in Seroconversion Panel

Panel	4th generation											3rd generation	
	Coulter p24 ^a	PCR ^a	ARCHITECT Combo	Genscreen Ultra	Modular Combi	AxSYM Combo	Murex Combo	Genscreen Plus	Cobas Combi	Vironostika Ag/Ab	Enzygnost Integral	Genscreen v2	Ortho Capture
923 W	37	35	37	37	37	37	37	47	37	47	47	84	47
932-AG	27	27	27	27	27	27	27	27	27	27	27	27	27
941-AQ	9	4	9	9	18	18	18	18	18	18	18	18	18
944-AT	2	0	2	2	7	2	7	7	7	14	14	9	14
952 BB	10	7	10	10	10	7	10	10	10	17	17	14	14
953 BC	7	0	3	7	7	3	7	7	7	10	10	7	10
954 BD	21	10	17	17	17	17	17	21	17	21	21	21	21
957 BG	16	14	23	23	23	23	23	23	23	23	23	23	23
958 BH	7	0	7	7	9	9	7	15	9	15	17	15	15
9,013	23	18	23	25	a	25	25	a	a	a	a	a	a
9,016	30	27	30	30	34	34	a	34	34	a	a	a	a
9,017	28	0	24	14	21	21	24	14	28	28	28	7	10
6,243	25	18	25	25	32	25	25	27	25	32	32	27	27
SVO251	22	4	15	18	22	11	18	18	18	22	18	18	18
SVO261	10	3	10	10	10	10	10	10	10	17	10	13	13
SVO281	8	1	8	34	34	8	8	34	34	34	34	34	34
SVO321	8	1	8	8	8	8	8	8	8	12	12	12	12
SVO331	13	6	13	13	13	13	13	13	13	20	15	20	20
SVO371	17	17	17	17	17	17	17	17	17	17	17	22	22
SVO401	8	1	8	8	12	8	12	12	12	15	15	12	12
SVO404	19	1	19	19	23	23	19	23	23	a	a	a	23
4888	1	ND	1	1	6	1	6	6	1	8	6	6	6
37,748	1	ND	1	1	1	1	1	1	1	1	1	1	6
122,399	6	ND	8	8	13	8	8	13	8	13	13	13	13
No. of positive/total samples			92/168	91/168	77/168	89/168	83/168	78/168	80/168	59/168	62/168	70/168	68/168
%			54.8	54.2	45.8	53.0	49.4	46.4	47.6	35.1	36.9	41.7	40.5

^a negative result on all bleeds of panel
^b p24 Ag data from BBI, NABI, PCB and Diagnostic Specialties

Table 4. Time Delay for Detection of Primary HIV Infection in Comparison With the Most Sensitive Assay for Each Panel (last negative sample plus 1 day: Paul Erlich Institute Method)

Panel	4th generation											3rd generation	
	Coulter p24 ^a	PCR ^a	ARCHITECT Combo	Genscreen Ultra	Modular Combi	AxSYM Combo	Murex Combo	Genscreen Plus	Cobas Combi	Vironostika Ag/Ab	Enzygnost Integral	Genscreen v2	Ortho Capture
923 W	1	0	1	1	1	1	1	3	1	3	3	13	3
932-AG	0	0	0	0	0	0	0	0	0	0	0	0	0
941-AQ	1	0	1	1	6	6	6	6	6	6	6	6	6
944-AT	1	0	1	1	3	1	3	3	3	10	10	8	10
952 BB	1	0	1	1	1	0	1	1	1	8	8	4	4
953 BC	4	0	1	4	4	1	4	4	4	8	8	4	8
954 BD	8	0	5	5	5	5	5	8	5	8	8	8	8
957 BG	1	0	3	3	3	3	3	3	3	3	3	3	3
958 BH	3	0	3	3	8	8	3	10	8	10	16	10	10
9,013	1	0	1	6	8	6	6	8	8	8	8	8	8
9,016	1	0	1	1	4	4	4	4	4	8	8	8	8
9,017	25	0	22	11	18	18	22	11	25	25	25	4	8
6,243	4	0	4	4	10	4	4	8	4	10	10	8	8
SVO251	12	0	8	12	15	5	12	12	12	15	12	12	12
SVO261	1	0	1	1	1	1	1	1	1	11	1	8	8
SVO281	6	0	6	8	8	6	6	8	8	8	8	8	8
SVO321	1	0	1	1	1	1	1	1	1	1	8	8	8
SVO331	3	0	3	3	3	3	3	3	3	10	8	10	10
SVO371	0	0	0	0	0	0	0	0	0	0	0	1	1
SVO401	5	0	5	5	8	5	8	8	8	12	12	8	8
SVO404	16	0	16	16	19	19	16	23	19	23	23	23	19
4888	0	ND	0	0	1	0	1	1	0	6	1	1	1
37,748	0	ND	0	0	0	0	0	0	0	0	0	0	5
122,399	0	ND	1	1	3	1	1	3	1	3	3	3	3
Total no. of days	95	0	85	88	130	98	115	129	125	196	189	166	167
Mean no. of days	3.96	0	3.54	3.68	5.41	4.08	4.79	5.37	5.21	8.17	7.87	6.92	6.96

^a Data from BBI, NABI et BCP.
ND = no data

Using a positive scoring system (i.e., number of positive bleeds for each assay out of a total number of 168 bleeds from 24 panels) calculated based on mean (100x positive bleeds per panel divided by total number of bleeds per panel), differences in assay sensitivities were observed instead of grading. Based on percentage of positive samples (Table 3), the assays can be ranked in the following order: ARCHITECT HIV Ag/Ab Combo (54.8%), Genscreen Ultra (54.2%), AxSYM HIV Ag/Ab Combo (53%), Murex HIV Ag/Ab Combo (49.4%), Cobas Core (47.6%), Genscreen Ag/Ab Plus (46.4%), Modular (45.8%), Genscreen v.2 (41.7%), Ortho Capture (40.5%), Enzygnost