ARCHITECT HIV Ag/Ab Combo Assay for Simultaneous Detection of HIV p24 Antigen and Anti-HIV-1 Group M and Group O and Anti-HIV-2 Antibody*

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*In development, not available for sale in the U.S.

Abstract

Background: Currently, there are no approved HIV assays on the market in the United States that allow for the simultaneous detection of HIV p24 antigen and anti-HIV immunoglobulin. HIV antigen/antibody combination assays allow clinicians to detect the presence of p24 antigen prior to seroconversion, providing for earlier detection of infection and improving the ability of laboratories to diagnose HIV infection compared with antibody-only assays. The ARCHITECT HIV Ag/Ab Combo assay, launched outside the U.S. in 2004, is currently being developed for approval in the U.S. The ARCHITECT HIV Ag/Ab Combo assay is a two-step chemiluminescent microparticle immunoassay (CMIA) for the simultaneous qualitative detection of HIV p24 antigen (Ag) and antibodies (Ab) to human immunodeficiency virus type 1 (HIV-1 group M and group O) and/or type 2 (HIV-2) in human serum or plasma.

Method: The purpose of this study was to evaluate the performance of the ARCHITECT HIV Ag/Ab Combo assay in development for specificity, sensitivity, and imprecision by testing: a) diagnostic specimens (n=2,500), b) blood donor specimens (n=2,000), c) known antibody positive specimens: HIV-1 group M subtypes (n=500) (A, B, C, D, F, G, circulating recombinant forms, and unique recombinant forms), HIV-1 group O (n=65), and HIV-2 (n=125), d) commercial HIV seroconversion panels (n=10), e) HIV-1 p24 antigen panels derived from HIV-1 group M and HIV-1 group O viral isolates (n=38), f) AFSSAPS (Agence française de sécurité sanitaire des produits de santé) HIV antigen panel and g) precision over 5 days.

Results: The ARCHITECT HIV Ag/Ab Combo assay demonstrated 100% antibody sensitivity with HIV-1 group M subtypes, HIV-1 group O and HIV-2 specimens. The assay also showed earlier seroconversion detection compared to HIV antibody assays for 8 of the 10 seroconversion panels tested. The seroconversion window was reduced by 0 to 9 days based on these 10 panels. HIV p24 antigen sensitivity was < 20 pg/mL based on the AFSSAPS HIV antigen panel and similar sensitivity was demonstrated with the panel of HIV-1 p24 antigen derived from tissue culture of 38 unique HIV-1 group M and HIV-1 group O viral isolates. Observed specificity was 99.55% for the diagnostic specimens (2,460/2,471; 29 confirmed HIV positive) and 99.95% for the donor specimens (1,999/2,000). Total imprecision (within run, between run, and between day %CV) ranged between 3.5% to 8.4% for Ag and Ab analytes at various levels.

Conclusion: The ARCHITECT HIV Ag/Ab Combo assay provides HIV antigen and antibody detection in a single test on an automated, high throughput random-access analyzer. The assay demonstrates excellent specificity, improved sensitivity, and reduced the seroconversion window, compared to HIV antibody assays, by up to 9 days based on the seroconversion panels tested.

Background

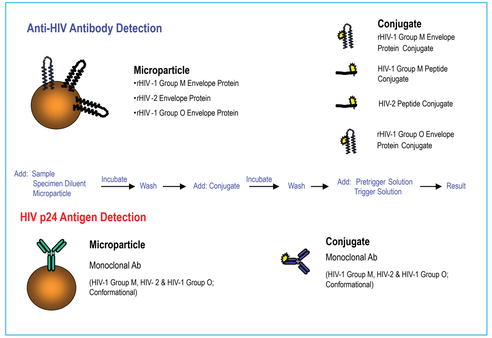
Currently, there are no approved HIV assays on the market in the United States that allow for the simultaneous detection of HIV p24 antigen and anti-HIV immunoglobulin. HIV antigen/antibody combination assays allow clinicians to detect the presence of p24 antigen prior to seroconversion, providing for earlier detection of infection and improving the ability of laboratories to diagnose HIV infection compared with antibody-only assays. The ARCHITECT HIV Ag/Ab Combo assay, launched outside the U.S. in 2004, is currently being developed for approval in the U.S.

The ARCHITECT HIV Ag/Ab Combo assay is a two-step chemiluminescent microparticle immunoassay (CMIA) for the simultaneous qualitative detection of HIV p24 antigen (Ag) and antibodies (Ab) to human immunodeficiency virus type 1 (HIV-1 Group M and Group O) and/or type 2 (HIV-2) in human serum and 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.

E000250

Methods and Procedures

ARCHITECT HIV Ag/Ab Combo Format



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.
- 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 2,500 diagnostic specimens.
- Evaluated by testing 2,000 blood donor 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).

Results

Seroconversion Sensitivity: Commercial Panels (n=10)

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
ANT-9013	1	0	0.06	0.342	0.09	p51(vf) p66(f)	<50
	2	7	0.07	0.171	0.06	p51(vf) p66(f)	<50
	3	9	0.07	0.171	0.09	p51(vf) p66(f)	<50
	4	14	0.07	0.171	0.08	p51(vf) p66(f)	<50
	5	18	0.09	0.171	0.09	p51(vf) p66(f)	58
	6	23	**0.93**	0.171	**1.56**	p51(vf) p66(f)	56350
	7	25	**3.94**	0.171	**7.84**	p51(vf) p66(f)	185800
ANT-9032	1	0	0.11	0.35	0.191	No Bands	<50
	2	2	0.06	0.21	0.391	No Bands	<50
	3	7	0.07	0.22	0.191	No Bands	<50
	4	10	0.08	0.23	0.330	No Bands	<50
	5	14	0.07	0.23	0.183	No Bands	134
	6	17	0.08	0.23	0.148	No Bands	1507
	7	22	0.47	0.30	0.557	No Bands	29006
	8	24	**0.91**	**2.24**	0.744	No Bands	40815
	9	29	**1.33**	**6.56**	0.348	p24	20395
	10	36	**1.47**	**2.19**	0.157	p24, p65	2425
	11	38	**1.26**	**2.00**	0.148	p24, p65	3161
	12	49	**1.32**	**3.17**	0.191	p24, p65	4052
	13	51	**1.35**	**3.89**	0.183	**p24, p65, gp160**	4967
	14	56	**1.96**	**5.49**	0.148	**p24, p65, gp160**	1377
PRB941	1	0	0.09	0.1	0.0	NEG (no bands)	BLD
	2	4	0.14	0.1	0.0	NEG (no bands)	3000
	3	9	0.40	0.2	**1.4**	NEG (no bands)	50000
	4	18	**8.97**	**15.9**	**2.3**	IND (24)	70000
	5	21	**8.18**	**11.3**	0.2	IND (24)	10000
	6	25	**7.16**	**6.7**	0.1	IND (24)	900
SV-0401	1	0	0.08	0.26	0.27	IND (p24)	4035
	2	4	0.31	0.33	0.48	IND (p24)	10090
	3	7	**1.52**	0.29	**2.57**	IND (p24)	63770
	4	11	**8.62**	**2.29**	**9.36**	IND (p24)	207600
	5	14	**15.38**	**6.26**	**12.06**	IND (p24)	435900
	6	18	**14.59**	**7.21**	**2.42**	IND (p24)	52200
	7	22	**30.91**	**4.91**	**1.47**	IND (p24)	53760
PRB944	1	0	0.31	0.1	0.0	NEG (no brands)	7000
	2	2	0.83	0.1	**0.9**	NEG (no brands)	80000
	3	7	**20.70**	0.1	**10.9**	NEG (no brands)	>800000
	4	9	**14.19**	0.6	**12.6**	NEG (no brands)	>800000
	5	14	**12.34**	**11.7**	**8.7**	IND (24)	600000
	6	16	**24.04**	**14.4**	**3.3**	**POS (24, 160)**	300000
PRB952	1	0	0.09	0.2	0.2	NEG (no brands)	BLD
	2	7	0.44	0.2	0.5	NEG (no brands)	5000
	3	10	**11.81**	0.2	**7.5**	NEG (no brands)	300000
	4	14	**4.24**	**1.0**	**2.3**	IIND (24)	100000
	5	17	**3.30**	**6.2**	**1.1**	**POS (24, 41, 51, f160)**	100000
	6	21	**4.09**	**5.4**	0.4	**POS (24, 41, 51, 160)**	50000
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
SV0404	1	0	0.41	0.41	0.45	IND (p24)	2326
	2	8	0.42	**0.94**	0.48	IND (p24)	2672
	3	11	0.38	0.36	0.56	IND (p24)	3079
	4	15	0.48	0.40	0.52	IND (p24)	7190
	5	18	**1.51**	0.32	**1.48**	IND (p24)	54380
	6	22	**6.85**	**1.00**	**4.84**	IND (p24)	2798000

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 First Detected Positive at:	ARCHITECT HIV Ag/Ab Combo
2 pg/mL	5
5 pg/mL	28
10 pg/mL	5
25 pg/mL	0
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

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

		ARCHITECT HIV Ag/Ab Combo	
Subtype	Number of Samples	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 0	65	0	65
Total	**565**	**0**	**565**

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

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

Specificity

Donor and Diagnostic Populations

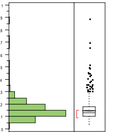
Population	Total	Negative	Initial Reactive (IR)[1]	% IR	Repeat Reactive (RR)[2]	% RR	PCR Positive	Rapid Antibody Test (MOZN) Positive	Non-Confirmed RR[3]	% Specificity
Donor	2000	1999	1	0.05	1	0.05	0	0	1	**99.95% (1999/2000)**
Diagnostic	2500	2460	40	1.60	40	1.60	11	29	11	**99.55% (2460/2471)**

[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] Non-confirmed RR specimens were negative by PCR and the rapid Ab test

Donor and Diagnostic Population Distributions

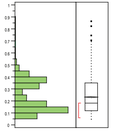
Donor Population*

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

Assay Imprecision

			Within-Run		Between-Run		Between-Day		Total[1]	
Sample	N	Mean	SD	% CV	SD	% CV	SD	% CV	SD	% 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

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 13.27 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 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.

Conclusion

The ARCHITECT HIV Ag/Ab Combo assay provides HIV antigen and antibody detection in a single test on an automated, high throughput random-access analyzer. The assay demonstrates excellent specificity, improved sensitivity, and reduced the seroconversion window, compared to HIV antibody assays, by up to 9 days based on the seroconversion panels tested.

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