Seven Human Immunodeficiency Virus (HIV) Antigen-Antibody Combination Assays: Evaluation of HIV Seroconversion Sensitivity and Subtype Detection

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In this study, we evaluated the performance of two prototype human immunodeficiency virus (HIV) antigen-antibody (Ag-Ab) combination assays, one from Abbott Laboratories (AxSYM HIV Ag-Ab) and the other from bioMérieux (VIDAS HIV Duo Ultra), versus five combination assays commercially available in Europe. The assays were Enzygnost HIV Integral, Genscreen Plus HIV Ag-Ab, Murex HIV Ag-Ab Combination, VIDAS HIV Duo, and Vironostika HIV Uniform II Ag-Ab. All assays were evaluated for the ability to detect p24 antigen from HIV-1 groups M and O, antibody-positive plasma samples from HIV-1 groups M and O, HIV-2, and 19 HIV seroconversion panels. Results indicate that although all combination assays can detect antibodies to HIV-1, group M, subtypes A to G, circulating recombinant form (CRF) A/E, and HIV-1 group O, their sensitivity varied considerably when tested using diluted HIV-1 group O and HIV-2 antibody-positive samples. Among combination assays, the AxSYM, Murex, and VIDAS HIV Duo Ultra assays exhibited the best antigen sensitivity (at ~25 pg of HIV Ag/ml) for detection of HIV-1 group M, subtypes A to G and CRF A/E, and HIV-1 group O isolates. However, the VIDAS HIV Duo Ultra assay had a lower sensitivity for HIV-1 group M and subtype C, and was unable to detect subtype C antigen even at 125 pg of HIV Ag/ml. The HIV antigen sensitivity of the VIDAS HIV Duo and Genscreen Plus combination assays was ~125 pg of HIV Ag/ml for detection of all HIV-1 group M isolates except HIV-1 group O while the sensitivity of Vironostika HIV Uniform II Ag-Ab and Enzygnost HIV Integral Ag-Ab assays for all the group M subtypes was >125 pg of HIV Ag/ml. Among the combination assays, the AxSYM assay had the best performance for detection of early seroconversion samples, followed by the Murex and VIDAS HIV Duo Ultra assays.

Transmission of human immunodeficiency virus (HIV) through blood transfusion and diagnosis of infection in hospitals and public health settings continues to be a worldwide concern. Detection of HIV antigen (Ag) and antibodies (Ab) during the early viremic window period between HIV infection and seroconversion continues to be a challenge. Over the past 16 years, considerable progress has been made in serologic detection of HIV infection. The first generation of HIV assays relied on the detection of antibody to HIV viral proteins. These assays used the solid phase coated with viral antigens and polyclonal antibodies to human immunoglobulins conjugated to an enzyme for detection of HIV-specific antibodies (1, 24). The second-generation assays used HIV recombinant antigens instead of viral lysate as the source of antigen on the solid phase and also incorporated recombinant antigen for HIV-2 (6, 10, 11, 12, 13, 16). These assays had improved specificity though the overall sensitivity remained similar to that of the first-generation assays. Third-generation assays used the solid phase coated with recombinant antigens and/or peptides and similar recombinant antigens and peptides conjugated to a detection enzyme or hapten that could detect HIV-specific antibodies bound to a solid phase. These assays

could detect immunoglobulin M, early antibodies to HIV, in addition to immunoglobulin G, thus resulting in a reduction of the seroconversion window (9, 14, 21, 26). At the same time, assays were also developed to detect HIV-1 antigen using anti-p24 antibodies (either polyclonal or monoclonal) on the solid phase and p24 specific antibodies conjugated to an enzyme for detection of HIV antigen bound to the solid phase (4, 7, 8, 18). Many studies have demonstrated that detection of HIV antigen prior to the development of detectable immune response results in a further reduction of the seroconversion window by approximately 9 days (4, 5). The use of these two independent assays (Ag and Ab) could reduce the seroconversion window significantly (4, 7, 18).

While some developed countries have mandated HIV antigen assays for blood screening, there is also a demand to reduce the number of tests that have to be performed in order to identify and eliminate contaminated blood. The development of assays that would detect both antigens and antibodies in a single assay would be preferable over performing two separate assays, one for antigen and the other for antibody. Several reports have demonstrated that the Ag-Ab combination assays are more sensitive than antibody assays alone (3, 15, 19, 20, 22, 23, 25). In the present study, we evaluate two newly developed prototype Ag-Ab combination assays developed at Abbott Laboratories and bioMérieux that have improved antigen detection and compared their performance to five com-

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bination assays commercially available in Europe. The comparative evaluation was performed using a well-characterized, 217-member HIV panel. This panel was developed to evaluate (i) the sensitivity of various assays to detect p24 antigen from diverse virus isolates, including HIV-1 group M subtypes and HIV-1 group O, (ii) the ability to detect antibodies to HIV-1 groups M and O and HIV-2, and (iii) seroconversion sensitivity using 19 HIV seroconversion panels.

MATERIALS AND METHODS

Panels. A panel of well-characterized samples was used for the evaluation of various serologic assays. The panel had 34 HIV viral antigen-positive samples. The HIV-1 group M subtype A (UG 273), subtype B (US 2), subtype C (SM 145), subtype D (UG 274), CRF A/E (POC 30506), subtype F (BZ 163), and subtype G (MIKAG) virus isolates were kindly provided by Nelson Michael and Merlin Robb (The Walter Reed Army Institute of Research, Rockville, Md.) (17). The HIV-1 group O virus was obtained from Serologicals Corporation, Clarkston, Ga. All viruses were propagated in tissue culture by SRA Technologies, Rockville, Md. The virus stocks were diluted in negative human plasma to 2, 5, 10, and 25 pg of p24/ml using the Abbott HIV antigen assay (HIV Ag-1 Monoclonal) and Abbott HIV-1 p24 antigen (Viral) quantitation panel (Abbott Laboratories, Abbott Park, Ill.). Subsequent comparison of the p24 antigen concentrations by using the antigen panel of the French Society of Blood Transfusion (Ag VIH SFTS96') revealed that there was a fivefold difference between the Abbott panel and the SFTS panel. The values presented in the manuscript were adjusted to 10, 25, 50, and 125 pg of HIV Ag/ml to be equivalent to the SFTS panel. In addition, the antigen panel also included two HIV-1 group M, subtype C, antigen-positive plasma samples identified in a South African blood bank.

The antibody-positive panel consisted of 31 plasma samples that were collected from various areas of the world where HIV is endemic and that represent HIV-1 group M subtypes A to G and CRF A/E. These samples were characterized based on sequence analysis of the envelope and the p24 gene described previously (2). The panel also included four neat (undiluted) and nine diluted (1:100 in negative human plasma) HIV-1 group O samples obtained from 13 independent donors, and six HIV-2 antibody-positive samples. The HIV-2 samples were obtained from six individuals and diluted (1:50 or 1:100 or 1:1000 in negative human plasma). Each of the 13 HIV-1 group O and 6 HIV-2 samples was characterized by genomic sequence and phylogenetic analyses. In addition, 19 seroconversion panels (133 samples) purchased from North American Biologicals, Inc. (Boca Raton, Fla.) (NABI HIV-1 SV 0251, 0261, 0281, 0321, 0331, 0371, 0401, 0404, and 4888; 37748; and 122399); Bioclinical Partners (Franklin, Mass.) (BCP 6243, 9013, 9016, and 9017); and Boston Biomedica Inc. (West Bridgewater, Mass.) (BBI 932, 941, 944, and 952) were included in the panel.

Serologic evaluation. The serologic assays used in this study have the capability to detect both HIV antigen and antibody in a single assay. The assays included the following: VIDAS HIV Duo and VIDAS HIV Duo Ultra assays (bioMérieux, Marcy l'Etoile, France), Murex HIV Ag-Ab Combination assay (Murex Biotech Ltd., Dartford, United Kingdom), Enzygnost HIV Integral assay (Dade Behring, Penzberg, Germany), Vironostika HIV Uniform II Ag-Ab assay (Organon Teknika, Boxtel, The Netherlands), Genscreen Plus assay (Sanofi Pasteur, Marnes La Coquette, France), and a prototype AxSYM HIV Ag-Ab Combination assay (Abbott Laboratories). All the assays on the 217-member blinded panel were performed at the Laboratoire Claude Lévy, Ivry Sur Seine, France, according to protocols provided by the manufacturers.

AxSYM HIV Ag-Ab assay. This assay is performed on the AxSYM instrument, an automated immunoassay system that incorporates continuous access, random access, and mid-range daily testing capacity as a stand-alone analyzer. The AxSYM HIV Ag-Ab assay utilizes a blend of microparticles (solid phase) coated with HIV recombinant antigens (HIV-1 group M gp41, HIV-1 group O gp41, and HIV-2 gp36) for the capture of antibodies and microparticles coated with HIV-1 p24-specific monoclonal antibodies for the capture of HIV antigen in a test sample. A serum or plasma sample (95 μ l) is incubated with antigen- and monoclonal antibody-coated particles for 9 min at 34°C. This step is followed by dispensing the microparticle sample onto a matrix cell, followed by a wash with specimen diluent buffer. Next, a probe mixture containing recombinant HIV-1 group M, subtype B gp41, HIV-1 group O gp41, HIV-2 gp36, and HIV-1 and HIV-2 synthetic peptide antigens as well as HIV p24 monoclonal antibodies conjugated with biotin is added to the matrix cell. After a 9-min incubation, the microparticles are washed two times with a specimen diluent buffer to remove unbound conjugate. The biotinylated probes bound to solid phase are next

incubated with rabbit anti-biotin antibody conjugated to alkaline phosphatase for 9 min, followed by five buffer washes. The alkaline phosphatase activity is determined by addition of substrate, 4-methylumbelliferyl phosphate, that is converted to methylumbelliferone by alkaline phosphatase and is quantitated based on a fluorescent signal measured by the AxSYM instrument. The HIV p24 antigen concentration and antibody titer in a sample are proportional to the alkaline phosphatase activity. The cutoff value was calculated as the index calibration mean rate +27.5.

Murex antigen/antibody assay. The Murex HIV Ag-Ab assay utilizes microtiter plates coated with HIV recombinant antigens and peptides from HIV-1 group M gp41 and polymerase (*pol*), HIV-1 group O gp41, and HIV-2 gp36 for the capture of antibodies and monoclonal antibodies for the capture of HIV antigen in test samples. In a three-step assay protocol, the sample (100 μ l) is incubated for 60 min with antigen- and antibody-coated plates, followed by five buffer washes. In the next step, conjugate is added to the well and incubated for 30 min, followed by five buffer washes. The conjugate contains recombinant antigens and peptides from HIV-1 group M gp41 and *pol*, HIV-1 group O gp41, HIV-2 gp36, and monoclonal antibodies to HIV-1 p24 conjugated with horseradish peroxidase. The amount of conjugate bound is determined by incubation with hydrogen peroxide-3,3',5,5'-tetramethylbenzidine (TMB) substrate for 30 min, termination of incubation with H_2SO_4 , and absorbance measurement at 450 nm. All incubations are carried out at 37°C and addition of each reagent is monitored by a color change that can be either visually observed or monitored by a spectrophotometer. The simultaneous detection of HIV p24 antigen and HIV antibody in a sample is achieved based on binding of the antigen and an antibody to the solid phase and is proportional to horseradish peroxidase activity. The cutoff is calculated as the mean of negative control values plus 0.15. The overall mean must be less than 0.150 for the run to be valid.

Genscreen HIV Plus assay. Genscreen Plus HIV Ag-Ab is a microplate double-sandwich enzyme-linked immunosorbent assay. In the first step, the sample (75 μ l) is incubated with biotinylated anti-p24 polyclonal antibodies (conjugate 1) in a microtiter plate coated with anti-p24 monoclonal antibodies and purified HIV-1 and HIV-2 antigens (recombinant gp160, artificial functional consensus group O gp41 polypeptide, and synthetic gp36 polypeptide). After a first washing step, avidin and HIV-1 and HIV-2 antigens (HIV-1 group M, gp41 synthetic polypeptides, artificial functional consensus group O gp41 polypeptide, and synthetic gp36 polypeptide) labelled to horseradish peroxidase (conjugate 2) are added. After a second washing step, a TMB substrate solution is added. The presence of HIV antigen or antibody is proportional to the binding of the conjugate and peroxidase activity.

VIDAS HIV Duo Ultra assay. VIDAS HIV Duo Ultra is based on enzyme-linked fluorescence assay (ELFA) technology. The sample (200 μ l) is incubated simultaneously with an anti-p24 polyclonal antibody within the entire solid-phase receptacle (SPR). Antibodies against HIV bind to the antigens (gp160 of HIV-1, immunodominant region peptides of HIV-2 and HIV-1 group O) coated in the lower part of the SPR. The p24 antigen in the sample binds to the monoclonal anti-p24 antibody coated in the upper part of the SPR and is recognized by biotinylated anti-p24 polyclonal antibody. After a wash step to remove unbound material, biotinylated antigens (the same used in the solid phase) are incubated in the lower part of the SPR to detect the presence of antibodies. Following a wash step, streptavidin coupled to alkaline phosphatase is incubated in the entire SPR, which binds to the biotin. Again after a washing step, the substrate (4-methylumbelliferyl-phosphate) is incubated first in the lower part of the SPR and then an initial measurement of fluorescence is performed to reveal the presence or absence of HIV antibodies. Following this step, the substrate is then incubated in the entire SPR, and a second measurement of fluorescence is then performed, which detects presence or absence of HIV p24 Ag. All the steps performed were automated.

VIDAS HIV Duo, Enzygnost, and Vironostika. Procedures used for VIDAS HIV Duo, Enzygnost, and Vironostika assays have been described in detail previously (3, 22, 25).

RESULTS

Detection of viral antigen. HIV-1 antigen sensitivity was evaluated using a panel of viral isolates diluted in normal HIV-negative human plasma. Results from the four assays with the highest sensitivity for antigen detection are presented in Fig. 1. Based on detection of all virus strains, the Abbott AxSYM, Murex, and VIDAS Duo Ultra assays exhibited the best sensitivity among all the Ag-Ab combination assays at an

FIG. 1. Sensitivity for HIV-1 antigen detection. Antigen detection sensitivity for HIV-1 isolates was determined as described in Materials and Methods. The graphs designate (left to right) group M virus subtypes A, B, C, D, CRF A/E, F, and G and HIV-1 group O. The S/CO values for each virus strain are shown on the y axis. The viral antigen concentrations of 10, 25, 50, and 125 pg of HIV Ag/ml were equivalent to the antigen panel of the French Society of Blood Transfusion (Ag VIH SFTS96').

antigen concentration of approximately 25 pg of HIV Ag/ml. The AxSYM Ag-Ab assay detected HIV-1 group M, subtypes A, C, CRF A/E, and G at 25 pg of HIV Ag/ml while the sample-to-cutoff (S/CO) ratios for HIV-1 group M, subtypes B, D, and F, and group O were close to the cutoff, ranging from 0.8 to 0.99. The Murex combination assay detected all the HIV-1 group M subtypes and HIV-1 group O at 25 pg of HIV Ag/ml, except HIV-1 group M, subtype G, that had an S/CO ratio of 0.96 at 25 pg of HIV Ag/ml. The VIDAS Duo Ultra could detect HIV-1 group M, subtypes A and B, and CRF A/E at 25 pg of HIV Ag/ml, while subtypes G and group O had an S/CO value of 0.92 and 0.96, respectively, at this concentration. However, HIV-1 group M, subtype F, was detected only at 50 pg of HIV Ag/ml and subtype C was not detected even at 125 pg of HIV Ag/ml. The Genscreen Plus assay could not detect HIV-1 group M, subtypes A and B, nor HIV-1 group O antigen in the range tested (10 to 125 pg/ml); however, subtypes A and B had an S/CO signal in the range of 0.90 to 0.97 at the concentration of 125 pg of HIV Ag/ml. The Genscreen Plus assay required 125 pg of HIV Ag/ml or higher concentrations of HIV antigen for detection (Fig. 1). The VIDAS Duo combination assay detected all HIV strains at a concentration of ~125 pg of HIV Ag/ml (data not shown). Enzygnost Ag-Ab combination assays detected only CRF A/E at 125 pg of HIV Ag/ml, whereas the Vironostika HIV Uniform II Ag-Ab assay did not detect any of the HIV strains in the HIV antigen range tested (data not shown).

Antibody sensitivity to HIV variants. A panel of 50 HIV antibody-positive samples was used to evaluate the sensitivity of the various assays. The panel included 31 HIV-1 group M, subtypes A to G, samples collected from diverse areas of the world where HIV is endemic. The panel also included 4 neat and 9 diluted (1:100) HIV-1 group O-positive samples obtained from 13 donors, and 6 diluted (1:50, or 1:100, or 1:1000) HIV-2 antibody-positive samples from six individuals. The HIV-1 group M, antibody-positive samples are readily detected by all assays except for the Enzygnost and Vironostika assays that each missed a subtype C samples (Table 1). However, not all the assays could detect diluted HIV-1 group O or HIV-2 samples (Table 1). The AxSYM HIV Ag-Ab and Enzygnost assays detected all 13 HIV-1 group O samples, whereas the VIDAS Duo, VIDAS Duo Ultra, Genscreen Plus, Vironostika, and Murex Ag-Ab assays detected either 11 or 12 samples. The AxSYM Ag-Ab assay detected five out of six and the Enzygnost detected 4 out of 6 diluted HIV-2 antibody-positive samples. The Murex assay detected one out of six whereas VIDAS Duo, VIDAS Duo Ultra, Genscreen Plus, and Vironostika detected three out of six diluted HIV-2-positive samples. While the diluted samples show differences among assays, efficient identification of neat samples indicated that these assays have the ability to detect antibodies to all HIV strains as claimed in their package inserts.

Seroconversion sensitivity. The seroconversion sensitivity of the various HIV Ag-Ab combination assays was evaluated using 19 seroconversion panels. Data on six representative seroconversion panels are presented in Table 2. In two (SV0401 and BCP9013) of the six panels, the AxSYM, Murex, and VIDAS Duo Ultra Ag-Ab combination assays detected earlier bleeds than the Genscreen Plus, Vironostika, and Enzygnost

TABLE 1. Performance of assays of antibody-positive samples

Panel	No. of positive samples detected by:						
	AxSYM	Murex	Vidas Duo Ultra	Vidas Duo	Genscreen Plus	Enzygnost	Vironostika
HIV-1 Group M Ab (<i>n</i> = 31)	31	31	31	31	31	30	30
HIV-1 Group O Ab (<i>n</i> = 4)	4	4	4	4	4	4	4
HIV-1 Group O diluted Ab (<i>n</i> = 9)	9	7	8	8	8	9	7
HIV-2 Ab diluted (<i>n</i> = 6)	5	1	3	3	3	4	3
Total (<i>n</i> = 50)	49	43	46	46	46	47	44

assays. In one (SV0281) of the six panels, the AxSYM and Murex assays detected earlier bleeds than all other assays.

The seroconversion sensitivity of each assay can be measured based on the bleed day with the first positive result (Table 3). The comparative data on all 19 seroconversion panels shows that the AxSYM assay has the best seroconversion sensitivity. The AxSYM assay detected 3 of 19 panels (PRB932, PRB952, and SVO251) earlier than all other combination assays. The AxSYM and Genscreen assays identified the first positive result (bleed 5, day 15) for panel BCP017, earlier than the other assays. The AxSYM, Murex, and VIDAS Duo Ultra combination assays detected the first positive bleed on the same day in 10 of 19 panels (Table 3). The AxSYM and VIDAS Duo Ultra combination assays identified the first positive result (bleed day 1) at the same time for panel 4888. For panel SVO281, the AxSYM and Murex Ag-Ab assays detected a bleed earlier than the other combination assays. The VIDAS Duo Ultra assay detected the first positive sample in panel 122399 and the Murex assay detected the first positive sample in panel BCP9016 earlier than other combination assays. The results on these seroconversion panels are in agreement with the data on HIV antigen sensitivity (Fig. 1) that demonstrated superior antigen detection by the Murex, AxSYM, and VIDAS Duo Ultra combination assays.

DISCUSSION

The 217-member panel used for the performance evaluation in this study had 34 HIV antigen-positive specimens, 32 derived from 8 genetically diverse HIV isolates propagated in tissue culture and diluted to 10 to 125 pg of HIV Ag/ml and 2 HIV-1 group M, subtype C antigen-positive human plasma samples identified in a South African blood bank. The viral antigen panel consisted of HIV-1 group M, subtypes A to G and CRF A/E, and HIV-1 group O viruses diluted in HIV-negative plasma. The Murex and Abbott AxSYM Ag-Ab combination assays detected all HIV variants evaluated (25 and 22 panel members out of 34, respectively) with a sensitivity of approximately 25 pg of HIV Ag/ml for all HIV strains. Among the other Ag-Ab assays, VIDAS Duo Ultra detected 19 out of 34, while VIDAS Duo detected 9 out of 34 samples. The Genscreen Plus detected only 7 out of 34 panel members, with a sensitivity of ~125 pg of HIV Ag/ml. Notably, the Genscreen Plus assay failed to detect HIV-1 group M, subtypes A and B, and HIV-1 group O p24 antigen in the range tested. The Enzygnost assay detected only one panel member, while the Vironostika assay failed to detect any of the panel members.

In terms of detection of antibodies, this evaluation revealed that each of the assays performed well against all of the HIV

strains by using neat plasma samples. All assays detected 31 out of 31 HIV-1 group M, subtype samples with the exception of Enzygnost and Vironostika, each of which failed to detect one subtype C sample. However, diluted panel members were not efficiently detected by all assays. Of 13 HIV-1 group O samples, 4 neat and 9 diluted, the range of detection varied from 11 to 13 depending on the assay (Table 1). Similarly, out of six diluted HIV-2 samples, the range of detection varied from one to five (Table 1). Reduced detection of diluted samples may be correlated with the reagents used, assay conditions, and the configuration of the assays. In general, assays that are driven by short peptides have reduced sensitivity for detection of diluted samples compared to assays that use large recombinant antigens with multiple antibody binding sites (data not shown). However, as shown in this study, the ability of combination assays to detect antibodies in neat samples from HIV-1 group M and HIV-1 group O demonstrate that in general, all the assays had good sensitivity for the detection of antibodies to HIV variants.

Seroconversion sensitivity of assays for detection of HIV infections can be best assessed using seroconversion panels. Based on the present evaluation, the AxSYM, VIDAS Duo Ultra, and Murex Ag-Ab assays have the best sensitivity among the combination assays. The AxSYM assay detected the first positive bleed in 16 out of 19 seroconversion panels (80 out of 133 samples from 19 panels). The VIDAS Duo Ultra and the Murex assays detected the first positive bleed from 13 out of 19 seroconversion panels (Table 3). The analytical sensitivity based on a tissue culture derived viral antigen panel is directly reflected in the clinical sensitivity observed with seroconversion panels. However, results on seroconversion panels revealed differences between the three assays for the detection of early seroconversion samples. The differential performance among various assays may be dependent on the efficiency of antigen detection. In the present study, only four antigen concentrations were tested, 10, 25, 50, and 125 pg of HIV Ag/ml. Based on the plots of antigen concentration and S/CO values for each HIV strain, the average analytical sensitivity for the Murex assay was 16 pg/ml while the sensitivities of the AxSYM and the VIDAS Duo Ultra assays were at 25 of and 26 pg of HIV Ag/ml, respectively. The differences in antigen sensitivity observed using seroconversion panels and the virus strains diluted in negative human plasma may be attributed to differences in viral subtypes present in the sample. All the seroconversion samples are HIV-1 group M, subtype B, while the tissue culture-derived antigen panel included several HIV strains in addition to subtype B. Moreover, in the seroconversion panels, the presence of antigen and an immune response

TABLE 2. Performance of various assays on seroconversion panels

Panel members	Days	S/CU ratio							
		HIV p24 Ag ^a	AxSYM	Murex	Vidas Duo Ultra	Vidas Duo	Genscreen Plus	Enzygnost	Vironostika
SV0401									
A	1	0.39	0.47	0.39	0.40	0.24	0.28	0.19	0.44
B	5	0.46	0.57	0.44	0.40	0.31	0.35	0.10	0.46
C	8	1.01	1.11	1.52	1.80	0.72	0.46	0.14	0.48
D	12	3.84	6.33	5.37	7.36	3.04	2.13	0.88	0.91
E	15	6.08	11.72	> ^b	9.24	4.92	9.06	3.26	2.07
F	19	1.80	9.73	9.89	3.92	54.88	>	>6	5.60
G	23	0.92	8.51	8.67	3.84	60.72	>	>6	5.91
SV0281									
A	1	0.39	0.57	0.28	0.40	0.20	0.22	0.10	0.37
B	6	0.46	0.47	0.46	0.40	0.16	0.24	0.10	0.38
C	8	1.03	1.05	1.42	0.80	0.48	0.36	0.16	0.43
D	34	1.10	4.83	6.61	3.08	55.76	>	>6	4.85
E	36	1.03	5.38	8.02	3.36	46.20	>	>6	4.64
F	41	0.69	5.00	11.83	6.08	44.32	>	>6	5.58
G	43	0.62	5.42	10.14	6.96	61.28	>	>6	5.72
H	48	0.59	7.74	13.12	9.56	45.24	>	>6	7.70
SV0261									
A	1	0.39	0.48	0.33	0.44	0.08	0.31	0.10	0.39
B	3	0.32	0.58	0.33	0.56	0.20	0.24	0.10	0.43
C	10	6.45	9.01	12.74	18.04	2.96	3.12	1.32	0.42
D	13	13.27	13.37	>	27.44	8.40	10.34	2.83	0.97
E	17	19.87	19.60	>	23.86	11.48	13.63	>6	1.73
F	20	30.52	20.03	>	17.80	39.40	>	>6	6.73
G	24	5.87	9.32	>	9.32	56.76	>	>6	8.37
BCP9013									
1	1	0.34	0.57	0.28	0.40	0.20	0.74	0.10	0.44
2	8	0.34	0.39	0.31	0.40	0.20	0.20	0.10	0.42
3	10	0.29	0.48	0.30	0.40	0.25	0.27	0.10	0.42
4	15	0.33	0.49	0.38	0.40	0.12	0.28	0.10	0.39
5	19	0.28	0.99	0.32	0.40	0.12	0.30	0.10	0.46
6	24	0.46	0.64	0.93	0.44	0.36	0.35	0.10	0.39
7	26	1.10	1.52	2.93	1.84	1.04	0.71	0.10	0.42
SV0321									
A	1	0.46	0.87	0.76	0.68	0.28	0.38	0.10	0.37
B	4	3.89	5.77	7.24	7.76	2.20	1.74	0.46	2.12
C	8	1.60	7.63	10.56	13.88	3.08	>	4.51	3.87
D	11	0.59	7.53	12.51	15.00	38.80	>	>6	6.04
E	15	0.26	6.25	>	15.08	42.36	>	>6	9.53
BCP9017									
1	1	0.71	0.41	0.46	0.50	0.27	0.21	0.10	0.39
2	4	0.62	0.43	0.32	0.50	0.16	0.31	0.10	0.46
3	8	0.40	0.71	0.55	0.36	0.08	0.55	0.10	0.38
4	11	0.55	0.62	0.40	0.40	0.12	0.87	0.10	0.45
5	15	0.67	1.27	0.56	0.42	0.08	1.29	0.10	0.54
6	18	0.44	2.84	0.82	0.45	0.20	1.66	0.10	0.56
7	22	0.64	1.45	0.62	0.64	0.80	4.64	0.20	0.68
8	25	0.69	2.09	0.98	1.08	4.08	13.25	0.45	0.76
9	29	0.92	3.06	2.25	1.68	10.16	18.88	2.39	1.01
10	33	1.00	3.96	3.37	2.44	25.78	19.23	3.70	1.26
11	36	0.88	4.08	4.30	2.60	33.12	>	5.86	1.69

^a HIV p24 antigen data (Abbott Laboratories) are from the package insert of the seroconversion panel.^b >, values above the detectable range.

can be detected simultaneously and may contribute to a higher signal, unlike the viral strain panel. The sensitivity and seroconversion performance correlation is clearly evident for the other combination assays evaluated in the study. The Enzygnost, Vironostika, Genscreen Plus, and VIDAS Duo assays

have lower antigen sensitivities and detected a lower number of seroconversion samples (Table 4).

Most seroconversion panels exhibit antigen-positive bleeds followed by the development of antibodies in the later bleeds. The combination assays are expected to provide enhanced

TABLE 3. Assay performance to detect seroconversion panel members

Panel	Bleed day with first positive result						
	AxSYM	Murex	Vidas Duo Ultra	Vidas Duo	Genscreen Plus	Enzygnost	Vironostika
PRB932	14	28	28	28	28	28	28
PRB932	8	11	11	11	11	18	18
SVO251	8	18	18	22	18	18	22
BCP9017	15	29	25	25	15	29	29
SVO261	10	10	10	10	10	10	17
SVO331	13	13	13	13	13	15	20
SVO371	17	17	17	17	17	17	17
BCP9013	26	26	26	26	Neg	Neg	Neg
37748	1	1	1	1	1	1	1
PRB944	3	3	3	8	8	15	15
SVO401	8	8	8	12	12	15	15
SVO321	8	8	8	8	8	12	8
SVO404	23	19	19	23	Neg	Neg	Neg
6243	26	26	26	33	28	33	33
4888	1	6	1	6	6	6	8
SVO281	8	8	34	34	34	34	34
122399	8	8	6	8	13	13	13
BCP9016	35	31	35	35	35	Neg	Neg
PRB941	19	19	19	22	19	19	19

sensitivity compared to assays that detect only antibody in a sample due to antigen detection prior to the development of an immune response. However, BCP 9017 is an unusual seroconversion panel where the early bleeds are positive for antibody and not antigen (Table 2). Among all the combination assays evaluated, the AxSYM and Genscreen assays identified early seroconversion bleeds (bleed 5, day 15), resulting in a 10-day early detection of first positive result than other assays. These data indicate that the AxSYM and the Genscreen assays have better antibody detection compared to other combination assays evaluated in this study. However, the Genscreen assay has significantly lower sensitivity for detection of HIV antigen (Fig. 1). The Genscreen assay detected the first positive bleed from 7 out of 19 seroconversion panels (67 out of 133 seroconversion samples) compared to the AxSYM assay, which detected the first positive bleed from 16 out of 19 seroconversion panels (80 out of 133 seroconversion samples). Thus, the AxSYM assay has the best antibody detection among all combination assays evaluated in this study.

Specificity is yet another issue that needs to be addressed when discussing the utility of an Ag-Ab combination assay. It has been reported that combining an antigen assay with an antibody assay results in reduced specificity (19, 25). Preliminary data on 2,020 samples from blood banks and hospital populations indicated that the AxSYM HIV Ag-Ab assay has a

specificity of 99.85%. The data on the Murex HIV Ag-Ab combination assay based on an evaluation of 9,289 samples showed a specificity of 99.78% (R. Bristow et al., personal communication). There are no comparative side-by-side studies to evaluate the specificity of various combination tests on the same panel of samples; consequently, additional studies are needed to address this issue. Maintaining similar specificity for combination assays as compared to current antibody assays would be ideal for blood banks and plasma centers.

The present evaluation indicates that among all the combination assays, the AxSYM combination assay has the best overall sensitivity, followed by the Murex and VIDAS Duo Ultra assays (Table 4). The AxSYM combination assay identified the highest number of panel members, 151 out of 217, and exhibited the best sensitivity among all combination assays evaluated in this study. AxSYM assay detected the first positive bleed in 16 out of 19 seroconversion panels (detecting 80 out of 133 seroconversion panel members) compared to Murex and VIDAS Duo Ultra assays that detected 13 out of 19 panels (Table 3). The AxSYM assay demonstrated the best seroconversion sensitivity, compared to the VIDAS Duo Ultra that identified 73 out of 133 while the Murex assay detected 72 out of 133 seroconversion samples from 19 seroconversion panels. In order to detect HIV infection early in a diagnostic setting, and to maintain a safe blood supply, an assay with improved

TABLE 4. Summary of assay performance

Panel	No. of positive samples detected by:						
	AxSYM	Murex	Vidas Duo Ultra	Vidas Duo	Genscreen Plus	Enzygnost	Vironostika
Seroconversion ($n = 133$)	80	72	73	62	67	54	50
HIV Ab ($n = 50$) ^a	49	43	46	46	46	47	44
HIV Ag variants ($n = 34$) ^b	22	25	19	9	7	1	0
Total ($n = 217$)	151	140	138	117	120	102	94

^a Data are from Table 1.

^b Panel included two plasma samples.

sensitivity and automation, such as the AxSYM HIV Ag-Ab assay, will be a superior alternative to the currently available combination assays.

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