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4 **Assessment of the Ability of a Fourth Generation Immunoassay for HIV Antibody**
5 **and p24 Antigen to Detect both Acute and Recent HIV Infection in a High-Risk**
6 **Setting**

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9 Mark W. Pandori¹, John Hackett Jr.² Brian Louie¹, Ana Vallari², Teri Dowling¹, Sally
10 Liska¹, Jeffrey D. Klausner^{1,3}

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- 13 1. San Francisco Department of Public Health, San Francisco, CA, USA
14 2. Abbott Diagnostics, Abbott Park, IL, USA
15 3. University of California at San Francisco, San Francisco, CA, USA

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18 Corresponding Author: Mark Pandori
19 San Francisco Department of Public Health
20 101 Grove St. Room 412
21 San Francisco, CA, 94102
22 Phone: (415) 554-2800
23 FAX: (415) 431-0651
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Abstract

An immunoassay (IA) that simultaneously detects both antibody to HIV and HIV p24 antigen (ARCHITECT® HIV Ag/Ab Combo) was evaluated for its ability to detect HIV infection using a panel of specimens collected from individuals recently infected with HIV-1. This IA was found to be capable of detecting the majority (89%) of infections, including 79.5% of those considered as 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 generation IAs as well as the capacity to detect acute infections have important implications for HIV prevention strategies.

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

1 documented (1, 3, 12-15, 21, 23, 26–31). However, given the paucity of data available
2 on performance of fourth generation assays relative to HIV RNA detection algorithms in
3 the diagnostic setting, it is of considerable interest from a public health perspective to
4 assess the ability of these assays to detect acute HIV infections.

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

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1 As shown in Table 1, 57 of the 64 specimens from recently-infected individuals
2 were found to be reactive in the HIV Combo assay. Amongst the 57 specimens found
3 reactive by the HIV Combo were 28 of the 35 previously determined to contain no
4 detectable HIV antibody by any other antibody-based method evaluated. These data
5 confirm the ability of the HIV Combo to detect HIV antigen and therefore provide a
6 reactive (positive) result, even when antibody to HIV is not detectable. Moreover, these
7 data demonstrate that the HIV Combo assay is able to identify acutely infected
8 individuals in the majority of the cases where antibody testing fails to do so. HIV Combo
9 detected all 7 specimens reactive on only one of the antibody tests as well as all 22
10 reactive by multiple but not all antibody tests. The HIV Combo assay found all of the
11 positive controls to be reactive and all negative controls to be non-reactive.

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13 Seven of the 64 panel members were non-reactive in the HIV Combo assay.
14 These specimens (A, E, G, H, J, N, and BJ) are notable because each was found to be
15 uniformly non-reactive for HIV antibody while being shown to contain measurable viral
16 RNA. Of interest, the HIV-1 RNA levels measured in these specimens were relatively
17 low (mean = 7,716 copies/ml; median = 6,373 copies/ml; range 1,177 to 14,062
18 copies/ml). Of the antibody-negative specimens detected by the HIV Combo, the lowest
19 RNA value was 30,734 copies/ml, while the highest RNA value for a specimen not
20 detected by HIV Combo was 14,062 copies/ml. These data suggest that the limit of
21 detection for the HIV Combo assay, with regard to virus detection, is between 14,000 and
22 30,000 RNA copies/ml. This is consistent with recent estimates for this assay based on
23 analysis of multiple cultured viral isolates (8). Additionally, these data provide further

1 evidence that the HIV Combo non-reactive specimens are derived from individuals who
2 were so recently infected with HIV at the time of collection, that they had not yet
3 mounted an antibody response. This supposition is substantiated by data obtained from
4 follow-up specimens, available for 5 of the 7 individuals, where viral RNA levels
5 dramatically increased between the first and second visit and antibodies to HIV had
6 become detectable (data not shown).

7

8 The HIV Combo assay's ability to detect HIV antigen empowers the test with the
9 capacity to detect recent HIV infections during the antibody-negative window period. In
10 the present study, it detected approximately 89% of infected individuals who had been
11 missed by an initial antibody-screening test (Table 2). With regard to specimens from
12 acutely infected individuals that were initially screened and missed by a third generation
13 IA, HIV Combo detected 6 out of 7 (85.7%). For specimens from acutely infected
14 individuals who were initially screened by a rapid, Point-of-care test or first generation
15 IA, HIV Combo detected 51 of 57 (89.5%). Thus, the incremental yield in detection of
16 recently-infected individuals by HIV Combo relative to immunoassays currently used in
17 the U.S. is substantial. These data, combined with the relatively low cost and lower labor
18 requirements as compared to RNA-based testing argue that fourth generation IA will be a
19 useful addition to available HIV diagnostic tests. This may be particularly true for
20 regions where there exists some reasonable concern about the detection of recent,
21 window period infections (i.e. high risk, high incidence populations). However it should
22 be recognized that performance of fourth-generation assays vary substantially with
23 respect to p24 antigen sensitivity, antibody sensitivity, HIV group/subtype detection, and

1 specificity (9, 14). Comparative evaluations have revealed that the HIV Combo assay is
2 among the most sensitive for detection of p24 antigen across genetically divergent strains
3 and has high specificity (14). Thus, viral RNA detection levels observed for HIV Combo
4 likely are not directly translatable to all fourth generation assays. For communities of
5 high HIV incidence where a significant percentage of tested, infected individuals might
6 be within the antibody negative window period it is reasonable to consider the possibility
7 that ARCHITECT HIV Ag/Ab Combo, with automation and high throughput, may be an
8 appropriate replacement for testing algorithms that combine HIV RNA testing with
9 antibody screening.

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11 **Tables**

12

13 **Table 1. Summary of assay performances with specimens from acutely and recently**
14 **infected individuals.**

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16 **Table 2. Percentages of acute/recent specimens from Table 1 found positive by**
17 **different assay methods.**

Table 1: Comparative HIV detection test results with acute infection panel

ID	Initial Test Screen	1st/2ND Gen. EIA S/C Avg	1st/2ND Gen. EIA Result	WB	3rd Gen. EIA S/C Avg	3rd Gen. EIA Result	OQ RT	UG RT	SR RT	MS RT	Viral Load copies/ml	4th Gen IA S/C	4th Gen IA Result
A	1st Gen EIA	0.351	NR	I	0.127	NR	N	N	N	N	5770†	0.37	NR
B	1st Gen EIA	0.602	NR	I	0.955	NR	N	N	N	N	≥500,000†	611.12	R
C	1st Gen EIA	0.440	NR	I	≥14.658	R	N	R	N	N	12183†	1.62	R
E	1st Gen EIA	0.368	NR	I	0.233	NR	N	N	N	N	6373†	0.65	NR
F	1st Gen EIA	0.329	NR	I	13.433	R	N	R	N	R1	≥500,000†	85.73	R
G	1st Gen EIA	0.317	NR	N	0.084	NR	N	N	N	N	12852†	0.74	NR
H	1st Gen EIA	0.338	NR	I	0.109	NR	N	N	N	N	14062†	0.68	NR
I	1st Gen EIA	0.646	NR	I	≥14.658	R	R	R	R	R1	≥500,000†	67.70	R
J	1st Gen EIA	0.358	NR	N	0.106	NR	N	N	N	N	3921†	0.23	NR
K	1st Gen EIA	0.346	NR	N	4.574	R	N	R	N	N	≥500,000†	43.92	R
L	1st 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	1st Gen EIA	0.337	NR	N	0.113	NR	N	N	N	N	1177†	0.21	NR
O	OQ RT-FS	0.301	NR	N	0.127	NR	N	N	N	N	≥500,000†	61.32	R
P	1st Gen EIA	0.755	NR	N	≥14.658	R	N	R	N	R1	≥500,000†	136.62	R
Q	1st Gen EIA	0.311	NR	N	0.277	NR	N	N	N	N	43173†	1.80	R
R	OQ RT-FS	0.642	NR	I	0.117	NR	N	N	N	N	30734†	2.05	R
S	1st 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	1st 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	1st Gen EIA	0.378	NR	N	0.201	NR	N	N	N	N	102288†	2.09	R
AA	1st Gen EIA	0.343	NR	N	0.327	NR	N	N	N	N	327333†	6.34	R
AB	1st 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	1st 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	389850†	12.24	R
AF	OQ RT-FS	0.761	NR	I	0.907	NR	N	R	N	R1	413186†	13.89	R
AG	3rd Gen EIA	0.436	NR	I	0.165	NR	N	N	N	N	446770†	19.21	R
AH	3rd Gen EIA	0.371	NR	N	0.195	NR	N	N	N	N	358030†	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	427490†	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	3rd Gen EIA	0.45	NR	N	9.634	R	N	N	N	N	≥500,000†	22.83	R
AP	3rd 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	3rd 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	3rd 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	3rd 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

1st Gen. EIA - BioMérieux Vironostika HIV-1 Microelisa
 2nd Gen. EIA - BioRad Genetic Systems rLAV HIV-1 EIA
 3rd Gen. IA - BioRad Genetic Systems HIV-1/2 plus O EIA
 4th Gen. IA - Abbott Architect HIV Ag/Ab Combo
 WB - BioRad Genetic Systems HIV-1 Western Blot
 OQ RT-OF - OraQuick Rapid Test - Oral Fluid
 OQ RT-FS - OraQuick Rapid Test - Fingertick
 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-SpotHIV-1/2 rapid test
 † viral load determined by Siemens Versant
 HIV-1 RNA v. 3.0 (otherwise tested by Abbott RealTime HIV-1)
 R - Reactive
 NR - NonReactive
 R1 - Reactive for HIV-1
 P - Positive
 N - Negative
 I - Indeterminate

Table 2: Comparative Test Method Detection Rates

Method	% Detected
1st/2nd 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
3rd Generation IA	42.2
4th Generation IA	89.1