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Performance Characteristics of the ADVIA Centaur HIV Ag/Ab Combo (CHIV) Assay for the Simultaneous Qualitative Detection of HIV p24 Antigen and Antibodies to HIV-1 (Groups M and O) and HIV-2 in Human Serum or Plasma

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Abstract

Objective: The automated ADVIA Centaur® HIV Ag/Ab Combo (CHIV) assay* is designed to simultaneously detect both antibody to human immunodeficiency virus (HIV) and HIV p24 antigen on the ADVIA Centaur systems. This study evaluated the sensitivity and specificity of the ADVIA Centaur CHIV assay.

Methods: The diagnostic sensitivity of the CHIV assay was evaluated with 728 HIV-positive samples and specificity was determined by testing 9191 unique random donor samples. The results were reported in Index values as reactive (Index ≥ 1.0) or nonreactive (Index < 1.0). A total of 326 samples from 35 disease groups of potential cross-reactants were tested on the CHIV assay. Forty-eight HIV-infected viral lysate (antigen) isotypes that included isotypes A, B, C, D, F, G, O, AE, and AG were tested on CHIV for reactivity. Forty-five commercially available HIV seroconversion panels were tested. HIV-1 p24 Antigen (1st International Reference Reagent) was used to evaluate analytical sensitivity to the p24 antigen. Precision was evaluated in a study involving 20 days, two runs per day.

Results: All the positive samples showed reactivity by the CHIV assay, resulting in 100% (728/728) sensitivity. Specificity determined by testing 9191 unique random donor samples was 99.81% (9171/9188). Specificity calculation was based on the 9188 confirmed negative samples remaining after removal of 3 confirmed positive samples. Testing of the potentially cross-reactive samples (n = 326) on the CHIV assay yielded no reactive samples. All HIV-infected viral lysates (n = 48), including isotypes A, B, C, D, F, G, O, AE, and AG, as well as HIV-2 strain NHIZ, tested reactive on the CHIV assay. The seroconversion sensitivity of the CHIV assay on all 45 panels tested was equivalent to that of the reference methods as per vendor certificate of analysis. The observed mean analytical sensitivity of HIV-1 p24 antigen across three lots on the ADVIA Centaur system was 1.17 IU/mL. The CHIV assay had a within-run CV of <10% and a total CV of <12%.

Conclusions: The results of this study show that the ADVIA Centaur HIV Ag/Ab Combo (CHIV) assay is a reliable and accurate, fully automated qualitative method to simultaneously detect the presence of both HIV p24 antigen and HIV antibodies in human serum or plasma.

*Not available for sale in the U.S. This assay is CE marked. ADVIA Centaur HIV Ag/Ab Combo assay is developed, manufactured, and sold by Siemens Healthcare Diagnostics Inc. for Ortho-Clinical Diagnostics Inc.

Background

Human immunodeficiency virus is the causative agent of acquired immunodeficiency syndrome (AIDS). AIDS was first described in the United States in 1981 and has become one of the leading causes of death worldwide. Although the percentage of people living with HIV globally has stabilized since 2000, the overall number of people infected with HIV has steadily increased to an estimated 33.3 million in 2009.[1]

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 subfamily Lentivirinae, 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.[2,3] 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).

Both HIV-1 and HIV-2 viruses can be classified into different groups according to origin. The different groups of HIV-1, namely M, N, O, and P), and of HIV-2, that is A through H, are the results of cross-species transmission events from different primate sources in West Central Africa. Classification of each HIV strain has aided in tracking the spread of the HIV virus. The HIV-2 virus has been mainly restricted to West Africa, where the two variants are primarily the ones represented.[1,4] However, HIV-2 infections have been identified in North America and Europe at a low frequency.[5–8] While the HIV-1 group O is primarily endemic to Cameroon, where it represents about 1% of HIV infections, there have been documented infections in Europe and the U.S.[9,10] Additionally, the HIV-1 group O sequence is highly diverse, impacting its diagnosis. Of the classified HIV groups, only the HIV-1 group M, of which there are nine genetic subtypes (A–D, F–H, J, and K), have spread across Africa and to all the other continents.

Early diagnosis is essential for optimal outcomes in patients infected with either HIV-1 or HIV-2 because it facilitates timely initiation of appropriate care and decreases the rate of HIV transmission by three- to fivefold.[4] HIV combo assays detect both HIV antibodies and the p24 antigen. These assays provide an advantage for detection of infection prior to seroconversion—during the window period—because they are not dependent solely on the detection of HIV antibodies. Therefore, a highly specific and sensitive test that can detect HIV-1 groups M, O, and subtypes, and HIV-2 during the window period may assist in early diagnosis.

The ADVIA Centaur HIV Ag/Ab Combo assay uses yeast recombinant derived antigens corresponding to the viral envelope and core proteins. Recombinant antigens include 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 two monoclonal antibodies specific to HIV p24 antigen to capture and detect this antigen in patient samples.

The primary purpose of the ADVIA Centaur HIV Ag/Ab Combo assay is to aid in the diagnosis of HIV infection and AIDS. Specimens that are initially reactive should be retested in duplicate. Repeat reactivity is highly predictive of the presence of antibody to HIV-1 and/or HIV-2 in specimens from people at risk for HIV infection. Therefore, these specimens should be followed up with appropriate supplemental tests for HIV-1 and HIV-2 antibody and/or p24 antigen before making a diagnosis of HIV infection.

Materials and Methods

Performance characteristics were evaluated at two sites: Yhtyneet Medix Laboratoriot (YML), Espoo, Finland, and Siemens Healthcare Diagnostics (SHD), Tarrytown, NY. Patient and seroconversion panel samples tested at SHD were compared to an FDA-approved HIV method and/or vendor certificate of analysis (CoA). Patient samples tested at YML were compared to a CE-marked HIV combo assay, and samples reactive by either method were confirmed by a second CE-marked combo assay and immunoblot. Previously mentioned assays were performed according the manufacturer's instructions.

Assay principle

The ADVIA Centaur HIV Ag/Ab Combo (CHIV) assay is an in vitro diagnostic immunoassay for the simultaneous qualitative detection of human immunodeficiency virus p24 antigen and antibodies to human immunodeficiency viruses type 1 (including group O) and type 2 in serum and plasma (potassium-EDTA) to aid in the diagnosis of HIV infection using the ADVIA Centaur systems.

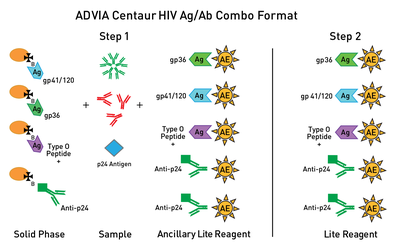


Figure 1. Schematic of the ADVIA Centaur CHIV assay.

Sensitivity

The diagnostic sensitivity of the assay was evaluated using 728 characterized HIV-positive samples from Baystate Biologicals, SeraCare, and YML. This population included samples reactive for HIV-1, HIV-1 group O, HIV-2, and p24 antigen. Forty-eight HIV-1 viral particle (antigen) isolates, which included isotypes A, B, C, D, F, G, O, AE, and AG, as well as HIV-2 strain NHIZ, were obtained from SeraCare, Zeptometrix, and Advanced Biotechnology. These samples tested positive in the Western blot, HIV-1 p24 Ag EIA, and/or bDNA assays.

Specificity

Specificity was determined by testing 9191 (3774 fresh, 5417 frozen) unique random donors obtained from various vendors.[†] Specificity calculation was based on the 9188 confirmed negative samples remaining after removal of 3 confirmed positive samples. Samples included 209 hospitalized patient samples. The results were reported in Index values as reactive (Index ≥ 1.0) or nonreactive (Index < 1.0). Reactive samples were tested using an FDA-approved HIV 1/O/2 assay (Siemens), HIV1/2/O EIA (Bio-Rad, WA, U.S.), p24 Ag EIA (Zeptometrix, NY, U.S.), and Western blot (Bio-Rad, WA, U.S.) assays.

[†]Continental Services, Interstate Blood Bank, Research Sample Bank, ProMedx, Institute für Infusion und Immunologie

Precision

The precision study was evaluated with two reagent lots according to CLSI protocol EP5-A2.[11] The study was performed for each reagent lot by using two ADVIA Centaur systems with two runs per day for 20 days. The instrument was calibrated on the first run of day 1 and recalibrated every 21 days as per the ADVIA Centaur CHIV assay's instructions for use.

Seroconversion sensitivity

Forty-five commercially available HIV seroconversion panels obtained from SeraCare, NABI and Bioclinical Partners were tested on ADVIA Centaur CHIV assay. The results were compared to the vendor CoA.

Analytical sensitivity

Dilutions of World Health Organization (WHO) 1st International Standard HIV-1 p24 Antigen (90/636) were serially diluted with negative plasma pool and tested with the ADVIA Centaur CHIV assay. The quantitative value of WHO standard detected at the cutoff (Index = 1.00) was calculated using linear regression analysis.

High-dose hook effect

Six HIV-1 high-positive samples and six HIV-2 high-positive samples with an initial concentration above an Index of 1000 obtained from Montefiore Hospital and Seracare were serially diluted into the negative basepool and assayed to evaluate the high-dose hook effect.

Cross-reactivity (disease state specimens)

A total of 326 samples from 35 groups of potential cross-reactants were obtained from various sources[†] and assayed on the CHIV as well as an FDA-approved HIV 1/O/2 assay. All specimens were assayed in singleton using two separate CHIV lots.

[†]ProMedx, Bioreclamation, SCIPAK, Zeptometrix, Keystone Biologicals, Nova Biologicals, Siemens Healthcare Diagnostics, SRL Research Group, Good Work Biomins.

Results

Sensitivity

The 728 presumably positive samples and 48 viral particle (antigen) isolates, which included isotypes A, B, C, D, F, G, O, AE, and AG, as well as HIV-2 strain NHIZ, were tested by the ADVIA Centaur CHIV assay. All samples were reactive, and the observed sensitivity of the assay was 100% with an exact 95% confidence interval of 99.58%–100% (Table 1).

Table 1. Sensitivity of the ADVIA Centaur CHIV assay.

Sample Classification	Number of Samples Tested	ADVIA Centaur CHIV Assay		Vendor CoA (HIV Reactive)	ADVIA Centaur CHIV Sensitivity
		Non Reactive	Reactive		
Characterized HIV-Positive Samples	538	0	538	538	100% (538/538)
p24 Core Antigen–Reactive Samples	131	0	131	131	100% (131/131)
HIV Type 2–Specific Samples	54	0	54	54	100% (54/54)
HIV-1 Group O–Reactive Samples	5	0	5	5	100% (5/5)
					95% Confidence Interval
Total	**728**	**0**	**728**	**728**	**99.58%–100% (728/728)**

Lysate Classification	Number of Lysates Tested	ADVIA Centaur CHIV Assay		Vendor CoA (HIV Reactive)	ADVIA Centaur CHIV Sensitivity
		Non Reactive	Reactive		
HIV-1 Viral Lysates	47	0	47	47	100% (47/47)
HIV-2 Viral Lysates	1	0	1	1	100% (1/1)
					95% Confidence Interval
Total	**48**	**0**	**48**	**48**	**93.95%–100% (48/48)**

Specificity

A total of 9191 HIV-negative unique random donors were tested by the ADVIA Centaur CHIV assay. Of the 9191 samples tested, 20 samples were found to be reactive on the Centaur CHIV assay. Three of the CHIV-reactive samples were confirmed as true positives and were omitted from the final calculations. In addition, 12 out of the 17 CHIV-reactive samples were tested on a selected FDA-approved HIV 1/O/2 assay. Of the 12 CHIV reactive samples tested, 6 were confirmed reactive on the selected FDA-approved HIV 1/O/2 assay. The overall resolved specificity of the ADVIA Centaur CHIV assay was 99.81% (Table 2).

Table 2. Resolved specificity of the ADVIA Centaur CHIV assay.

Sample List	Number Tested	ADVIA Centaur CHIV Assay		Number of Confirmed Reactive on an FDA-Approved HIV 1/O/2 Assay	Resolved ADVIA Centaur CHIV Assay Specificity	95% CI
		Nonreactive	Reactive			
Fresh Donors (US)	1866	1863	3	2	99.83% (1863/1866)	99.53%–99.96%
Fresh Donors (Finland)	1908[a]	1900	5	N/A	99.73% (1900/1905)	99.38%–99.91%
Frozen	5210	5201	9	4	99.83% (5201/5210)	99.67%–99.92%
Hospitalized Patients	207	207	0	0	100.0% (207/207)	98.56%–100%
Total	**9191**	**9171**	**17**	**6**	**99.81% (9171/9188)**	**99.70–99.89%**

[a]Three samples were confirmed to be true positives. The final number of confirmed negative samples tested was 1905; the three positives were omitted from the calculations.
N/A = Not applicable

Precision

The pooled within-run, among-run, and among-date CVs were no greater than 10.1% for all positive sample levels (Index ≥ 1.0). The pooled total CVs were no greater than 11.6% for all positive samples across the range of the assay.

Table 3. ADVIA Centaur CHIV assay precision summary.

Centaur CHIV Verification Lot 3 Precision Summary

Total Replicates (n=160)		Within-Run		Among-Run		Among-Date		Total	
Sample	MEAN (Index)	SD	CV	SD	CV	SD	CV	SD	CV
	(RLUs)								
Low Calibrator (RLUs)	**4397**	216	**4.9**	106	2.4	155	3.5	286	**6.5**
Negative Control (RLUs)	**6482**	302	**4.7**	123	1.9	401	6.2	517	**8.0**
	(Index)								
High Calibrator	**2.03**	0.06	**2.8**	0.04	1.8	0.06	3.1	0.09	**4.6**
HIV-1 Control	**2.88**	0.08	**2.7**	0.04	1.5	0.07	2.3	0.11	**3.9**
HIV-2 Control	**2.03**	0.06	**3.0**	0.03	1.5	0.03	1.6	0.07	**3.7**
p24 Ag Control	**2.10**	0.08	**3.7**	0.00	0.0	0.03	1.6	0.08	**4.0**
HIV-1 Group O Control	**3.05**	0.20	**6.5**	0.09	2.8	0.15	5.0	0.27	**8.7**
HIV Ag Low Positive	**2.15**	0.06	**3.0**	0.03	1.5	0.04	1.7	0.08	**3.7**

Centaur CHIV Verification Lot 4 Precision Summary

Total Replicates (n=160)		Within-Run		Among-Run		Among-Date		Total	
Sample	MEAN	SD	CV	SD	CV	SD	CV	SD	CV
	(RLUs)								
Low Calibrator (RLUs)	**3064**	120	3.9	65	2.1	159	5.2	210	**6.8**
Negative Control (RLUs)	**3986**	188	4.7	56	1.4	216	5.4	292	**7.3**
	(Index)								
High Calibrator	**1.83**	0.06	3.0	0.03	1.8	0.10	5.2	0.12	**6.3**
HIV-1 Control	**2.96**	0.08	2.7	0.06	2.2	0.15	5.1	0.18	**6.2**
HIV-2 Control	**2.06**	0.06	3.0	0.04	1.9	0.15	7.2	0.16	**8.0**
p24 Ag Control	**1.85**	0.05	2.5	0.01	0.7	0.17	9.0	0.17	**9.4**
HIV-1 Group O Control	**2.26**	0.12	5.5	0.04	1.6	0.23	10.1	0.26	**11.6**
HIV Ag Low Positive	**1.94**	0.04	2.2	0.02	1.1	0.17	8.7	0.18	**9.0**

Seroconversion sensitivity

The seroconversion panel testing results on the ADVIA Centaur CHIV assay are summarized in Table 4. The ADVIA Centaur CHIV assay demonstrated sensitivity equivalent to that reported in the vendor CoA on 40 of the 45 seroconversion panels. Of the five nonequivalent results, the ADVIA Centaur CHIV assay reported the first positive result at least one bleed earlier than one of the two HIV Ag assays represented on the vendor's CoA.

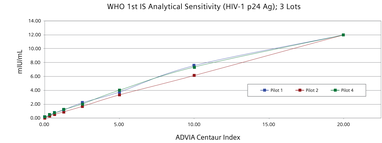
Table 4. ADVIA Centaur CHIV assay seroconversion panel summary.

Panel ID	Vendor ID	Days with the First Positive Results by				
		Vendor CoA				ADVIA Centaur CHIV
		Abbott HIV1/2	Gen-Sys HIV1/2	Abbott HIV Ag	Coulter HIV Ag	
RP018	BBI	29	29	16	N/A	16
6242	BCP	8	15	0	N/A	0
SV-0271	NABI	15	20	8	8	8
PR8903	BBI	7	21	0	N/A	0
PR8904	BBI	92	92	>99	N/A	92
PR8905	BBI	91	126	84	N/A	84
PR8909	BBI	14	16	7	N/A	0
PR8910	BBI	26	26	14	N/A	14
PR8912	BBI	0	9	0	N/A	0
PR8913	BBI	22	22	>22	N/A	22
PR8916	BBI	30	30	15	N/A	15
PR8922	BBI	0	>11	0	N/A	0
PR8923	BBI	47	84	37	N/A	37
PR8925	BBI	44	49	44	N/A	44
PR8926	BBI	27	27	7	N/A	7
PR8927	BBI	28	40	28	28	28
PR8928	BBI	111	120	111	111	111
PR8929	BBI	25	28	18	14	18
PR8930	BBI	7	10	3	0	0
PR8932	BBI	27	34	27	27	27
PR8937	BBI	21	>21	21	21	14
PR8940	BBI	11	15	7	7	7
PR8942	BBI	>14	>14	14	14	14
PR8943	BBI	14	>21	12	7	7
PR8944	BBI	14	>21	2	14	7
PR8945	BBI	13	13	13	13	13
PR8946	BBI	>11	>11	7	7	7
PR8947	BBI	9	20	9	9	9
PR8948	BBI	>23	>23	23	23	23
PR8950	BBI	28	28	21	18	18
PR8951	BBI	19	19	8	8	8
PR8952	BBI	14	>21	10	10	10
PR8953	BBI	7	10	7	7	7
PR8954	BBI	>21	>21	17	21	17
PR8955	BBI	12	>14	7	3	3
PR8956	BBI	>50	>50	47	47	47
PR8958	BBI	15	15	9	7	7
PR8959	BBI	9	14	7	0	0
PR8960	BBI	>30	N/A	N/A	28	28
PR8961	BBI	>29	N/A	N/A	27	27
PR8962	BBI	>17	N/A	N/A	14	14
PR8963	BBI	>21	N/A	N/A	17	17
PR8964	BBI	>22	N/A	N/A	22	22
PR8965	BBI	21	N/A	21	21	7
PR8966	BBI	48	N/A	N/A	44	44

N/A = Not available

Analytical sensitivity

The calculated average analytical sensitivity as determined on the ADVIA Centaur system with the WHO International Standard HIV-1 p24 Antigen (90/636) was 1.17 IU/mL.



HIV-1 p24 ID	mIU/mL	Pilot 1 ADVIA Centaur Index	Pilot 2 ADVIA Centaur Index	Pilot 4 ADVIA Centaur Index
WHOp24-20	20.00	>12	>12	>12
WHOp24-10	10.00	7.56	6.13	7.35
WHOp24-5	5.00	3.75	3.34	4.01
WHOp24-2.5	2.50	2.20	1.67	2.03
WHOp24-1.3	1.25	**1.14**	**0.89**	**1.21**
WHOp24-0.63	0.63	0.72	0.52	0.67
WHOp24-0.31	0.31	0.42	0.29	0.38
WHOp24-0	0.00	0.13	0.00	0.09

HIV1-p24 Antigen Sensitivity via NIBSC WHO Reagent (Lot 90/636) at Assay Index 1.0 CHIV (ADVIA Centaur)			
Pilot 1	Pilot 2	Pilot 4	Avg
1.074 IU/mL	1.393 IU/mL	1.042 IU/mL	1.167 IU/mL

Figure 2. Analytical sensitivity on the ADVIA Centaur CHIV assay, three lots.

High-dose hook effect

Serial dilution of the six HIV-1 and six HIV-2 high-positive samples showed Index values of >12.0 for the neat samples. The assay did not show negative results with high-positive samples (Figures 3 and 4).

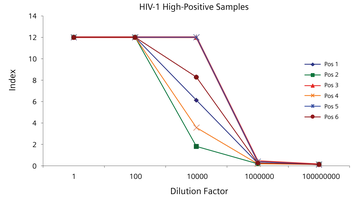


Figure 3. Six HIV-1 high-positive samples do not hook below the cutoff level.

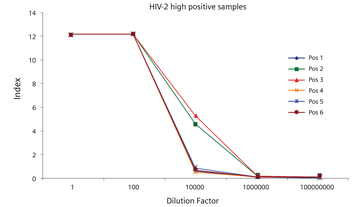


Figure 4. Six HIV-2 high-positive samples do not hook below the cutoff level.

Cross-reactivity (disease state specimens)

Table 5 shows the summary of the cross-reactive samples tested on two lots of the ADVIA Centaur CHIV assay. There was no interpretation change for the potential cross-reactants. Results from the CHIV assay show 100% agreement with an FDA-approved HIV assay.

Table 5. Summary of potential cross-reactive samples tested with two lots of the ADVIA Centaur CHIV assay.

Sample Category	Number of Positive Disease State Samples Tested				Agreement
	Number Tested	ADVIA Centaur CHIV Lot 1	ADVIA Centaur CHIV Lot 2	FDA-Approved HIV 1/O/2 Assay	CHIV vs. FDA-Approved HIV 1/O/2 Assay
High Human Immunoglobulin IgA	11	0	0	0	100%
High Human Immunoglobulin IgM	9	0	0	0	100%
High Human Immunoglobulin IgG	13	0	0	0	100%
HAMA	18	0	0	0	100%
Ulcerative Colitis	9	0	0	0	100%
Fibromyalgia Condition	10	0	0	0	100%
Systemic Lupus Erythematosus (SLE)	9	0	0	0	100%
Graves' Disease	8	0	0	0	100%
Scleroderma	10	0	0	0	100%
Crohn's Disease	10	0	0	0	100%
Flu Vaccine Recipient	22	0	0	0	100%
Rheumatoid Factor Positive	10	0	0	0	100%
Hepatitis C Virus (HCV) Ab	10	0	0	0	100%
Hepatitis C Virus (HCV) Ag	5	0	0	0	100%
Hepatitis B Virus (HBV) IgM	10	0	0	0	100%
Heptitis B Surface Ag (HBsAg)	10	0	0	0	100%
Hepatitis A Virus (HAV) IgM	5	0	0	0	100%
Human T-cell Lymphotropic Virus (HTLV III) IgM	10	0	0	0	100%
Anti-Nuclear Antibody (ANA)	9	0	0	0	100%
Varicella Zoster Virus (VZV) IgG	10	0	0	0	100%
Herpes Simplex Virus (HSV1/2) IgM	10	0	0	0	100%
Herpes Simplex Virus (HSV1/2) IgG	5	0	0	0	100%
Epstein–Barr Virus (EBV) IgG	5	0	0	0	100%
Epstein–Barr Virus (EBV) IgM	10	0	0	0	100%
Cytomegalovirus IgM	10	0	0	0	100%
Cytomegalovirus IgG	5	0	0	0	100%
Syphilis IgG	9	0	0	0	100%
Syphilis IgM	10	0	0	0	100%
Rubella IgM	10	0	0	0	100%
Rubella IgG	10	0	0	0	100%
Toxo IgG	10	0	0	0	100%
Toxo IgM	12	0	0	0	100%
Diabetes	10	0	0	0	100%
E. coli Ag	1	0	0	0	100%
Staphylococcus Ag	1	0	0	0	100%
Total	**326**	**0**	**0**	**0**	**100%**

Conclusions

- The ADVIA Centaur HIV Ag/Ab Combo (CHIV) assay is useful for the simultaneous qualitative detection of human immunodeficiency virus p24 antigen and antibodies to human immunodeficiency viruses type 1 (including group O) and type 2, in serum and plasma.
- This fully automated assay is accurate and reliable, comparable to existing assays, and provides excellent sensitivity and specificity to aid in the diagnosis of HIV infection.

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