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Performance of the ADVIA Centaur HIV Ag/Ab Combo (CHIV) Assay for the Detection of p24 Antigen and HIV Types, Subtypes, and Circulating Recombinant Forms

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Abstract

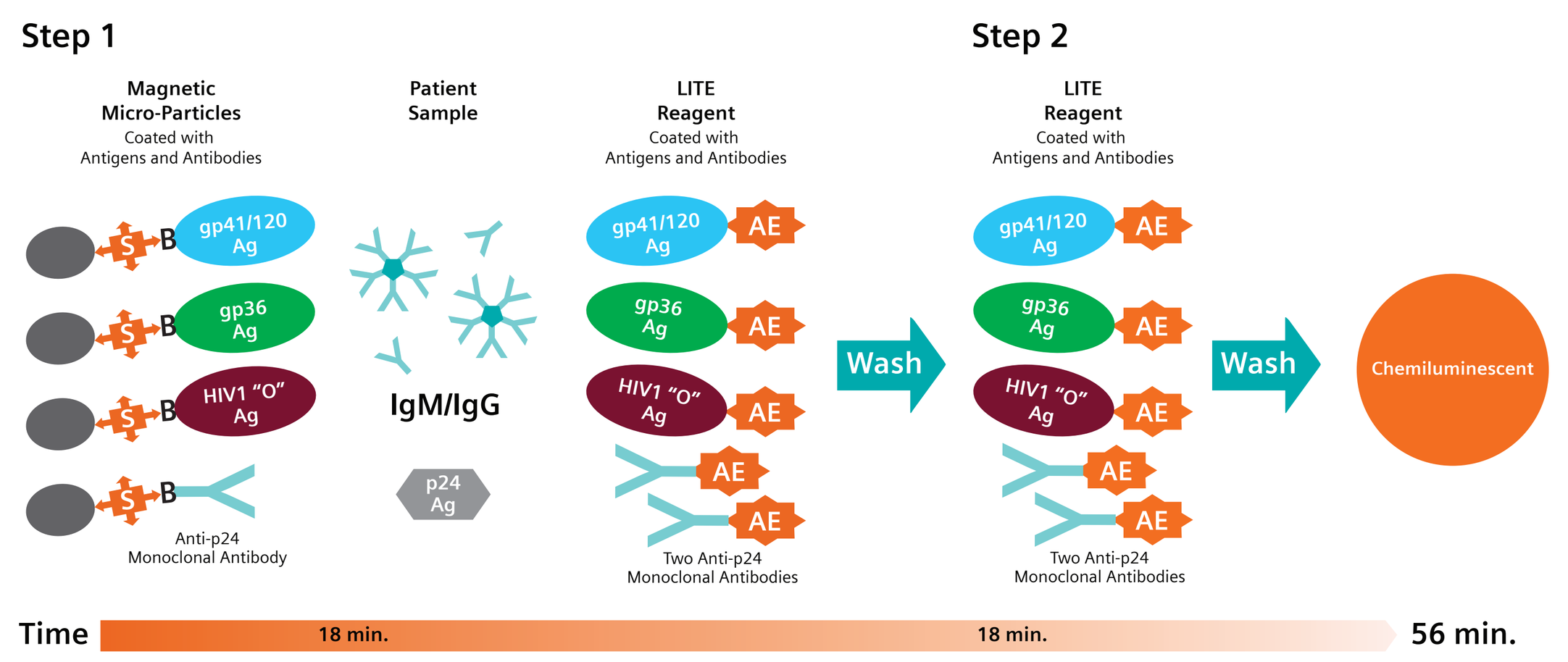
Objective: The ability of the ADVIA® Centaur HIV Ag/Ab Combo (CHIV) assay to detect p24 antigen and common and less-frequent circulating HIV antibody subtypes was assessed. The assay is designed to simultaneously recognize human immunodeficiency virus (HIV) antibody and HIV p24 antigen.

Methods: p24 sensitivity (IU/mL) was evaluated by diluting the NIBSC HIV-1 p24 Antigen 1st International Reference Reagent in HIV-negative serum as per WHO specifications. Regression analysis was performed and sensitivity calculated. Additionally, p24 sensitivity was determined and reported in pg/mL by diluting a quantified p24 standard of a research-use-only quantitative p24 EIA. Genotype sensitivity was evaluated using 45 specimens known to be infected with subtypes (clades) descended from either the HIV-1 group M or group O genotype. HIV-infected viral lysates (n=48), including HIV-1 strains Mn, BA-L, and IIB; HIV-2 strain NIH-Z; and a purified HIV-1 p24 native antigen sample were tested. In addition, 1102 HIV-1 and 103 HIV-2 samples and seroconversion panels were also tested with the CHIV assay.

Results: The observed mean analytical sensitivity of HIV-1 p24 across five lots of CHIV on the ADVIA Centaur was 1.05 IU/mL. Quantitation of p24 in pg/mL was determined with a standard from a Zeptometrix (Buffalo, NY) HIV p24 EIA Kit. Sensitivity to the p24 antigen quantified using the EIA standard was determined to be 9.04 pg/mL. All known-positive samples showed reactivity by the CHIV assay, resulting in 100% (1298/1298) sensitivity. All HIV-infected viral lysates, including group M subtypes (A, B, C, D, F, G), group O, and circulating recombinant forms (CRFs) AE and AG, as well as HIV-2 strain NHIZ, tested reactive on the CHIV assay. The CHIV assay seroconversion sensitivity on all panels tested was equivalent to the reference methods as per vendor certificate of analysis.

Conclusion: The ADVIA Centaur HIV Ag/Ab Combo (CHIV) assay is a reliable, accurate method to simultaneously detect the presence of both HIV p24 antigen and HIV antibodies, including common and less frequent circulating forms.

Figure 1: Principle of the assay



Background

Human immunodeficiency virus type 1 (HIV-1) has been identified as the primary cause of acquired immunodeficiency syndrome (AIDS). This retrovirus, a member of the Lentivirinae subfamily, is spread by sexual contact, exposure to infected blood or blood products, and perinatal transmission. In 1986, human immunodeficiency virus type 2 (HIV-2) was isolated from AIDS patients in West Africa. These viruses share epitopes of the core proteins, but exhibit little or no cross-reactivity between the envelope glycoproteins.[1,2]

Comparison of the nucleic acid sequences for HIV-1 and HIV-2 shows approximately 60% homology in the conserved genes, such as gag and pol (encoding core proteins), and 30% to 40% homology in less conserved regions (encoding envelope proteins). HIV-1 has been subdivided into group M (subtypes A–H) and group O.[3]

The routes of transmission of HIV-1 and HIV-2 are the same; however, the transmission and viral replication rate, and therefore disease progression, are much lower in HIV-2 infections. In HIV-2 infections there is a slower rate in the decline of CD4 T cells and reduced viremia. Individuals infected with HIV-2 generally have a better clinical outcome.[1,4]

The ADVIA Centaur HIV Ag/Ab Combo (CHIV) assay uses yeast-derived recombinant antigens corresponding to the viral envelope proteins: an HIV-1 envelope protein (gp41/120) and an HIV-2 envelope protein (gp36). A synthetic peptide is added for the detection of antibodies to HIV-1 group O. The assay uses three monoclonal antibodies specific to HIV p24 antigen to capture and detect HIV p24 antigen in a sample.

Methods

HIV p24 Antigen Sensitivity (WHO) (Table 1)

- The p24 antigen WHO International Standard (09/636) from NIBSC was reconstituted in 1 mL of diH2O to an assigned potency of 1000 International Units (IU) per mL.
- The reconstituted standard was further diluted with HIV-negative plasma pool to target a range of 0 to 20 IU/mL.
- Each dilution was assayed as an unknown on the ADVIA Centaur system(s) with five ADVIA Centaur CHIV assay lots.
- ADVIA Centaur Index values were plotted against the calculated values of each sample based on the known dilution factor.
- p24 antigen sensitivity was calculated as IU/mL at an index of 1.00 based on the linear regression of the dilution series.

HIV p24 Antigen Sensitivity (Recombinant) (Table 2)

- Detergent-disrupted, heat-inactivated viral antigen from HIV-1 IIIB was commercially obtained from Zeptometrix Corporation (Buffalo, NY).
- The viral antigen was diluted with HIV-negative plasma pool to target a range of 0 to 25 pg/mL.
- Each dilution was assayed as an unknown on the ADVIA Centaur system(s) with five ADVIA Centaur CHIV assay lots.
- ADVIA Centaur Index values were plotted against the calculated values of each sample based on the known dilution factor.
- p24 antigen sensitivity was calculated as pg/mL at an index of 1.00 based on the linear regression of the dilution series.

Genotype Sensitivity (Table 3 and 4)

- 45 worldwide specimens known to be infected with subtypes (clades) were sourced from various commercial vendors.
- The following subtypes from HIV-1 group M clades A, B, C, D, E, F, G, H, J, K, AE, and AG were tested.
- Five samples from the HIV-1 group O genotype were tested.
- All samples were tested with a single ADVIA Centaur CHIV assay lot.
- Samples with Index values ≥1.0 with the ADVIA Centaur CHIV assay were considered positive.

Viral Lysate Sensitivity (Tables 5 and 6)

- 48 HIV-infected viral lysates were tested with the ADVIA Centaur CHIV assay.
- 43 of the 48 viral lysates representing subtypes A, B, C, D, F, G, AE, AG, and O were assayed without dilution.
- Included in the 48 samples were three strains of HIV-1 viral lysates (Mn, BA-L, and IIB), a single strain of HIV-2 viral lysate (NIH-Z), and a single sample of purified HIV-1 p24 native antigen.
- All samples were tested with a single ADVIA Centaur CHIV assay lot.
- Samples with Index values ≥1.0 with the ADVIA Centaur CHIV assay were considered positive.

Seroconversion Sensitivity (Table 7)

- Fifteen commercially available seroconversion panels were divided among three ADVIA Centaur testing sites and were tested with the ADVIA Centaur CHIV assay.
- Reference testing was performed with a reference assay for comparison.
- Testing at all sites was performed in singleton and reported as such.
- Samples with Index values ≥1.0 with the ADVIA Centaur CHIV assay were considered positive.

HIV-1 / HIV-2 Sensitivity (Table 8)

- A total of 1102 characterized HIV type 1 and 103 HIV type 2 positive samples obtained from various vendors were assayed using two separate lots of the ADVIA Centaur CHIV assay.
- Sensitivity was calculated as percent agreement between the ADVIA Centaur CHIV assay and either the vendor certificate of analysis (CoA) and/or R&D HIV-2 screening results.

Results

Table 1. Calculated p24 antigen sensitivity of the ADVIA Centaur CHIV assay, relative to the WHO HIV p24 Ag International Standard (09/636) from NIBSC, ranged from 0.74 to 1.39 IU/mL, with an average of 1.05 IU/mL.

CHIV (CO=1.0)	Sensitivity Equivalent to NIBSC in IU/mL	
	ADVIA Centaur	R^2
Lot 1	1.07	0.99
Lot 2	1.39	0.99
Lot 3	1.04	0.99
Lot 4	1.00	0.99
Lot 5	0.74	0.99
Average (mean)	**1.05**	**NA**

Table 2. Calculated p24 antigen sensitivity of ADVIA Centaur CHIV assay, relative to the commercially available Zeptometrix p24 Ag standard, ranged from 6.1 to 11.4 pg/mL, with a mean of 9.04 pg/mL.

CHIV (CO=1.0)	Sensitivity Equivalent to Zeptometrix p24 in pg/mL	
	ADVIA Centaur	R^2
Lot 1	10.4	0.99
Lot 2	11.4	0.99
Lot 3	8.60	0.99
Lot 4	8.70	0.99
Lot 5	6.10	0.99
Average (mean)	**9.04**	**NA**

Table 3. A total of 45 HIV-positive samples representing the indicated genotypes were tested with the ADVIA Centaur CHIV assay. All viral lysates were positive compared to the CoA. Genotype K was not available in these samples.

HIV-1 Subtype	Number Tested	Number Reactive
A	2	2
B	2	2
C	3	3
D	3	3
E	4	4
F	4	4
G	4	4
H	1	1
J	1	1
A1	2	2
F2	2	2
O	5	5
CRF01_AE	4	4
CRF02_AG	4	4
CRF06	2	2
CRF11	1	1
CRF13	1	1
Total	**45**	**45**

Table 4. Individual results of 45 HIV-positive samples by the ADVIA Centaur CHIV assay representing the indicated genotypes, compared to the CoA.

Panel Member	Genotype	ADVIA Centaur	VendorCoA
		CHIV Index	Anti-HIV1/2
WWRB304-01	A	>12	Reactive
WWRB304-02	A	11.4	Reactive
9249313	A1	>12	Reactive
9249722	A1	11.3	Reactive
WWRB304-03	B	>12	Reactive
WWRB304-04	B	>12	Reactive
9249734	C	>12	Reactive
WWRB304-05	C	5.2	Reactive
WWRB304-06	C	>12	Reactive
9249721	CRF01 (AE)	>12	Reactive
9249728	CRF01 (AE)	>12	Reactive
WWRB304-07	CRF01 (AE)	11.2	Reactive
WWRB304-08	CRF01 (AE)	>12	Reactive
9249731	CRF02 (AG)	11.7	Reactive
9249736	CRF02 (AG)	>12	Reactive
WWRB304-09	CRF02 (AG)	>12	Reactive
WWRB304-10	CRF02 (AG)	>12	Reactive
9249316	CRF06	>12	Reactive
9249732	CRF06	>12	Reactive
9249318	CRF11	10.4	Reactive
9249310	CRF13	>12	Reactive
9249725	D	>12	Reactive
9249730	D	>12	Reactive
WWRB304-11	D	>12	Reactive
WWRB301-06	E	>12	Reactive
WWRB301-12	E	3.8	Reactive
WWRB301-34	E	1.2	Reactive
WWRB301-40	E	7.4	Reactive
9249726	F	>12	Reactive
9249729	F	>12	Reactive
WWRB304-12	F	>12	Reactive
WWRB304-13	F	>12	Reactive
9249312	F2	>12	Reactive
9249724	F2	6.0	Reactive
9249723	G	>12	Reactive
9249727	G	>12	Reactive
WWRB304-14	G	>12	Reactive
WWRB304-15	G	>12	Reactive
WWRB304-16	H	>12	Reactive
WWRB304-17	J	7.9	Reactive
9243004	O	>12	NA
PRD301-01	O	1.6	Reactive
PRD301-02	O	4.9	Reactive
PRD301-03	O	1.2	Reactive
PRD301-04	O	1.1	Reactive

Table 5. A total of 48 HIV viral lysates representing the indicated genotypes were tested with the ADVIA Centaur CHIV assay and were positive compared to the CoA.

Viral Lysate Subtype	Number Tested	Number positive
A	2	2
B	10	10
C	7	7
D	3	3
F	4	4
G	2	2
AE	10	10
AG	3	3
O	2	2
HIV-1 IIIB	2	2
HIV-1 strain Mn & BA-L	2	2
HIV-2 NIHZ	1	1
Total	**48**	**48**

Table 6. Individual results of the 48 HIV viral lysates representing the indicated genotypes comparing the ADVIA Centaur CHIV assay to the CoA.

Vendor CoA			ADVIA Centaur CHIV Assay	
Diluted Viral Lysates				
Sample ID	HIV1 Subtype	Dilution	Dose	Sensitivity
HIV-1 BA-L	B	1:10	>12	100%
HIV-1 IIIB	B	1:5000	>12	100%
Purified p24 (IIIB)	B	1:50000	>12	100%
HIV-1 Mn	B	1:10	>12	100%
HIV-2 NIHZ	NA	1:5000	>12	100%
Undiluted Viral Lysates				
Sample ID	HIV1 Subtype	Viral Particle Counts (/ml)	Dose	Sensitivity
BV-5039P	A	1.4E+09	>12	100%
BV-5047p	A	3.1E+08	>12	100%
BV-5006P	AE	2.0E+08	>12	100%
BV-5008P	AE	2.5E+08	>12	100%
BV-5016P	AE	3.3E+09	>12	100%
BV-5017P	AE	4.2E+08	>12	100%
BV-5021P	AE	5.6E+08	>12	100%
BV-5023P	AE	4.8E+08	>12	100%
BV-5027P	AE	3.6E+08	>12	100%
BV-5029P	AE	3.9E+08	>12	100%
BV-5035P	AE	1.0E+08	>12	100%
BV-5038P	AE	2.3E+08	>12	100%
BV-5005P	AG	3.3E+07	>12	100%
BV-5036P	AG	8.6E+08	>12	100%
BV-5043P	AG	2.4E+08	>12	100%
BV-5009P	B	2.9E+08	>12	100%
BV-5022P	B	1.3E+08	>12	100%
BV-5028P	B	3.0E+08	>12	100%
BV-5030P	B	1.9E+09	>12	100%
BV-5032P	B	1.0E+08	>12	100%
BV-5041P	B	3.3E+08	>12	100%
BV-5044P	B	7.4E+08	>12	100%
BV-5045P	B	1.6E+08	>12	100%
BV-5019p	B	2.6E+08	>12	100%
BV-5040p	B	1.0E+07	>12	100%
BV-5007P	C	2.7E+08	>12	100%
BV-5010P	C	7.0E+08	>12	100%
BV-5013P	C	8.3E+08	>12	100%
BV-5020P	C	2.2E+08	>12	100%
BV-5025P	C	7.1E+08	>12	100%
BV-5037P	C	2.5E+08	>12	100%
BV-5048P	C	3.9E+09	>12	100%
BV-5004P	D	1.9E+08	>12	100%
BV-5014P	D	1.1E+08	>12	100%
BV-5015P	D	1.7E+08	>12	100%
BV-5012P	F	1.3E+08	>12	100%
BV-5031P	F	1.0E+08	>12	100%
BV-5034P	F	1.4E+08	>12	100%
BV-5042P	F	1.4E+07	>12	100%
BV-5046P	G	2.9E+08	>12	100%
BV-5011P	G	4.8E+08	>12	100%
BV-5003P	O	4.1E+08	>12	100%
BV-5024P	O	3.1E+08	>12	100%

Table 7. Seroconversion panel testing results. The data of the first reactive results were compared for the reference assay and ADVIA Centaur HIV Ag/Ab Combo (CHIV) Assay. If the first reactive test result occurred on the same day, then the difference is 0; if the ADVIA Centaur CHIV assay had an earlier date, then the difference is positive; if the ADVIA Centaur CHIV assay had a later date, then the difference is negative.

Panel ID	Number of Panel Members Tested	Number of Reactive Panel Members		Days to First Reactive Result		Difference in Days to First Reactive Result (Based on Bleed Date)
		ADVIA Centaur CHIV Assay	Reference Assay	ADVIA Centaur CHIV Assay	Reference Assay	
PRB926	6	4	2	7	27	20
PRB940	8	7	7	7	7	0
PRB942	4	1	0	14	0†	0†
PRB943	7	4	2	12	19	7
PRB946	4	2	0	7	0†	0†
PRB948	4	1	0	23	0†	0†
PRB954	7	2	1	17	21	4
PRB955	5	3	1	7	14	7
PRB956	5	2	1	47	50	3
PRB960	9	2	0	28	–†	–†
PRB961	9	2	0	27	–†	–†
PRB962	6	2	0	14	–†	–†
PRB963	7	2	0	17	–†	–†
PRB964	6	1	0	22	–†	–†
PRB966	10	3	2	44	48	4
Total		**38**	**16**			

†All bleeds in these panels were nonreactive with the reference assay.

Table 8. A total of 1205 (1102 HIV-1, 103 HIV-2) samples were tested with the ADVIA Centaur CHIV assay. All viral lysates were positive compared to the CoA or internal R&D screening results. Total HIV-1 and HIV-2 sensitivity was calculated as 100%.

HIV Types	Sample Sources	HIV Positive (COA)	CHIV Positive	Sensitivity
HIV-1	Montefiore	704	704	100% (704/704)
HIV-1	Baystate	398	398	100% (398/398)
HIV-2	Montefiore	51	51	100% (51/51)
HIV-2	SeraCare	52	52	100% (52/52)
Total		**1205**	**1205**	**100% (1205/1205)**

Conclusion

- The ADVIA Centaur HIV Ag/Ab Combo (CHIV) assay detected all the HIV-positive samples and viral lysates tested, encompassing multiple HIV genotypes.
- Linear regression comparing the ADVIA Centaur CHIV assay to the WHO HIV p24 Ag International Standard (09/636) yielded a mean p24 Ag sensitivity of 1.05 IU/mL across multiple lots.
- Linear regression comparing the ADVIA Centaur CHIV assay to a commercially available p24 Ag standard yielded an average p24 Ag sensitivity of 9.04 pg/mL across multiple lots.
- Overall, the ADVIA Centaur CHIV assay detected more reactive bleeds (n=38) than the reference assay across the tested seroconversion panels.
- The ADVIA Centaur CHIV assay showed very good sensitivity on the HIV-1 and HIV-2 populations tested.

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