Detection of Acute and Chronic HIV Infections by an HIV Antigen/Antibody Combination Assay

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Abstract

Background: Individuals with acute HIV infection (antibody (Ab)-negative) have a substantially elevated risk of transmission and represent an important driver of the ongoing epidemic. Reliable and cost-effective methods to detect acute infections have important implications for HIV prevention strategies. The ARCHITECT® HIV Ag/Ab Combo assay, in development for the US (not FDA approved), is a two-step chemiluminescent microparticle immunoassay for the simultaneous qualitative detection of HIV p24 antigen (Ag) and antibodies to HIV-1 groups M and O, and HIV-2.

Method: This study assessed the performance of the ARCHITECT HIV Ag/Ab Combo assay for specificity, sensitivity, and imprecision by testing specimens from diagnostic patients, blood donors, known Ab positive HIV-1 group M (N=500), O (N=65), and HIV-2 (N=125) infections, HIV-1 p24 Ag, and HIV seroconversion panels. In addition, we determined the viral RNA level corresponding to the cutoff of the Combo assay and assessed performance on genetically divergent HIV-1 strains.

Results: The ARCHITECT HIV Ag/Ab Combo assay demonstrated 100% Ab sensitivity with HIV-1 group M, O, and HIV-2 specimens. Compared to HIV Ab assays, the assay showed earlier detection for 8 of 10 seroconversion panels tested, reducing the seroconversion window by 0 to 9 days. HIV p24 Ag sensitivity was <20 pg/mL based on the AFSSAPS panel and no subtype-related differences in sensitivity were observed for 38 unique HIV-1 group M and O isolates. Observed specificity for the Combo assay was 99.55% (2460/2471) for diagnostic and 99.95% (1999/2000) for donor specimens. Total imprecision (within run, between run, and between day) ranged between 3.5% to 8.4%. An S/CO of 1.0 in the Combo assay corresponded to 13,000, 18,000, and 30,000 RNA copies/mL based on the AMPLICOR HIV-1 Monitor v1.5, VERSANT HIV-1 RNA 3.0, and RealTi*m*e HIV-1 assays, respectively.

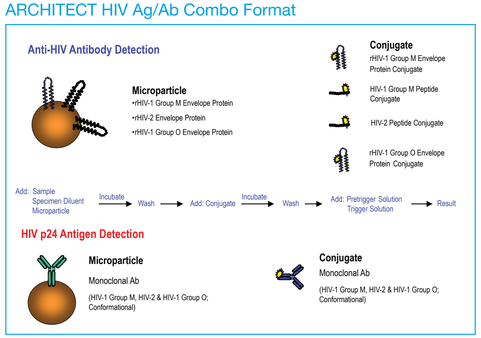
Conclusions/Implications: The ARCHITECT HIV Ag/Ab Combo assay demonstrated sensitive detection of HIV p24 antigen and antibodies across divergent HIV strains and earlier detection of acute infections than HIV Ab assays. With automation, individual sample testing, high throughput, and excellent specificity, the ARCHITECT HIV Ag/Ab Combo assay could provide a cost-effective alternative to pooled nucleic acid testing strategies for detection of acute HIV infections.

Background

Individuals with acute HIV infection (antibody (Ab)-negative) have a substantially elevated risk of transmission and represent an important driver of the ongoing epidemic. Reliable and cost-effective methods to detect acute infections have important implications for HIV prevention strategies.

The ARCHITECT HIV Ag/Ab Combo assay, in development for the US (not FDA approved), is a two-step chemiluminescent microparticle immunoassay for the simultaneous qualitative detection of HIV p24 antigen (Ag) and antibodies to HIV-1 groups M and O, and HIV-2 in human serum or plasma. The ARCHITECT HIV Ag/Ab Combo assay is intended to be used as an aid in the diagnosis of acute HIV-1/HIV-2 infection.

ARCHITECT HIV Ag/Ab Combo Format



Methods

Sensitivity

- Seroconversion Sensitivity
  - Evaluated by testing 10 commercially available seroconversion panels.
- Antigen Sensitivity
  - Quantitative antigen sensitivity assessed by testing the AFSSAPS (Agence française de sécurité sanitaire des produits de santé) HIV antigen panel (5 to 500 pg/mL).
  - Quantitative antigen sensitivity also assessed by testing an HIV-1 p24 antigen Clade panel derived from tissue culture of 38 unique HIV-1 group M and HIV-1 group O viral isolates diluted from 25 pg/mL to 2 pg/mL (quantitated relative to an Abbott internal standard).
  - Evaluated by testing 9 HIV antigen known positive specimens from Africa.

Methods (continued)

- Antibody Sensitivity
  - Evaluated by testing 500 HIV-1 group M subtype specimens: A, B, C, D, F, G, circulating recombinant forms (CRF), and unique recombinant forms (URF).
  - Evaluated by testing 65 HIV-1 group O specimens.
  - Evaluated by testing 125 HIV-2 specimens.

Specificity

- Evaluated by testing 2000 blood donor and 2500 diagnostic specimens.
- Initial reactive specimens were retested in duplicate after centrifugation. Repeat reactive specimens were tested with PCR and a rapid Ab test to confirm.

Imprecision

- Assessed by testing one reagent lot on one instrument over five days (2 runs per day; 4 replicates of each control and panel per run).

Comparison of Viral Load and S/CO

- Serial dilutions of 13 genetically divergent HIV-1 isolates were tested in the ARCHITECT HIV Ag/Ab Combo assay and quantified using Roche AMPLICOR HIV-1 Monitor v1.5, Bayer VERSANT HIV-1 RNA 3.0, and Abbott RealTi*m*e™ HIV-1.

Results

Seroconversion Sensitivity: Representative Panels (n=4)

Seroconversion Panel	Panel Member	Days Since First Bleed	ARCHITECT HIV Ag/Ab Combo S/CO	HIV Ab S/CO	HIV-1 p24 Ag S/CO	Western Blot	PCR
PRB944	1	0	0.31	0.1	0.0	NEG (no bands)	7000
	2	2	0.83	0.1	0.9	NEG (no bands)	80000
	3	7	20.70	0.1	10.9	NEG (no bands)	>800000
	4	9	14.19	0.6	12.6	NEG (no bands)	>800000
	5	14	12.34	11.7	8.7	IND (24)	60000
	6	16	24.04	14.4	3.3	POS (24, 160)	30000
PRB954	1	0	0.19	0.1	0.5	NEG (no bands)	BLD
	2	2	0.17	0.1	0.4	NEG (no bands)	BLD
	3	7	0.21	0.3	0.3	NEG (no bands)	BLD
	4	10	0.16	0.1	0.3	NEG (no bands)	1000
	5	14	0.79	0.1	0.3	NEG (no bands)	60000
	6	17	**6.80**	0.1	0.6	NEG (no bands)	600000
	7	21	**94.64**	**12.0**	**3.1**	NEG (no bands)	>800000
PRB955	1	0	0.25	0.1	0.3	IND (f24)	1000
	2	3	**1.83**	0.1	**1.8**	IND (f24)	70000
	3	7	**19.24**	0.2	**14.0**	IND (f24)	400000
	4	12	**33.52**	**2.6**	**22.4**	IND (f24)	>800000
	5	14	**35.67**	**>16.9**	**21.3**	IND (24)	700000
SV0321	1	1	0.55	.016	0.44	NEG	20000 RUO
	2	8	**6.97**	0.16	**5.19**	NEG	130000 RUO
	3	12	**6.06**	**8.47**	**2.26**	IND	70000 RUO
	4	15	**7.35**	**11.90**	0.26	IND	2000 RUO
	5	21	**29.18**	**6.70**	0.00	**POS**	1500 RUO

= S/CO> 1.0 = Grayzone

HIV Antigen Sensitivity – Determined Using the AFSSAPS HIV Antigen Panel

AFSSAPS Panel Member ID	HIV p24 Ag Concentration (pg/mL)	ARCHITECT HIV Ag/Ab Combo (S/CO)
2023	500	25.75
2024	250	12.57
2025	100	5.43
2026	50	2.90
2027	25	1.55
2028	10	0.92
2029	5	0.65
Slope 0.050, Y-Intercept 0.330, R^2 1.000		
Sensitivity (pg/mL) 13.27		

HIV Antigen Sensitivity – HIV-1 p24 Antigen Clade Panel

Derived from 38 HIV-1 group M and HIV-1 group O viral isolates diluted from 25 pg/mL to 2 pg/mL (quantitated relative to an Abbott internal standard).

Number of Panels Detected Positive at:	ARCHITECT HIV Ag/Ab Combo
2 pg/mL	5
5 pg/mL	33
10 pg/mL	38
25 pg/mL	38
Total Number of Panels Tested	38

Subtype	Number of Samples Included
A	2
A/G/G	1
B	7
B/A/B	1
C	6
D	4
F	4
G	1
CRF01 AE	8
CRF02 AG	2
Group O	2
Total	38

Results (continued)

HIV Antigen Sensitivity – HIV Antigen Known Positive Specimens from Africa (n=9)

Source	Subtype	HIV p24 EIA S/CO	ARCHITECT HIV Ag/Ab Combo S/CO
S. Africa	B	2.83	17.71
S. Africa	B	4.79, 4.03, 4.00	491.44
S. Africa	C	5.81	22.8
S. Africa	C	2.25	11.77
S. Africa	C	2.01	404.33
S. Africa	C	1.27	17.87
S. Africa	C	1.15	445.19
S. Africa	C	17.0, 14.3, 14.1	502.1
Cameroon	CRF02	NT*	31.2

* NT = Not Tested

HIV-1 Antibody Sensitivity – HIV-1 Group M Subtype (A, B, C, D, F, G, CRF, and URF) (n=565) and HIV-1 Group O (n=65) Specimens

Subtype	Number of Samples	ARCHITECT HIV Ag/Ab Combo	
		Not Reactive	Reactive
A	65	0	65
B	44	0	44
C	44	0	44
D	39	0	39
F	24	0	24
G	20	0	20
CRF01 AE	92	0	92
CRF02 AG	50	0	50
CRF09	1	0	1
CRF11	11	0	11
CRF13	4	0	4
URF	106	0	106
Group O	65	0	65
Total	**565**	**0**	**565**

HIV-2 Antibody Sensitivity – HIV-2 Specimens (n=125)

Number of Samples	ARCHITECT HIV Ag/Ab Combo	
	Not Reactive	Reactive
125	0	125

Specificity

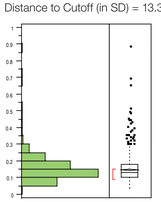
Donor and Diagnostic Populations

Population	Total	Negative	Initial Reactive (IR)[1]	% IR	Repeat Reactive (RR)[2]	% RR	Confirmed Positive[3]	Non-Confirmed RR[4]	% Specificity
Donor	2000	1999	1	0.05	1	0.05	0	1	**99.95% (1999/2000)**
Diagnostic	2500	2460	40	1.60	40	1.60	29	11	**99.55% (2460/2471)**

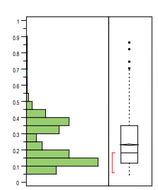
[1] Specimens with an initial result of ≥0.90 S/CO were centrifuged and retested in duplicate
[2] Specimens with 2 of 3 results ≥1.00 S/CO were considered repeat reactive and tested with PCR and a rapid Ab test
[3] Confirmed positive based on Ab and/or PCR reactivity
[4] Non-confirmed RR specimens were negative by PCR and the rapid Ab test

Donor and Diagnostic Population Distributions

Donor Population*
Serum and Plasma
Mean S/CO = 0.15; SD = 0.064
Distance to Cutoff (in SD) = 13.35



Diagnostic Population*
Serum and Plasma
Mean S/CO = 0.23; SD = 0.127



*Repeat reactive specimens not included in distributions

Imprecision

Sample	N	Mean	Within-Run SD	Within-Run % CV	Between-Run SD	Between-Run % CV	Between-Day SD	Between-Day % CV	Total[1] SD	Total[1] % CV
Calibrator[2]	40	5802	284.3	4.9	0.000	0.0	0.000	0.0	284.3	4.9
Negative Control	40	0.11	0.034	30.0	0.019	16.9	0.000	0.0	0.040	34.5
HIV p24 Ag Positive Control	40	3.22	0.173	5.4	0.084	2.6	0.000	0.0	0.193	**6.0**
HIV p24 Ag Grayzone Panel	40	0.90	0.046	5.1	0.037	4.1	0.048	5.3	0.075	**8.4**
HIV-1 Ab Positive Control	40	4.79	0.126	2.6	0.064	1.3	0.089	1.9	0.167	**3.5**
HIV-1 Ab Low Positive Panel	40	1.11	0.055	5.0	0.055	5.0	0.033	3.0	0.085	**7.7**
HIV-2 Ab Positive Control	40	4.22	0.144	3.4	0.112	2.7	0.088	2.1	0.203	**4.8**
HIV-2 Ab Low Positive Panel	40	1.13	0.064	5.7	0.060	5.3	0.015	1.3	0.089	**7.9**
HIV-1 Group O Ab Positive Control	40	2.54	0.084	3.3	0.084	3.3	0.067	2.6	0.136	**5.4**
Group O Ab Positive Panel	40	2.57	0.089	3.5	0.098	3.8	0.000	0.0	0.132	**5.1**
Group O Ab Grayzone Panel	40	0.95	0.052	5.5	0.043	4.5	0.042	4.4	0.080	**8.4**
HIV-1 gp41 Cluster I MAb Positive Panel	40	2.58	0.097	3.8	0.053	2.1	0.085	3.3	0.139	**5.4**
HIV-1 gp41 Cluster II MAb Positive Panel	40	1.93	0.128	6.6	0.000	0.0	0.073	3.8	0.147	**7.6**

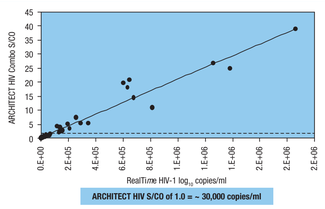
[1]Total imprecision includes within-run, between-run, and between-day
[2]Data for Calibrator based on RLUs since it is used to generate cutoff

Comparison of Viral Load and S/CO: Quantified RNA Panel

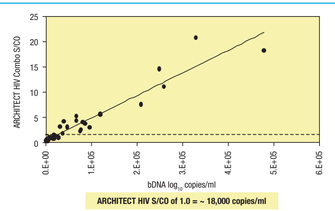
Virus Dilution	A* (UG95-327)	B (US1)	B (US3)	C (ZAM18)	C (ETH2220)	C (SE364)	D (UG270)	F (BZ162)	F (BZ163)	G (HH8793)	CRF01 (NP1465)	CRF02 (DJ258)	CRF02 (DJ263)
					ARCHITECT HIV Ag/Ab Combo (S/CO)								
Neat	39.0	3.6	2.1	18.2	14.5	19.6	2.9	5.4	3.0	11.0	20.8	24.9	26.8
1:5	7.5	0.8	0.6	3.9	3.0	4.3	0.7	1.3	0.8	2.4	4.1	5.6	5.2
1:25	1.7	0.3	0.2	0.9	0.8	0.9	0.3	0.4	0.3	0.7	0.9	1.3	1.3
1:125	0.4	0.2	0.1	0.3	0.3	0.3	0.2	0.2	0.2	0.3	0.4	0.5	0.4
					Viral Load (copies/ml): RealTi*m*e HIV-1, bDNA v3.0, Monitor v1.5								
Neat	1.9E6	2.1E5	1.3E5	6.3E5	6.8E5	6.0E5	1.5E5	3.5E5	1.4E5	8.1E5	6.4E5	1.4E6	1.2E6
Neat	>5E5	8.7E4	7.6E4	4.8E5	2.5E5	>5E5	9.7E4	1.2E5	4.8E4	2.6E5	3.3E5	>5E5	>5E5
Neat	>7.5E4	>7.5E4	5.6E4	6.9E4	>7.5E4	>7.5E4	7.3E4	>7.3E4	6.5E4	6.7E4	5.9E4	NA	>7.5E4
1:5	2.6E5	3.9E4	2.7E4	1.4E5	1.4E5	1.2E5	3.2E4	6.2E4	3.5E4	1.4E5	1.3E5	2.9E5	1.9E5
1:5	2.1E5	2.8E4	1.9E4	8.2E4	3.2E4	6.8E4	1.6E4	2.1E4	9.8E3	7.5E4	3.9E4	1.2E5	6.8E4
1:5	7.1E4	2.6E4	1.7E4	2.9E4	5.9E4	3.8E4	3.5E4	4.9E4	3.8E4	4.6E4	1.3E4	>7.5E4	6.9E4
1:25	6.8E4	7.6E3	5.5E3	1.9E4	3.2E4	2.2E4	7.6E3	1.5E4	6.4E3	1.9E4	2.1E4	5.5E4	3.6E4
1:25	3.8E4	5.3E3	3.8E3	1.1E4	7.2E3	1.8E4	2.8E3	5.4E3	2.9E3	1.8E4	7.2E3	2.1E4	1.8E4
1:25	5.1E4	5.3E3	8.6E3	1.3E4	2.1E4	1.2E4	4.5E3	2.3E4	8.3E3	1.3E4	4.6E3	2.2E4	1.2E4
1:125	1.2E4	1.2E3	1E3	4.4E3	5.9E3	4.5E3	1.6E3	2.7E3	1.2E3	5.0E3	4.8E3	8.1E3	8.5E3
1:125	7.3E3	1.1E3	8.2E2	2.9E3	1.7E3	3.4E3	9.0E2	1.2E3	5E2	3E3	2.3E3	2.4E3	2.9E3
1:125	1.1E4	1.2E3	1.5E3	3.9E3	3.7E3	4.6E3	6.9E2	4.9E3	1.0E3	3.4E3	1.2E3	5.9E3	4.8E3

*Group M subtype (Isolate ID)
Highlight = ARCHITECT HIV S/CO >1.0

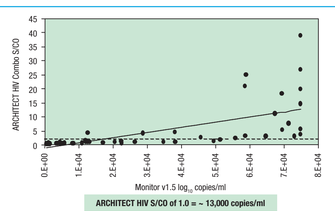
ARCHITECT HIV Ag/Ab Combo vs RealTi*m*e HIV-1: Quantified RNA Panel



ARCHITECT HIV Ag/Ab Combo vs bDNA v3.0: Quantified RNA Panel



ARCHITECT HIV Ag/Ab Combo vs Monitor v1.5: Quantified RNA Panel



Summary of Results

Seroconversion Sensitivity

- The ARCHITECT HIV Ag/Ab Combo showed earlier seroconversion detection compared to HIV antibody assays for 8 of the 10 seroconversion panels tested.
- The seroconversion window was reduced by up to 9 days based on the panels tested.

Antigen Sensitivity

- The quantitative antigen sensitivity against the AFSSAPS HIV-1 subtype B p24 quantitative antigen panel (5 to 500 pg/mL) was less than 20 pg/mL.
- Quantitative antigen sensitivity (relative to an Abbott internal standard) ranged from 2 to 10 pg/mL with the HIV-1 p24 antigen Clade panel derived from tissue culture of 38 unique HIV-1 group M and HIV-1 group O viral isolates.
- Sensitivity with HIV antigen positive specimens from Africa was 100%.

Antibody Sensitivity

- Antibody sensitivity with HIV-1 group M subtypes (A, B, C, D, F, G, CRF, and URF), HIV-1 group O and HIV-2 positive specimens was 100%.

Specificity

- Observed specificity for the assay in development for the U.S. was 99.55% for the diagnostic specimens (2460/2471; 29 confirmed HIV positive) and 99.95% for the donor specimens (1999/2000).

Imprecision

- Within-run percent coefficients of variation (%CV) ranged from 2.6 to 6.6%. Between-run and between-day %CVs ranged from 0.0 to 5.3%. Total imprecision %CVs (includes within-run, between-run, and between-day) ranged from 3.5 to 8.4% for Ag and Ab analytes at various levels.

Comparison of Viral Load and S/CO

- An S/CO of 1.0 in the ARCHITECT HIV Ag/Ab Combo assay corresponded to ~13,000, ~18,000, and ~30,000 RNA copies/mL based on the AMPLICOR HIV-1 Monitor v1.5, VERSANT HIV-1 RNA 3.0, and RealTime HIV-1 assays, respectively.

Conclusions/Implications

The ARCHITECT HIV Ag/Ab Combo assay demonstrated sensitive detection of HIV p24 antigen and antibodies across divergent HIV strains and earlier detection of acute infections than HIV Ab assays. With automation, individual sample testing, high throughput, and excellent specificity, the ARCHITECT HIV Ag/Ab Combo assay provides a cost-effective alternative for detection of acute and chronic HIV infections.

ASSAY IN DEVELOPMENT. NOT APPROVED BY FDA