

# Evaluation of the Siemens HIV Antigen-Antibody Immunoassay

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## Key Words

Human immunodeficiency virus-1 · Performance evaluation · Immunoassay

## Abstract

Fourth-generation assays for the simultaneous detection of human immunodeficiency virus (HIV) antigen and antibodies are available on the international market and are currently used for blood donor screening and for HIV diagnosis. In this study we evaluated the performance of the novel automated fourth-generation ADVIA Centaur<sup>®</sup> HIV Ag/Ab Combo assay. The assay detected seroconversion at the same bleed or at least one bleed earlier in panels with respect to other assays and showed a detection efficacy equal to those of other assays in a low-titer panel. Samples obtained from blood donors ( $n = 2,778$ ) or from HIV-positive patients (HIV-1 B subtype,  $n = 82$ ; non-B subtype,  $n = 71$ ) were also tested, showing a good correlation with other fourth-generation assays. We assessed the performance of 3 fourth-generation assays for detecting in utero transmitted anti-HIV antibodies and found a more specific detection efficiency with the ADVIA Centaur HIV Ag/Ab Combo assay compared to the other fourth-generation assays.

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## Introduction

Early diagnosis of human immunodeficiency virus (HIV) infection is important for the prevention of HIV, and it is essential for optimal outcomes in infected patients, facilitating the timely initiation of therapy and decreasing the rate of HIV transmission by 3- to 5-fold [1]. Therefore, HIV-specific antibody tests have been in continual development since the incipience of the HIV pandemic. The success of third-generation assays and immunoglobulin G- and immunoglobulin M-sensitive antibody tests in narrowing the window period for HIV diagnosis has been notable. However, diagnostic tests designed to detect antibodies alone are unable to identify individuals with acute infection who have not yet begun to produce HIV-specific antibodies. RNA detection methods have been used to detect acutely infected individuals, but RNA-based detection methods are expensive and time-consuming; the time to results could range from 2 to 7 days (depending on the laboratory equipment), an amount of time not ideal for prevention purposes. An alternative is to utilize antigen-antibody combination tests known as HIV Combo assays or fourth-generation assays. These assays, detecting both HIV antibodies and p24 antigen, provide an advantage for the detection of

infection prior to seroconversion because they are not dependent solely on the detection of HIV antibodies. Fourth-generation Combo assays are standard and automated immunoassays. Combo assays are easy to perform and have rapidly replaced third-generation assays. However, it has been reported that the sensitivity for p24 antigen and/or antibodies varies significantly among fourth-generation assays [2–7]. Several fourth-generation assays have sensitivities equivalent to those of antibody assays; however, in some instances they have shown lower antibody detection efficiency [4–6].

Recently, a CE-marked automated fourth-generation assay, i.e. ADVIA Centaur<sup>®</sup> HIV Ag/Ab Combo, was released by Siemens. The performance of this assay was evaluated in comparison with a predicate assay, and similar sensitivities and specificities were found [8]. In this study, we evaluated the performance of the ADVIA Centaur HIV Ag/Ab Combo assay using different types of samples and in comparison with other assays and confirmed the good performance of the ADVIA Centaur HIV Ag/Ab Combo assay.

## Materials and Methods

### *Sources of Specimens and Statistical Analysis*

Samples were collected from November 2009 to July 2010. This study included a total of 2,778 samples obtained from blood donors, 105 samples from HIV-negative patients with different autoimmune diseases, 153 samples from pregnant women at 30–36 weeks of gestation, 82 HIV-1 B subtype-positive samples, 71 non-B subtype-positive samples (provided by the Virology Unit of A.O. Cotugno, Naples, Italy), and 50 samples obtained from babies born from HIV-1-positive mothers.

For blood donors samples, the correlation between the index values obtained with the Architect HIV Combo assay and the ADVIA Centaur HIV Ag/Ab Combo assay was calculated using Spearman's correlation coefficient ( $\rho$ ) and a significance level ( $p$  value) was indicated. The data analysis was performed using SPSS 17.0.

### *ADVIA Centaur HIV Ag/Ab Combo Assay*

The ADVIA Centaur HIV Ag/Ab Combo assay is an antigen-bridging, magnetic microparticle chemiluminometric immunoassay that detects antibodies to HIV-1 groups M and O, HIV-2, and p24 antigen in serum or plasma.

Streptavidin-coated microparticles and biotinylated recombinant antigens are used to capture the antigens and antibodies, and acridinium ester-labeled recombinant antibodies/antigens are then added to generate relative light units for detection on the ADVIA Centaur analyzer [9].

### *Architect HIV Ag/Ab Combo Assay*

The Architect HIV Ag/Ab Combo assay (Abbott Laboratories GmbH, Delkenheim, Germany) is a chemiluminescent magnetic

microparticle-based immunoassay used to determine the presence of the HIV-1 p24 antigen and antibody to HIV-1 group M, HIV-1 group O, and HIV-2.

### *Elecsys 2010 HIV Combi Assay*

Elecsys 2010 HIV Combi (Roche, Switzerland) is an electrochemiluminescence immunoassay. It detects HIV-1/HIV-2 antibodies using recombinant antigens derived from the polymerase and envelope regions of HIV-1 and HIV-2; for detection of the HIV-1 p24 antigen, monoclonal antibodies are used.

### *Vidas HIV DUO ULTRA Assay*

Vidas HIV DUO ULTRA (bioMérieux, Marcy-l'Étoile, France) is an enzyme-linked fluorescent assay which permits the simultaneous detection of p24 antigen and antibodies against HIV-1 (including group O) and HIV-2.

### *Confirmatory Assay*

Inno-Lia HIV I/II Score (Innogenetics, Gent, Belgium), a line immunoassay, was used as a confirmatory assay. Assays were performed according to the manufacturer's instructions.

### *Seroconversion Panels*

Seroconversion sensitivity was evaluated using 4 seroconversion panels, i.e. PRB-953, PRB-940, PRB-943, and PRB-916, obtained from BBI Diagnostics, West Bridgewater, Mass., USA. A low-titer panel [PRB-109(M); Seracare Life Sciences, West Bridgewater, Mass., USA] was also used.

### *HIV-1 Subtyping*

Sequences were obtained from plasma samples via direct sequencing of HIV-1 PR and RT coding regions using the Celera Diagnostics ViroSeq HIV-1 Genotyping System (version 2.0) and an ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Foster City, Calif., USA). HIV-1 subtyping was performed with the REGA HIV-1 Subtyping Tool (version 2.0; REGA Institute, Leuven, Belgium).

## Results

### *Assay Performance on Serum Conversion Panels*

The ability of the HIV Combo assay to detect HIV antigens enables the test to detect recent HIV infections during the antibody-negative window period. Therefore, we used serum conversion panels (PRB-916, PRB-940, and PRB-953) to assess the diagnostic efficiency of the ADVIA Centaur HIV Ag/Ab Combo assay. A positive signal was detected at the same bleed with the Abbott HIV Ag assay, i.e. day 15 for PRB-916 and day 7 for PRB-940 and PRB-953. Conversely, for testing panel PRB-943 a positive signal was observed on day 7 with the ADVIA Centaur HIV Combo assay, whereas the Abbott HIV Ag assay reacted positively on day 12. These data confirm the good performance in the diagnosis of HIV infection previously reported [8].

### Detection of Low-Titer HIV-Positive Samples

It has been reported that the sensitivity of fourth-generation assays varies significantly for p24 antigen and antibodies; some fourth-generation assays show a lower anti-HIV antibody detection efficiency [4–6, 10]. To exclude that the ADVIA Centaur HIV Ag/Ab Combo assay does not correctly identify low-titer samples, a low-titer panel (PRB-108M) was analyzed. This panel consisted of aliquots with anti-HIV-1 reactivity near the sensitivity limits of anti-HIV screening tests. The ADVIA Centaur HIV Ag/Ab Combo assay correctly identified all low-titer samples with a performance similar to that of other fourth-generation assays (table 1).

### Specificity Analysis Using HIV-Negative and HIV-Positive Samples

Blood donor samples (n = 2,778) routinely tested with the Architect HIV Combo assay were analyzed with the ADVIA Centaur HIV Ag/Ab Combo assay. Eight samples tested positive with the ADVIA Centaur HIV Ag/Ab Combo assay, one of which had tested positive with the Architect HIV Combo assay. Three samples tested positive with the Architect HIV Ag/Ab Combo assay. Positive samples were confirmed as false positive upon further testing with the Inno-Lia HIV I/II Score assay (table 2). The results showed a concordance of 99.6% between the Architect HIV Ag/Ab Combo assay and the ADVIA Centaur HIV Ag/Ab Combo assay. Analysis of the index values showed a high correlation ( $\rho = 0.952$ ,  $p < 0.0001$ ) between the assays.

Next, we evaluated the specificity of the ADVIA Centaur HIV Ag/Ab Combo assay using selected HIV-negative samples routinely tested with the Elecsys 2010 HIV Combi. Sera were obtained from patients with autoimmune diseases, i.e. 47 patients with rheumatoid arthritis, 40 patients with systemic lupus erythematosus, 18 patients with lupus anticoagulant, and 153 pregnant women at 30–36 weeks of gestation. Eighty-two confirmed HIV-positive samples (B subtype) routinely tested with the Elecsys 2010 HIV Combi were also evaluated.

The ADVIA Centaur HIV Ag/Ab Combo assay correctly detected positive and negative samples (table 3).

A growing number of infections with the HIV-1 group M non-B subtype have been reported. However, studies have demonstrated that some fourth-generation HIV assays are unable to detect certain HIV-1 group M non-B subtypes, yielding false negatives [11, 12]. Therefore, it is essential to evaluate the capability of assays to detect the infection independently of the HIV subtype.

**Table 1.** Detection of low-titer HIV-positive samples using an anti-HIV-1 low-titer panel

PRB109	ADVIA Centaur® HIV Ag/Ab Combo	Architect HIV Ag/Ab Combo	Prism HIV Ag/Ab Combo	Murex EIA HIV Ag/Ab Combo	Elecsys 2010 HIV Combi	Abbott HIV 1/2	Biorad HIV 1/2 Plus
01	+	+	+	+	+	+	+
02	+	+	+	+	+	-	+
03	+	+	+	+	+	-	+
04	+	+	+	+	+	+	+
05	+	+	+	+	+	+	+
06	+	+	+	+	-	+	+
07	+	+	+	+	+	-	+
08	-	-	-	-	-	-	-
09	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+
11	+	+	+	+	+	+	+
12	+	+	+	+	+	+	+
13	+	+	+	+	+	-	-
14	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+
16	+	+	+	+	+	+	+
17	+	+	+	+	+	+	+
18	+	+	+	+	+	+	+
19	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+

We analyzed 71 non-B subtype samples which had tested positive with the Elecsys 2010 HIV Combi (tables 4, 5). Forty-nine negative samples were also tested in the same run. All samples tested positive with the ADVIA Centaur HIV Ag/Ab Combo assay, confirming that the sensitivity of the assay is unaffected by the HIV-1 subtype [8].

### Detection of Anti-HIV in utero Transmitted Antibodies

HIV-infected mothers passively transfer antibodies to babies in utero; therefore, assays based on antibody detection are of limited use until the mother's antibodies have been cleared from the infant's blood or the infected child develops its own antibodies against HIV. Clearance of the maternal antibodies is expected at about 18 months, and a noninfected child should test negative for HIV antibodies after this age [13–15]. HIV RNA testing is used in Western countries to diagnose vertical transmission; however, antibody tests are frequently performed after 18 months of age for conclusive evidence of a lack of HIV infection.

**Table 2.** Specificity analysis using sera collected from blood donors

ADVIA Centaur® HIV Ag/Ab Combo assay		Architect HIV Ag/Ab Combo assay	
non-reactive samples	reactive samples <sup>a</sup>	non-reactive samples	reactive samples <sup>a</sup>
2,770	7 <sup>+b</sup>	2,775	2 <sup>+b</sup>

The total number of blood donors was 2,778. There was a concordance of 99.6% between the Architect HIV Combo assay and the ADVIA Centaur HIV Ag/Ab Combo assay.

<sup>a</sup> Samples that tested positive with the ADVIA Centaur HIV Ag/Ab Combo assay and/or the Architect HIV Ag/Ab Combo assay and were found to be false positive with the confirmatory assay Inno-Lia.

<sup>b</sup> Samples that tested positive with the ADVIA Centaur HIV Ag/Ab Combo assay and were also found to be positive with Architect HIV Ag/Ab Combo assay.

**Table 3.** Specificity analysis using HIV-negative samples collected from patients with autoimmune diseases and HIV-positive samples collected from HIV B subtype individuals

ADVIA Centaur® HIV Ag/Ab Combo assay		Elecys 2010 HIV Combi	
positive samples <sup>a</sup>	negative samples <sup>b</sup>	positive samples <sup>a</sup>	negative samples <sup>b</sup>
82	258	82	258

The total number of samples was 340. There was a concordance of 100% for positive and negative samples between the Elecys 2010 HIV Combi and the ADVIA Centaur HIV Ag/Ab Combo assay.

<sup>a</sup> Collected from HIV-1 B subtype-infected patients.

<sup>b</sup> Collected from 47 patients with rheumatoid arthritis, 40 patients with systemic lupus erythematosus, 18 patients with lupus anticoagulant, and 153 pregnant women at 30–36 weeks of gestation.

**Table 4.** Specificity analysis using HIV non-B subtype samples

ADVIA Centaur® HIV Ag/Ab Combo assay		Elecys 2010 HIV Combi	
positive samples	negative samples	positive samples	negative samples
71	49	71	49

The total number of sera was 120. There was a concordance of 100% for positive and negative samples between the ADVIA Centaur HIV Combo assay and the Elecys 2010 HIV Combi.

**Table 5.** Non-B subtype and CRF-positive samples

	Samples, n
Non-B subtype	
A	11
C	7
F	2
G	5
K	1
J	1
CRF subtype	
CRF02_AG	26
CRF01_AE	4
Unassigned	14
Total	71

Fourth-generation assays can detect low-titer anti-HIV antibodies; therefore, we evaluated the performance of the ADVIA Centaur HIV Ag/Ab Combo assay for the detection of in utero transmitted anti-HIV antibodies. We analyzed 50 sera collected from babies aged >18 months (median age 28 months) and born from HIV-infected mothers. All of the babies had received an antiretroviral prophylaxis. HIV RNA testing was performed 3 times (at 21 days, at 2 months, and at 6 months) with negative results. Samples that were routinely evaluated with the Elecys 2010 HIV Combi and the bioMérieux HIV ULTRA DUO all tested positive for anti-HIV antibodies. Thirty-five samples tested positive with the ADVIA Centaur HIV Ag/Ab Combo assay and 15 tested negative (table 6).

## Discussion

Fourth-generation assays allow an earlier diagnosis of HIV infection than third-generation assays by detecting p24 antigen, which may be present in recent HIV infection prior to seroconversion, and comparative studies have provided evidence for the clinical use of HIV antigen/antibody combination assays. The detection of early bleeds by fourth-generation assays has been demonstrated on seroconversion panels, showing the advantage of these assays for reducing the seroconversion window [2–7, 15–17].

In the present study, we evaluated the performance of a novel fourth-generation assay, i.e. the ADVIA Centaur HIV Ag/Ab Combo.

**Table 6.** Detection of anti-HIV in utero transmitted antibodies

ADVIA Centaur® HIV Combo assay		Elecsys 2010 HIV Combi		bioMérieux HIV ULTRA DUO tests	
positive samples	negative samples	positive samples	negative samples	positive samples	negative samples
35	15	50	0	50	0

The total number of sera was 50. Sera were collected from HIV-negative 18- to 28-month-old babies born from HIV-positive mothers.

In a recent study, the relative sensitivity of the ADVIA Centaur HIV Ag/Ab Combo assay was 98.36% and its relative specificity was 99.74% [8], and in the same study the specificity of the HIV Ag/Ab Combo assay was 99.76% (7,506/7,524) among random blood donors and 99.2% (247/249) among hospitalized patients. Our data confirmed these previous observations.

That same study found that the performance of the ADVIA Centaur HIV Ag/Ab Combo assay for the detection of seroconversion was comparable to that of the predicate assay [8]. In the present study, we used 4 seroconversion panels which showed detection at the same bleed as the Abbott Ag HIV assay in 3 cases and an earlier detection in panel PRB-943, confirming the seroconversion detection efficacy of the ADVIA Centaur HIV Ag/Ab Combo assay. It is known that the performance of fourth-generation assays varies substantially with respect to p24 antigen sensitivity, antibody sensitivity, HIV group/subtype detection, and specificity [18]. A sensitivity of 1.15 IU/ml for p24 antigen with the ADVIA HIV Combo assay is reported by the manufacturer; the good performance on antigen detection can explain the difference observed with panel PRB-943. Good detection efficiency was observed using a low-titer panel, and we showed that the ADVIA Centaur HIV Ag/Ab Combo assay detects the infection independently of the HIV-1 subtype. Overall, our data confirmed the good seroconversion detection [8] and HIV-1 non-B subtype detection.

Vertical transmission of HIV infection represents a great challenge in developing countries, whereas in high-income countries antiretroviral prophylaxis and prevention have almost completely eliminated vertical HIV transmission.

In high-income countries, HIV RNA testing is easily accessible, allowing the use of algorithms based on HIV RNA testing for the diagnosis or exclusion of vertical transmission of HIV infection. However, HIV antibody testing at about 18 months of age is still used to confirm an HIV-negative status [19].

The kinetics of in utero transmitted maternal antibodies can vary, being about 2.5 months for rubella and varicella [20] or longer for other diseases. For anti-HIV maternal antibodies, a time of 18 months is assumed to be sufficient for the complete clearance of maternal antibodies [12–14]. Studies have shown that the persistence of maternal antibodies can vary by region, being stronger in Africa or in the third world, probably due to cofactors such as malnutrition or infections (malaria, etc.) which influence the rate of decline. Conversely, in Western countries a slow decay of specific immunoglobulin G has been observed [21]. Furthermore, the limit of 18 months has been based on the performance of previous anti-HIV antibody assays (second/third generation). However, as shown in the present study, fourth-generation assays can detect residual maternal antibodies over the limit of 18 months with positive results at up to 30 or even 36–40 months [Vallefuoco, unpubl. data]. The reactive results for HIV antibodies are potentially misleading and could extend the follow-up time, increasing the costs. Fourth-generation assays are now widely used in developed countries; therefore, in order to exclude vertical transmission of HIV infection, it is advisable to rely on HIV RNA testing and avoid HIV antibody testing or alternatively to postpone the HIV antibody testing to an age of 24–30 months.

Interestingly, the performance of the ADVIA Centaur HIV Ag/Ab Combo assay on the 50 sera obtained from subjects with in utero passive transfer of anti-HIV antibodies showed that only 35 out of the 50 sera collected after 18 months of age (median age 28 months) tested positive with the ADVIA Centaur HIV Ag/Ab Combo assay. Our data indicate that the ADVIA Centaur HIV Ag/Ab Combo assay has a more specific detection efficiency for in utero transmitted anti-HIV antibodies with respect to other fourth-generation assays and could represent a useful tool to monitor these subjects.

In conclusion, we evaluated the performance of the ADVIA Centaur HIV Ag/Ab Combo and confirmed the good correlation with other fourth-generation assays; moreover, we showed a more specific detection efficiency for in utero transmitted anti-HIV antibodies.

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