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Assessment of the Ability of a Fourth-Generation Immunoassay for Human Immunodeficiency Virus (HIV) Antibody and p24 Antigen To Detect both Acute and Recent HIV Infections in a High-Risk Setting▽

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An immunoassay (IA) that simultaneously detects both antibody to human immunodeficiency virus (HIV) and HIV p24 antigen (Architect HIV Ag/Ab Combo) was evaluated for its ability to detect HIV infection by using a panel of specimens collected from individuals recently infected with HIV type 1 (HIV-1). This IA was found to be capable of detecting the majority (89%) of infections, including 80% of those considered acute infections based on the presence of HIV RNA and the lack of detectable antibody to HIV. Substantial improvements in detection of recent infections by the Architect HIV Ag/Ab Combo relative to previous generations of IAs as well as the capacity to detect acute infections have important implications for HIV prevention strategies.

Immunoassays (IA) for the detection of human immunodeficiency virus (HIV)-specific antibodies have been in continual development since the incipience of the HIV pandemic. The recent success of immunoglobulin G (IgG)- and IgM-sensitive antibody tests (third-generation assays) in narrowing the window period of HIV diagnosis has been notable (2, 4, 5, 7, 10, 32). However, IA designed to detect antibody alone will not be able to identify individuals with acute infection who have not yet begun to produce HIV-specific antibodies. Attempts to detect acutely infected individuals have mostly involved RNA detection algorithms used on pooled HIV antibody-negative specimens. Such efforts have yielded significant returns in detection of recent HIV infection in certain communities (6, 16, 18, 22, 24). Evidence suggests that these individuals are at greatest risk for transmission of HIV and contribute disproportionately to the ongoing epidemic (17, 19, 20, 25). However, the use of RNA-based detection methods is expensive, laborious, and can be operationally daunting. Moreover, in most cases the time to results ranges from 7 to 14 days. This amount of time is less than ideal from an HIV prevention perspective. An alternative to the detection of acute HIV infections using RNA-based methods is to utilize antigen-antibody combination tests, also known as "fourth-generation" IA (12, 23, 26). Fourth-generation assays simultaneously function as both a third-generation IA (for the detection of IgG and IgM antibodies) and a capture IA for the direct detection of p24 antigen (the most abundant protein of HIV virions). Because fourth-generation IA are standard immunoassays, they are easy to perform, relatively inexpensive, and easily automated. At the time of preparation of this article, fourth-generation IA have not been cleared by the FDA within the United States, but their use and performance in other locations worldwide has been well-documented (1, 3, 12–15, 21, 23, 26–31). However, given the paucity of data available on performance of fourth-generation assays relative to HIV RNA detection algorithms in the diagnostic setting, it is of considerable interest from a public health perspective to assess the ability of these assays to detect acute HIV infections.

In the present study, performance of the Architect HIV Ag/Ab Combo (HIV Combo; Abbott Diagnostics, Wiesbaden, Germany [available for sale outside of the United States only]) was assessed. The HIV Combo is a chemiluminescent magnetic microparticle-based immunoassay run on an automated random access instrument. The assay is designed to detect HIV type 1 (HIV-1; groups M, O, and N) and HIV-2. Specimens with signal-to-cutoff (S/CO) ratios of 1.0 or greater are considered reactive. HIV Combo performance was evaluated on specimens from 64 recently infected individuals (tested in San Francisco, CA) identified based on an HIV-1 RNA testing algorithm (all specimens were HIV-1 RNA positive). This highly characterized panel, collected over a 5-year period, consists of a range of specimen types, including specimens from acutely infected individuals (HIV RNA positive/no detectable HIV antibody; $n = 35$), individuals reactive on a single antibody test ($n = 7$), and individuals who are reactive on multiple, but not all, antibody tests evaluated ($n = 22$) (10, 11). Sequence analysis was performed on 60 of the panel members. All were infected with subtype B virus (data not shown). The 64-member panel, interspersed with an additional 31 control specimens, was blinded prior to testing with HIV Combo. The 31 controls were known HIV antibody-positive ($n = 16$) and -negative ($n = 15$) specimens.

As shown in Table 1, 57 of the 64 specimens from recently

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TABLE 1. Summary of assay performance results with specimens from acutely infected and recently infected individuals[a]

Sample ID	Initial screen	First-gen or second-gen EIA		WB	Third-gen EIA		OQ	UG	SR	MS	Viral load (copies/ml)[b]	Fourth-gen IA	
		Avg S/C	Result		Avg S/C	Result	RT	RT	RT	RT		Avg S/CO	Result
A	First-gen EIA	0.351	NR	I	0.127	NR	N	N	N	N	5,770†	0.37	NR
B	First-gen EIA	0.602	NR	I	0.955	NR	N	N	N	N	≥500,000†	611.12	R
C	First-gen EIA	0.440	NR	I	≥14.658	R	N	R	N	N	12,183†	1.62	R
E	First-gen EIA	0.368	NR	I	0.233	NR	N	N	N	N	6,373†	0.65	NR
F	First-gen EIA	0.329	NR	I	13.433	R	N	R	N	R1	≥500,000†	85.73	R
G	First-gen EIA	0.317	NR	N	0.084	NR	N	N	N	N	12,852†	0.74	NR
H	First-gen EIA	0.338	NR	I	0.109	NR	N	N	N	N	14,062†	0.68	NR
I	First-gen EIA	0.646	NR	I	≥14.658	R	R	R	R	R1	≥500,000†	67.70	R
J	First-gen EIA	0.358	NR	N	0.106	NR	N	N	N	N	3,921†	0.23	NR
K	First-gen EIA	0.346	NR	N	4.574	R	N	R	N	N	≥500,000†	43.92	R
L	First-gen EIA	0.373	NR	N	0.175	NR	N	R	N	N	≥500,000†	39.55	R
M	OQ RT-FS	0.344	NR	N	1.5327	R	N	N	N	N	≥500,000†	368.21	R
N	First-gen EIA	0.337	NR	N	0.113	NR	N	N	N	N	1,177†	0.21	NR
O	OQ RT-FS	0.301	NR	N	0.127	NR	N	N	N	N	≥500,000†	61.32	R
P	First-gen EIA	0.755	NR	N	≥14.658	R	N	R	N	R1	≥500,000†	136.62	R
Q	First-gen EIA	0.311	NR	N	0.277	NR	N	N	N	N	43,173†	1.80	R
R	OQ RT-FS	0.642	NR	I	0.117	NR	N	N	N	N	30,734†	2.05	R
S	First-gen EIA	0.406	NR	N	13.276	R	N	R	N	R1	≥500,000†	219.97	R
T	OQ RT-OF	0.401	NR	N	4.929	R	N	N	N	N	≥500,000†	268.30	R
U	OQ RT-FS	0.325	NR	N	0.195	NR	N	N	N	N	≥500,000†	317.71	R
V	OQ RT-FS	0.512	NR	N	0.198	NR	N	N	N	N	≥500,000†	20.53	R
W	First-gen EIA	0.504	NR	I	≥12.403	R	N	R	N	R1	≥500,000†	121.68	R
Y	OQ RT-FS	0.340	NR	N	≥12.590	R	N	N	N	N	≥500,000†	237.06	R
Z	First-gen EIA	0.378	NR	N	0.201	NR	N	N	N	N	102,288†	2.09	R
AA	First-gen EIA	0.343	NR	N	0.327	NR	N	N	N	N	327,333†	6.34	R
AB	First-gen EIA	0.373	NR	N	0.189	NR	N	N	N	N	≥500,000†	168.87	R
AC	OQ RT-FS	0.396	NR	I	0.280	NR	N	N	N	N	≥500,000†	35.93	R
AD	First-gen EIA	0.426	NR	N	0.371	NR	N	N	N	N	≥500,000†	132.59	R
AE	OQ RT-FS	0.369	NR	N	0.145	NR	N	N	N	N	389,850†	12.24	R
AF	OQ RT-FS	0.761	NR	I	0.907	NR	N	R	N	R1	413,186†	13.89	R
AG	Third-gen EIA	0.436	NR	I	0.165	NR	N	N	N	N	446,770†	19.21	R
AH	Third-gen EIA	0.371	NR	N	0.195	NR	N	N	N	N	358,030†	10.66	R
AJ	OQ RT-FS	0.6	NR	N	1.528	R	N	N	N	N	≥500,000†	24.25	R
AK	OQ RT-FS	0.6	NR	N	0.147	NR	N	N	N	N	427,490†	3.45	R
AM	OQ RT-FS	0.41	NR	N	0.187	NR	N	N	N	N	≥500,000†	309.58	R
AN	OQ RT-OF	0.33	NR	N	0.17	NR	N	N	N	N	≥500,000†	17.55	R
AO	Third-gen EIA	0.45	NR	N	9.634	R	N	N	N	N	≥500,000†	22.83	R
AP	Third-gen EIA	0.34	NR	N	≥12.834	R	N	R	N	R1	≥500,000†	131.50	R
AR	OQ RT-OF	0.45	NR	N	0.097	NR	N	N	N	N	≥500,000†	10.57	R
AS	Third-gen EIA	0.48	NR	I	≥14.641	R	R	R	R	R1	≥500,000†	24.74	R
AT	OQ RT-OF	0.40	NR	N	0.174	NR	N	N	N	N	≥500,000†	176.23	R
AU	OQ RT-OF	1.48	R	P	13.09	R	R	R	R	R1	109,211	31.35	R
AV	OQ RT-OF	0.5	NR	N	0.271	NR	N	N	N	N	333,066	5.68	R
AW	OQ RT-OF	5.82	R	P	12.496	R	R	R	R	R1	335	14.33	R
AX	OQ RT-OF	0.36	NR	N	1.554	R	N	R	N	N	≥10,000,000	360.32	R
AY	OQ RT-OF	0.45	NR	N	0.215	NR	N	N	N	N	69,599	1.46	R
AZ	OQ RT-OF	0.59	NR	P	9.454	R	R	R	R	R1	2,915,309	80.82	R
BA	OQ RT-OF	0.31	NR	N	13.205	R	N	R	N	R1	518,434	9.13	R
BB	Third-gen EIA	0.32	NR	N	0.16	NR	N	N	N	N	317,609	3.81	R
BC	OQ RT-OF	6.65	R	P	≥13.943	R	R	R	R	R1	13,204	77.09	R
BD	OQ RT-FS	0.5	NR	P	10.7	R	R	R	R	R1	8,887,199	128.99	R
BE	OQ RT-FS	0.38	NR	I	0.243	NR	N	N	N	N	≥10,000,000	132.66	R
BF	OQ RT-OF	0.34	NR	N	5.443	R	N	N	N	N	≥10,000,000	430.39	R
BG	OQ RT-OF	5.69	R	P	≥13.514	R	R	R	R	R1	468,809	12.66	R
BH	OQ RT-OF	0.35	NR	N	0.232	NR	N	N	N	N	9,289,006	80.34	R
BI	OQ RT-FS	2.93	R	P	10.564	R	R	R	R	R1	355	3.99	R
BJ	Third-gen EIA	0.346	NR	I	0.123	NR	N	N	N	N	9,855	0.71	NR
BK	OQ RT-OF	0.294	NR	N	0.403	NR	N	N	N	N	650,629	6.08	R
BL	OQ RT-OF	5.13	R	P	≥14.493	R	R	R	R	R1	752	199.51	R
BM	OQ RT-FS	0.326	NR	N	0.345	NR	N	N	N	N	4,589,912	44.06	R
BN	OQ RT-FS	2.959	R	I	1.18	R	R	N	R	R1	4,571,787	48.35	R
BO	OQ RT-FS	0.22	NR	N	0.168	NR	N	N	N	N	1,531,891	18.05	R
BP	OQ RT-FS	1.015	R	N	4.139	R	N	R	N	R1	≥10,000,000	136.82	R
BQ	OQ RT-FS	0.15	NR	N	0.539	NR	N	N	N	N	3,427,483	14.92	R

[a] The first-generation (first-gen) EIA was the BioMérieux Vironostika HIV-1 micro-ELISA. The second-generation EIA was the Bio-Rad Genetic Systems rLAV HIV-1 EIA. The third-generation EIA was the Bio-Rad Genetic Systems HIV-1/2 plus O EIA. The fourth-generation IA was the Abbott Architect HIV Ag/Ab Combo. WB, Western blot assay using Bio-Rad Genetic Systems HIV-1; OQ RT-OF, OraQuick rapid test of oral fluid; OQ RT-FS, OraQuick rapid test of fingerstick sample; OQ-RT, Oraquick Advance HIV-1/2 rapid test; UG -RT, Uni-Gold Recombigen HIV-1 rapid test; SP-RT, Stat Pak HIV-1/2 rapid test; MS-RT, Multi-Spot HIV-1/2 rapid test. Results: R, reactive; NR, nonreactive; R1, reactive for HIV-1; P, positive; N, negative; I, indeterminate.

[b] †, viral load was determined with Siemens Versant HIV-1 RNA v. 3.0 (otherwise, samples were tested using the Abbott real-time HIV-1 test).

infected individuals were found to be reactive in the HIV Combo assay. Among the 57 specimens found reactive by the HIV Combo were 28 of the 35 previously determined to contain no detectable HIV antibody by any other antibody-based method evaluated. These data confirm the ability of the HIV Combo to detect HIV antigen and therefore provide a reactive (positive) result, even when antibody to HIV is not detectable. Moreover, these data demonstrate that the HIV Combo assay

TABLE 2. Comparative test method detection rates

Method	% Detected[a]
First- or second-generation IA	12.5
HIV-1 Western blot	12.5
OraQuick Advance HIV-1/2 rapid test	17.2
Stat-Pak HIV-1/2 rapid test	17.2
Multi-Spot HIV-1/2 rapid test	28.1
Uni-Gold HIV-1 rapid test	34.4
Third-generation IA	42.2
Fourth-generation IA	89.1

[a] Percentage of acute/recent specimens from Table 1 found positive by the indicated assay method.

is able to identify acutely infected individuals in the majority of the cases where antibody testing fails to do so. HIV Combo detected all 7 specimens reactive on only one of the antibody tests as well as all 22 reactive by multiple but not all antibody tests. The HIV Combo assay found all of the positive controls to be reactive and all negative controls to be nonreactive.

Seven of the 64 panel members were nonreactive in the HIV Combo assay. These specimens (A, E, G, H, J, N, and BJ) are notable because each was found to be uniformly nonreactive for HIV antibody while being shown to contain measurable viral RNA. Of interest, the HIV-1 RNA levels measured in these specimens were relatively low (mean, 7,716 copies/ml; median, 6,373 copies/ml; range, 1,177 to 14,062 copies/ml). Of the antibody-negative specimens detected by the HIV Combo, the lowest RNA value was 30,734 copies/ml, while the highest RNA value for a specimen not detected by HIV Combo was 14,062 copies/ml. These data suggest that the limit of detection for the HIV Combo assay, with regard to virus detection, is between 14,000 and 30,000 RNA copies/ml. This is consistent with recent estimates for this assay based on analysis of multiple cultured viral isolates (8). Additionally, these data provide further evidence that the HIV Combo nonreactive specimens are derived from individuals who were so recently infected with HIV at the time of collection that they had not yet mounted an antibody response. This supposition is substantiated by data obtained from follow-up specimens, available for five of the seven individuals, for which viral RNA levels dramatically increased between the first and second visit and antibodies to HIV had become detectable (data not shown).

The HIV Combo assay's ability to detect HIV antigen empowers the test with the capacity to detect recent HIV infections during the antibody-negative window period. In the present study, it detected approximately 89% of infected individuals who had been missed by an initial antibody screening test (Table 2). With regard to specimens from acutely infected individuals that were initially screened and missed by a third-generation IA, HIV Combo detected six out of seven (85.7%). For specimens from acutely infected individuals who were initially screened by a rapid, point-of-care test or first-generation IA, HIV Combo detected 51 of 57 (89.5%). Thus, the incremental yield in detection of recently infected individuals by HIV Combo relative to immunoassays currently used in the United States is substantial. These data, combined with the relatively low cost and lower labor requirements compared to RNA-based testing, argue that the fourth-generation IA will be a useful addition to available HIV diagnostic tests. This may be particularly true for regions where there exists some reasonable concern about the detection of recent, window period infections (i.e., high-risk, high-incidence populations). However, it should be recognized that performance of fourth-generation assays varies substantially with respect to p24 antigen sensitivity, antibody sensitivity, HIV group/subtype detection, and specificity (9, 14). Comparative evaluations have revealed that the HIV Combo assay is among the most sensitive for detection of p24 antigen across genetically divergent strains and has high specificity (14). Thus, viral RNA detection levels observed for HIV Combo likely are not directly translatable to all fourth-generation assays. For communities with high HIV incidence where a significant percentage of tested, infected individuals might be within the antibody-negative window period it is reasonable to consider that the Architect HIV Ag/Ab Combo, with automation and high throughput, may be an appropriate replacement for testing algorithms that combine HIV RNA testing with antibody screening.

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