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Performance evaluation of the new Access HIV Ag/Ab combo assay on the DxI 9000 Access Immunoassay Analyzer

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ABSTRACT

Fourth-generation HIV immunoassays have been developed to reduce the window period of detection during seroconversion period, allowing for the detection of early and established infections. The aim of this work was to evaluate a newly developed assay, Access HIV Ag/Ab combo on the novel high throughput DxI 9000 Access Immunoassay Analyzer (Beckman Coulter, Inc.). The assay allows for simultaneous qualitative detection and differentiation of HIV-1 p24 antigen and HIV-1/2 antibodies.

Assay performance was compared to two gold standard assays, the Abbott Architect HIV Ag/Ab Combo and Roche Elecsys HIV Duo, and assessed in a multicenter study, using a wide panel of samples ($n > 9000$, clinical samples and viral lysates) representative of genetic diversity for both antibodies and antigens, early phases of infection, negative, and cross-reacting samples.

The clinical sensitivity was 100 % for clinical samples as well as for viral lysates. Data on viral lysates and early detection on seroconversion panels showed a better result with the Access assay. Analytical sensitivity showed a limit of p24 detection determined around 0.2 IU/mL. The overall specificity was 99.91 %, and no interference was found using the potentially cross-reactive samples.

In conclusion, the Access HIV Ag/Ab combo assay demonstrated its ability for accurate diagnosis of chronic as well as primary HIV infections on the DxI 9000 Analyzer, despite the high level of genetic diversity of these viruses.

1. Introduction

Human immunodeficiency viruses (HIV) present genetic diversity that leads to classification in two types i) HIV type 1 (HIV-1), also classified into four groups, M (major), N (non-M and non-O), O (outlier) and P, and ii) HIV-2, also classified into nine groups A to I [1-5]. The continuous genetic evolution of HIVs over the past 40 years, has yielded an increasing number of variants which can be defined into subtypes, subgroups, Circulating and Unique Recombinant Forms (CRF and URF respectively) according to their genetic relationships [3]. This genetic plasticity has heightened the difficulties of serological diagnosis, with many variants not being detected due to their antigenic diversity; however, assay manufacturers have continuously attempted to correct this shortcoming, by adapting their assays to account for this wide antigenic diversity [1-6].

Fourth-generation HIV immunoassays have been developed to reduce the window period of detection during seroconversion period by 4–8 days [7,8], allowing the detection of early and established infections [8-10]. More than 10 different fourth-generation assays have been licensed in Europe; HIV automated fourth-generation tests are now

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Table 1
Summary of samples included in performances analyses.

Samples for specificity evaluation	6020 fresh blood samples 1198 frozen hospitalized patient samples 896 fresh hospitalized patient samples 260 samples from patients with potentially cross-reacting factors
Samples for sensitivity evaluation	948 HIV known positive samples (784 HIV-1 Ab, 100 HIV-2 Ab, 64 HIV-1 p24 Ag) 30 seroconversion panels 50 cell culture supernatants (50 distincts HIV isolates) WHO HIV-1 p24 (90/636) standard dilutions (0; 0.1; 0.3; 0.5; 0.7; 0.9; 1.0 IU/mL) in plasma and serum

routinely being used in diagnostic laboratories [11-13]. Thus, any new fourth-generation immunoassay needs to be assessed for determining both clinical sensitivity (genetic diversity and various clinical status) as well as analytical sensitivity and lot-to-lot reproducibility. Beckman Coulter recently developed the Access HIV Ag/Ab combo assay specifically for the novel high throughput DxI 9000 Access Immunoassay Analyzer. It allows for simultaneous qualitative detection and differentiation of HIV-1 p24 antigen and HIV-1/2 antibodies.

The aim of this work was to compare this new Access assay against the well-established, and considered here as gold standard, Abbott Architect HIV Ag/Ab Combo and Roche Elecsys HIV Duo assays, in a multicenter study. Performances were assessed using a wide panel of samples ($n > 9000$) representative of genetic diversity for both antibodies and antigens, of early phases of infection, negative samples and cross-reacting samples.

2. Study design

2.1. Assays

The Access HIV Ag/Ab combo assay (Beckman Coulter, Inc., Brea, California, USA) is a paramagnetic particle, chemiluminescent immunoassay for the simultaneous qualitative detection and differentiation of HIV-1 p24 antigen (Ag) and antibodies (Ab) to HIV-1/2, designed specifically for the DxI 9000 Immunoassay Analyzer. HIV-1 p24 antigen detection is performed in one reaction vessel using anti-p24 antibody coated paramagnetic particles (PMP) and anti-p24 antibody-alkaline phosphatase (ALP) conjugate. In parallel, in a second reaction vessel, anti-HIV antibody detection occurs using PMP coated with antigens

Table 2
Genetic diversity of HIV samples tested for clinical performances.

HIV Antibody positive samples			
Genotype			**Number**
HIV-1	group M	subtype A	26
		subtype B	72
		subtype C	24
		subtype D	18
		subtype F	17
		subtype G	20
		subtype H	9
		subtype J	3
		subtype K	1
		URF	24
		CRF01	37
		CRF02	128
		CRF03	2
		CRF04	1
		CRF06	13
		CRF07	1
		CRF09	1
		CRF11	11
		CRF12	3
		CRF13	5
		CRF14	1
		CRF18	8
		CRF22	5
		CRF25	3
		CRF37	12
		CRF43	2
		CRF45	2
		CRF49	1
		CRF60	1
		CRF94	1
	group O		11
HIV-2			100

mimicking immunodominant regions (gp41 and gp36) of HIV-1 group M, group O and HIV-2 proteins in association with these same antigens tagged with a flag and an anti-flag antibody conjugated to ALP. Testing result is reported as two sub-results for HIV-1 p24 antigen and HIV-1/HIV-2 antibody provided as both a signal-to-cutoff ratio (S/CO) and as a qualitative interpretation ("reactive" or "nonreactive"), and a combined result provided only as qualitative interpretation.

The Architect HIV Ag/Ab Combo assay (Abbott Laboratories, Chicago, Illinois, USA) is a chemiluminescent magnetic microparticle-based immunoassay dedicated to HIV-1 p24 antigen and antibody to HIV-1/2 detection on Architect I System. One unique result is reported without distinction between the detection of HIV-1 p24 antigen, HIV-1 antibody, or HIV-2 antibody [14].

The Elecsys HIV Duo assay (Roche Diagnostics GmbH, Mannheim, Germany) is an electrochemiluminescence assay intended for use on the Cobas e 801 Immunoassay Analyzer. It reports three results: HIV-1 p24 antigen and antibodies to HIV-1 and HIV-2 results as two separate determinations and an HIV Duo result automatically calculated by the analyzer [11,15].

2.2. Samples

Study was multicentric for both clinical and analytical studies, with collection of prospective and retrospective samples. A summary of samples included in performances analyses is presented in Table 1.

2.2.1. Sensitivity determinations

2.2.1.1. Clinical sensitivity (Appendix 1). Clinical sensitivity was evaluated through the testing of 884 HIV antibody positive samples; we included 563 samples representing different HIV-1 group M subtypes and CRFs, HIV-1 group O and HIV-2 samples (Table 2). The other samples (n = 321) were not genotyped.

Additional antigen positive samples (n = 114) were tested, corresponding to i) 64 clinical HIV-1 p24 samples (eight enrolled from patients during laboratory routine testing and 56 -one per panel- from seroconversion and performances panels) and ii) 50 positive viral lysates from HIV-1 group M (A-H subtypes, CRF01, CRF02, CRF06, CRF11, CRF14, CRF15, CRF18, CRF36), HIV-1 group O (H and T subgroups) and HIV-2 (group A and B), spiked into HIV negative human CPD plasma. All samples were tested on both Access and Architect assays.

2.2.1.2. Analytical sensitivity. Thirty commercial seroconversion panels acquired from ZeptoMetrix and LGC Clinical Diagnostics/SeraCare Life Sciences were assessed with the Access, Architect and Elecsys assays. They represented a total of 256 bleeds including 86 early seroconversion HIV specimens as defined by the European Common Specifications (2022/1107 of 4 July 2022, i.e., HIV-1 p24 antigen and/or HIV RNA positive, and not recognized by the antibody first-line assays and indeterminate or negative in confirmatory assays). Number of bleeds found reactive per panel and per assay was reported, as was the difference in days between the first bleed determined reactive with serological vs NAT assay.

Antigen analytical sensitivity of the Access assay was determined by testing a series of dilutions of the HIV-1 p24 Antigen World Health Organization (WHO) International Standard (NIBSC code: 90/636), spiked into four different HIV negative human serum or four different K2 EDTA plasma samples. These corresponded to 32 samples covering the 0, 0.1, 0.3, 0.5, 0.7, 0.9, 1.0 IU/mL range. The evaluation was performed using three reagent pack lots and three reagent calibrator lots.

2.2.2. Specificity determinations (Appendix 1)

2.2.2.1. Blood donors. Fresh samples from non-selected blood donors (n = 6020), collected by 60 different blood donation sites, were tested using the Access and Elecsys assays. For HIV reactive samples, status was confirmed by Immunoblot (IB) and HIV-1 RNA PCR (Procleix Ultrio Assay, Grifols Diagnostic Solutions Inc.), or HIV1/2 RNA PCR (Cobas 8800 MPX, Roche Molecular Systems Inc.).

2.2.2.2. Hospitalized patients. Frozen (n = 1198) and fresh (n = 896) HIV negative hospitalized patient samples were collected from five different French sites and tested with the Access and Architect assays.

2.2.2.3. Cross-reacting samples. Additionally, 260 potentially cross-reacting samples (10 individual samples covering each of 26 different categories (Table 3) were collected from commercial vendors and tested using the Access and Architect assays.

Table 3
List of potentially cross-reacting samples tested on the Access and Architect HIV combo assays.

Sample nature - potential cross-reaction	Number of samples determined negative/tested
Epstein-Bar Virus (EBV)	10
Cytomegalovirus (CMV)	10
Herpes Simplex Virus (HSV)	10
Varicella Zoster Virus (VZV)	10
Hepatitis A Virus (HAV)	10
Hepatitis B Virus (HBV)	10
Hepatitis C Virus (HCV)	10
Human T-cell Lymphotropic virus (HTLV)	10
Influenza A virus	10
Syphilis	10
E. coli (anti-*E. coli*) or *E. coli* urinary infection	10
Influenza Post Vaccination	10
Rheumatoid Factor (RF)	10
Anti-Nuclear Antibody (ANA)	10
Human Anti-Mouse Antibody (HAMA)	10
Graves' Disease	10
Crohn's Disease	10
Systemic Lupus Erythematosus (SLE)	10
Pregnancy first trimester	10
Pregnancy second trimester	10
Pregnancy third trimester	10
Pregnancy multiparous	10
Dialysis	10
Transplant/Transplant recipient	10
Toxoplasmosis	10
Hemophiliac/Clotting factor	10
Total	**260**

Table 4
Sensitivity results.

Enrollment Group	N	Access HIV Ag/Ab combo		Architect HIV Ag/Ab Combo	
		RR *	Sensitivity% (95 % CI)	RR	Sensitivity% (95 % CI)
HIV-1 Ab positive samples	784	784	100.00 % (99.51 – 100.0 %)	784	100.00 % (99.51 – 100.0 %)
HIV-2 Ab positive samples	100	100	100.00 % (96.30 – 100.0 %)	100	100.00 % (96.30 – 100.0 %)
HIV-1 p24 Ag positive samples	64	64	100.00 % (94.34 – 100.0 %)	64	100.00 % (94.34 – 100.0 %)
Total	**948**	**948**	**100.00 % (99.60 - 100.0 %)**	**948**	**100.00 % (99.60 - 100.0 %)**

* Repeatedly Reactive.

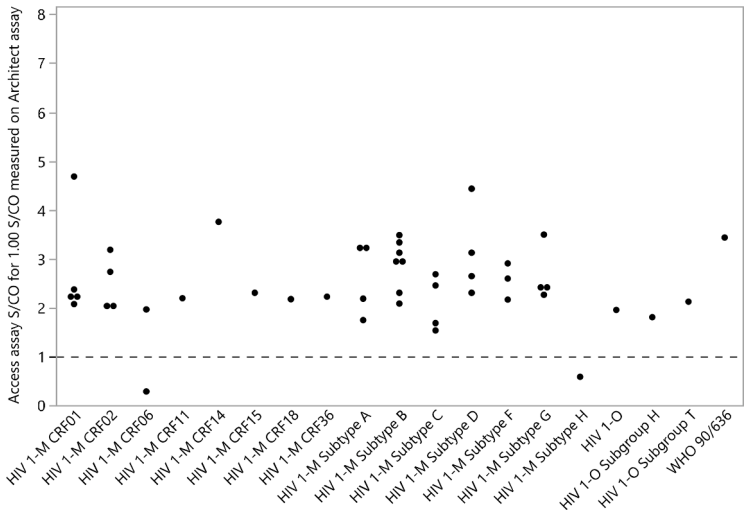

Fig. 1. HIV-1 p24 detection comparison between the Access and Architect HIV combo assays
A Weighted Deming analysis was used from the testing of different dilutions of viral lysate from 50 HIV strains (46 HIV-1 and 4 HIV-2), to determine which S/CO was obtained on the Access assay, when it was equal to 1.00 on the Architect assay. Even if HIV-2 strains were detected with the Access assay, results are not reported on the figure above since the Architect assay cannot detect p26.

Table 5
Seroconversion panel testing with the Access, Architect and Elecsys HIV combo assays.

Seroconversion Panel ID $N = 30$ 256 bleeds	Number of NAT positive panel bleeds*	Number of bleeds determined reactive per assay			Difference in days for first bleed determined reactive with serological vs NAT assay**		
		Access	Architect	Elecsys	Access	Architect	Elecsys
0600–0227	3 (3)	2 (2)	2 (2)	2 (2)	3	3	3
0600–0232	3 (2)	3 (2)	3 (2)	3 (2)	0	0	0
0600–0237	4 (2)	3 (1)	2 (0)	3 (1)	3	7	3
0600–0238	4 (3)	3 (2)	2 (1)	2 (1)	4	7	7
0600–0240	4 (3)	2 (1)	2 (1)	2 (1)	7	7	7
0600–0244	4 (4)	2 (2)	2 (2)	2 (2)	7	7	7
0600–0245	3 (2)	3 (2)	2 (1)	2 (1)	0	3	3
0600–0250	6 (2)	4 (0)	4 (0)	4 (0)	11	11	11
0600–0251	7 (4)	5 (2)	3 (0)	4 (1)	8	17	10
0600–0258	3 (2)	3 (2)	2 (1)	2 (1)	0	2	2
0600–0260	3 (3)	1 (1)	1 (1)	1 (1)	7	7	7
0600–0261	4 (4)	4 (4)	2 (2)	3 (3)	0	7	2
0600–0262	4 (2)	2 (0)	2 (0)	2 (0)	13	13	13
0600–0265	3 (3)	2 (2)	2 (2)	2 (2)	7	7	7
0600–0271	8 (4)	7 (3)	6 (2)	6 (2)	3	7	7
0600–0272	4 (2)	3 (1)	3 (1)	3 (1)	7	7	7
HIV6248	3 (2)	2 (1)	2 (1)	2 (1)	4	4	4
HIV9011	11 (9)	2 (0)	2 (0)	2 (0)	38 [8]	38 [8]	38 [8]
HIV9012	7 (4)	4 (1)	3 (0)	3 (0)	14	16	16
HIV9013	3 (2)	2 (1)	1 (0)	2 (1)	5	7	5
HIV9016	3 (3)	2 (2)	2 (2)	2 (2)	3	3	3
HIV9019	1 (0)	1 (0)	1 (0)	1 (0)	0	0	0
HIV9020	5 (3)	4 (2)	3 (1)	3 (1)	4	7	7
HIV9021	5 (3)	4 (2)	4 (2)	4 (2)	4	4	4
HIV9023	4 (3)	3 (2)	3 (2)	3 (2)	2	2	2
HIV9028	2 (2)	2 (2)	2 (2)	2 (2)	0	0	0
HIV9030	5 (3)	3 (1)	3 (1)	3 (1)	7	7	7
HIV9031	7 (4)	4 (1)	3 (0)	2 (0)	41 [7]	49 [15]	56 [22]
HIV9081	3 (0)	3 (0)	3 (0)	3 (0)	0	0	0
HIV9096	6 (3)	5 (2)	5 (2)	5 (2)	3	3	3
Total detected bleeds	**132 (86)**	**90 (44)**	**77 (31)**	**80 (35)**			
Average difference of days					**6.8 [4.7]**	**8.4 [6.3]**	**8.0 [5.9]**

Note: number of days between two bleeds can vary across bleeds of one panel.
* Number in brackets corresponds to #samples with early seroconversion sample status.
** Number in square brackets correspond to additional analysis considering second NAT assay results (panel HIV9011) or one unexpected NAT positive result removal (panel HIV9031 bleed 5 found NAT positive while bleeds 1 to 13 are NAT negative).

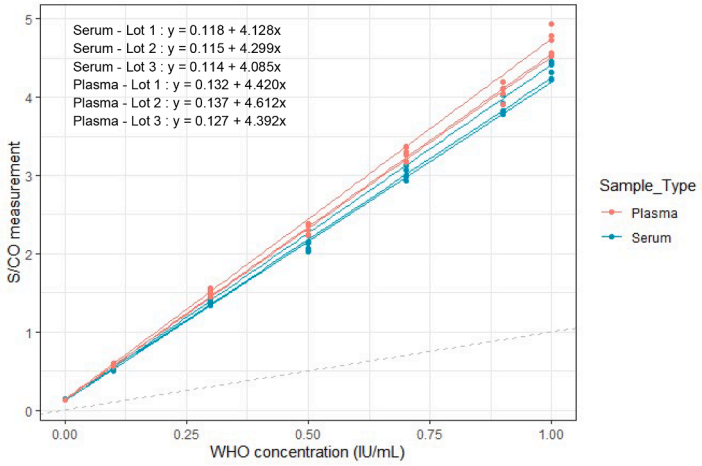

Fig. 2. WHO HIV-1 p24 analytical sensitivity of the Access HIV Ag/Ab combo assay (3 reagent pack lots; Passing Bablok Regressions)
Access assay analytical sensitivity for WHO HIV-1 p24 Ag spiked in serum and plasma (95 % CI) was determined with three reagent lots through Passing Bablok regressions as 0.22, 0.21, 0.22 IU/mL in serum and 0.20, 0.19, 0.20 IU/mL in plasma for lots 1, 2 and 3 respectively.

3. Results

3.1. Sensitivity performances

3.3.1. Clinical sensitivity

Sensitivity was 100 % with both the Access and Architect assays for the 884 known HIV Ab positive samples and the 64 known HIV-1 p24 Ag positive samples (Table 4).

Concerning the detection of the viral lysates representative of distinct HIV variants, the comparison of signal/cutoff (positive is ≥ 1.00) showed a higher sensitivity with the Access assay vs Architect assay for 44 of the 46 HIV-1 isolates, leading to an overall median S/CO = 2.45 with the 3 lots of the Access assay when 1.00 S/CO was measured on the Architect (Fig. 1). Tested in parallel of these supernatants, WHO HIV-1 p24 antigen led to S/CO = 3.51 on the Access assay when 1.00 S/CO was measured on the Architect.

Furthermore, both HIV-2 antigen groups A and B were detected with the Access assay.

3.3.2. Analytical sensitivity

Testing of 30 seroconversion panels demonstrated a higher number of detected bleeds (n) with the Access assay ($n = 90$) compared to both Elecsys ($n = 80$) and Architect ($n = 77$) (Table 5) assays: the Access assay detected eight panels one bleed earlier than the Elecsys assay and one panel two bleeds earlier. Compared to the Architect assay, the Access assay detected nine panels one bleed earlier and two panels two bleeds earlier. Separate antigen and antibody response of the Access assay demonstrated that earlier panel detection was allowed by HIV-1 p24 Ag detection.

From the 86 early seroconversion samples tested in this study, forty-four were found reactive with the Access assay compared to 35 with the Elecsys and 31 with the Architect assays. Average number of days between positive detection of a bleed previously identified NAT positive, was reduced using the Access assay (6.8 days) compared to the Elecsys and Architect assays (8.0 and 8.4 days respectively).

The data of p24 analytical sensitivity on the Access assay are presented in Fig. 2. The limit of detection was defined between 0.19 and 0.22 IU/mL (95 % CI) of WHO HIV-1 p24 antigen standard. Comparable results were obtained between the 3 lots, while a slightly better sensitivity was observed in plasma vs serum.

3.3.3. Clinical specificity

Analysis of 6020 blood donor samples demonstrated a 99.97 % specificity with the Access assay, compared to 99.93 % specificity measured with the Elecsys assay (Table 6), with two and four samples, respectively, identified as false positive, since their HIV negative status was confirmed by immunoblot and HIV-1 or HIV-1/2 PCRs. Higher specificity was measured with the Access assay compared to the Architect assay for the testing of 2094 HIV-negative hospitalized patient samples: 99.76% vs 99.09 % respectively (Table 6). This population included 893 fresh samples, for which 4 were detected as False Initial Reactive (found initial reactive but confirmed nonreactive after retest) with the Architect (0.46 %; 95 % CI: 0.18–1.16 %), but none with Access assay (0.00 %; 95 % CI: 0.00–0.43 %). Overall specificity determined with the Access assay on these two populations ($n = 8114$ HIV negative samples) was 99.91 %.

Table 6
Specificity results.

		Access HIV Ag/Ab combo assay				Reference assay[a]			
Enrollment Group	**N**	**NR**	**IR**	**RR**	**Specificity% (95 % CI)**	**NR**	**IR**	**RR**	**Specificity% (95 % CI)**
Blood donor samples	6020	6018	4	2[b]	**99.97 %** (99.88 – 99.99 %)	6016	4	4[c]	**99.93 %** (99.83 – 99.97 %)
Hospitalized patient samples	2094	2089	6	5[d]	**99.76 %** (99.44 – 99.90 %)	2075	23	19	**99.09 %** (98.59 – 99.42 %)
Fresh	896	893	3	3		879	21	17	
Frozen	1198	1196	3	2		1196	2	2	
Overall Specificity	8114	8107	10	7	**99.91 %** (99.82 – 99.96 %)				

[a] HIV Reference assay was the Abbott Architect HIV Ag/Ab Combo for the hospitalized patient samples and the Roche Elecsys HIV Duo for the blood donor samples.
[b] RR with the Access assay, Nonreactive (NR) with the Elecsys assay, NR on Immunoblot (IB) and HIV-1 PCR.
[c] 2 samples RR with the Elecsys assay, NR with the Access assay, NR on Immunoblot (IB) and HIV-1 PCR; 2 samples RR with the Elecsys assay, NR with the Access assay, NR on Immunoblot (IB) and HIV-1/2 PCR.
[d] RR with the Access assay, including 2 fresh samples RR with the Architect assay, Indeterminate WB and negative HIV-1 PCR result, and 3 samples (1 fresh and 2 frozen), NR with the Architect assay, negative IB/Western blot (WB) and negative HIV-1 PCR results.

Samples (n = 260) covering 26 different potentially cross-reactive factors were evaluated on the Access assay which showed no false positive reaction and a 100 % overall negative agreement with the Architect assay.

4. Discussion

This work is the first large scale evaluation of the new Access HIV Ag/Ab combo assay on the DxI 9000 Access Immunoassay Analyzer. Its performances were evaluated on a wide panel of positive samples representative of many variants found around the world (including HIV-2 and rarer group O), as well as a large panel of negative samples. The early detection capability of this kit was also evaluated using 30 seroconversions panels. Two major commercial kits from Abbott and Roche were used for comparison. The data are then robust.

Clinical sensitivity was found to be 100 % for clinical samples as well as for viral lysates. To note that this new test detected all group O samples (both antibodies and antigenic) for which antigenic diversity could be a difficulty [16]. To include the largest genetic diversity, we also added viral lysates representative of many variants that can be found in different geographical regions. The data showed a higher detection performance with the Access assay for the corresponding antigens, even for HIV-2 samples. However, it will be essential to evaluate many other variants that were not included in this work.

In details, the Access assay showed better clinical sensitivity on viral lysates and early detection on seroconversion panels. Indeed, the S/CO values were higher for 44 out the 46 lysates on the Access assay in comparison to Architect assay, and the p24 antigen was detected sooner for the Access assay by 1.2 and 1.6 days compared to the Elecsys and Architect assays, respectively. The limit of p24 detection determined around 0.22 IU/mL on the Access assay is also lower than the one reported in the literature for the Abbott Architect HIV Ag/Ab Combo (0.7–1.2 IU/mL) [17,18] and Roche Elecsys HIV Duo (0.3 IU/mL) assays [15]. These data are very interesting for a fourth-generation assay, which is now essential to diagnose very early infections and initiate patient care management. Furthermore, this assay developed for the new DxI 9000 analyzer now offers the advantage of independent reporting of the antigen assay result from that of the antibody assay, plus a final qualitative combo interpretation (reactive / nonreactive). Sub-results analysis, particularly for the antigen, could facilitate early detection of acute HIV infection. The overall specificity was 99.91 %, with a better result for this latter parameter compared to the Architect and Elecsys assays. No interference was found using a specific panel of 260 potentially cross-reactive samples.

This study was performed at multiples sites, an important point since it indicates that data were not produced by only one laboratory, with the question of a possible “local” bias. This emphasizes again the robustness of the Access HIV Ag/Ab combo assay dedicated to the DxI 9000 analyzer recently launched on the market.

Performances of this assay are likely associated with technological improvements made to the Beckman Coulter DxI 9000 Access Immunoassay Analyzer. The use of a new substrate (Lumi-Phos PRO) as well as the optimization of low-volume pipetting capabilities allows for increased sensitivity of immunoassays such as the Access HIV Ag/Ab combo test, while preserving valuable patient samples.

In conclusion, this work demonstrates that the new Access HIV Ag/Ab combo assay, presents performances allowing diagnosis of chronic as well as primary HIV infections, despite the high level of genetic diversity of these viruses.

CRediT authorship contribution statement

V. Lemée: Writing – review & editing, Writing – original draft, Investigation. **S. Gréaume:** Writing – review & editing, Writing – original draft, Investigation. **J. Gautier:** Writing – review & editing, Writing – original draft, Investigation. **S.A. Dzamitika:** Writing – review & editing, Writing – original draft, Investigation. **C. Coignard:** Writing – review & editing, Writing – original draft, Investigation. **S.A. Jortani:** Writing – review & editing, Writing – original draft, Investigation. **B. Grillet:** Writing – review & editing, Writing – original draft, Investigation. **M. Badawi:** Writing – review & editing, Writing – original draft, Investigation. **J-C. Plantier:** Writing – review & editing, Writing – original draft, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix 1

Testing and collection sites: CHU Rouen, Department of Virology, National Reference Center of HIV, Rouen, France; Etablissement Français du Sang (EFS) Hauts-de-France – Normandie (HFNO), Bois Guillaume, France; Cerba Xpert, Cergy-Pontoise, France; Eurofins Biomnis, Ivry-sur-Seine, France.

Testing only sites: Beckman Coulter Immunotech, Marseille, France; University of Louisville School of Medicine, Louisville, KY, USA; Kentucky Clinical Trials Laboratory (KCTL), Louisville, KY, USA.

Additional sample collection sites and commercial vendors : Biomex GmbH, LGC Clinical Diagnostics/SeraCare Life Sciences, Heidelberg, Germany; SlieaGen LLC, Austin, TX, USA; Boca Biolistics, FL, USA; Etablissement Français du Sang (EFS), Tours, France; Centre Hospitalier Universitaire (CHU) Amiens-Picardie, Amiens, France.

References

[1] T. Mourez, F. Simon, J.C. Plantier, Non-M variants of human immunodeficiency virus type 1, Clin. Microbiol. Rev. 26 (3) (2013) 448–461, https://doi.org/10.1128/CMR.00012-13.

[2] E. Alessandri-Gradt, A. Moisan, J.C. Plantier, HIV-1 non-group M strains and ART, Viruses 15 (3) (2023) 780, https://doi.org/10.3390/v15030780. PMID: 36992488. PMCID: PMC10058373.

[3] A. Williams, S. Menon, M. Crowe, N. Agarwal, J. Biccler, N. Bbosa, D. Ssemwanga, F. Adungo, C. Moecklinghoff, M. Macartney, V. Oriol-Mathieu, Geographic and population distributions of human immunodeficiency virus (HIV)-1 and HIV-2 circulating subtypes: a systematic literature review and meta-analysis (2010-2021), J. Infect. Dis. 228 (11) (2023) 1583–1591, https://doi.org/10.1093/infdis/jiad327. PMID: 37592824.

[4] Q. Le Hingrat, et al., Genetic variability of long terminal repeat region between HIV-2 groups impacts transcriptional activity, J. Virol. (2020). PMID: 31915276.

[5] N. Bbosa, P. Kaleebu, D. Ssemwanga, HIV subtype diversity worldwide, Curr Opin HIV AIDS 14 (3) (2019) 153–160, https://doi.org/10.1097/COH.0000000000000534. PMID: 30882484.

[6] Bharat S. Parekh, Chin-Yih Ou, Peter N. Fonjungo, Mireille B. Kalou, Erin Rottinghaus, Adrian Puren, Heather Alexander, Mackenzie Hurlston Cox, John N. Nkengasong, Diagnosis of human immunodeficiency virus infection, Clin. Microbiol. Rev. 32 (1) (2019), https://doi.org/10.1128/CMR.00064-18 e00064-18. Published online 2018 Nov 28PMCID: PMC6302353.

[7] A. Ayouba, P. Mauclère, P.M. Martin, P. Cunin, J. Mfoupouendoun, B. Njinku, S. Souquières, F. Simon, HIV-1 group O infection in Cameroon, 1986 to 1998, Emerg. Infect. Dis. 7 (3) (2001) 466–467, https://doi.org/10.3201/eid0703.010321. PMID: 11384531; PMCID: PMC2631804.

[8] H. Agut, D. Candotti, B. Rabanel, J.M. Huraux, G. Remy, D. Ingrand, T. Tabary, C. Chippaux, S. Chamaret, D. Guetard, et al., Isolation of atypical HIV-1-related retrovirus from AIDS patient, Lancet 340 (8820) (1992) 681–682, https://doi.org/10.1016/0140-6736(92)92226-6. PMID: 1355252.

[9] N.R. Faria, A. Rambaut, M.A. Suchard, G. Baele, T. Bedford, M.J. Ward, A.J. Tatem, J.D. Sousa, N. Arinaminpathy, J. Pépin, D. Posada, M. Peeters, O.G. Pybus, Lemey P.HIV epidemiology, The early spread and epidemic ignition of HIV-1 in human populations, Science 346 (6205) (2014) 56–61, https://doi.org/10.1126/science.1256739. Epub 2014 Oct 2. PMID: 25278604; PMCID: PMC4254776.

[10] A. Mühlbacher, H. Schennach, J. van Helden, T. Hebell, G. Pantaleo, P. Bürgisser, C. Cellerai, P. Permpikul, M.I. Rodriguez, A. Eiras, F. Alborino, P. Cunningham, M. Axelsson, S. Andersson, O. Wetlitzky, C. Kaiser, P. Möller, G. de Sousa, Performance evaluation of a new fourth-generation HIV combination antigen-antibody assay, Med Microbiol Immunol 202 (1) (2013) 77–86, https://doi.org/10.1007/s00430-012-0250-5. Epub 2012 Jun 17. PMID: 22706797; PMCID: PMC3562432.

[11] M.D. Krasowski, D. Wier, S. Smith, A. Riedel, Y. Lauseker-Hao, M. Kelner, S. Wang, Real-world clinical performance evaluation of a fourth-generation HIV Antigen/Antibody differentiation test, J. Appl. Lab. Med 6 (6) (2021) 1417–1432, https://doi.org/10.1093/jalm/jfab069. PMID: 34333654.

[12] D.R. Hardie, S.N. Korsman, N.Y. Hsiao, M.D. Morobadi, S. Vawda, D. Goedhals, Contamination with HIV antibody may be responsible for false positive results in specimens tested on automated platforms running HIV 4th generation assays in a region of high HIV prevalence, PLoS One 12 (7) (2017) e0182167, https://doi.org/10.1371/journal.pone.0182167. PMID: 28759622; PMCID: PMC5536287.

[13] S. Jurriaans, N.K. Back, K.C. Wolthers, Ten years of HIV testing with fourth generation assays: the Amsterdam experience, J. Clin. Virol. 52 (Suppl 1) (2011) S67–S69, https://doi.org/10.1016/j.jcv.2011.09.021. Epub 2011 Oct 12. PMID: 21995933.

[14] P. Chavez, L. Wesolowski, P. Patel, K. Delaney, S.M. Owen, Evaluation of the performance of the Abbott ARCHITECT HIV Ag/Ab Combo Assay, J. Clin. Virol. 52 (Suppl 1) (2011) S51–S55, https://doi.org/10.1016/j.jcv.2011.09.010. DecEpub 2011 Oct 7. PMID: 21983253.

[15] A. Mühlbacher, S. Sauleda, M. Piron, R. Rietz, P. Permpikul, M. Klinkicht, M. Gloeck, R. Imdahl, A multicentre evaluation of the Elecsys® HIV Duo assay, J. Clin. Virol. 112 (2019) 45–50, https://doi.org/10.1016/j.jcv.2018.11.005. Epub 2018 Nov 20. PMID: 30611626.

[16] J.C. Plantier, M. Djemai, V. Lemée, A. Reggiani, M. Leoz, L. Burc, A. Vessière, D. Rousset, J.D. Poveda, C. Henquell, A. Gautheret-Dejean, F. Barin, Census and analysis of persistent false-negative results in serological diagnosis of human immunodeficiency virus type 1 group O infections, J. Clin. Microbiol. 47 (9) (2009) 2906–2911, https://doi.org/10.1128/JCM.00602-09. Epub 2009 Jul 22. PMID: 19625478.

[17] M. Miedouge, M. Grèze, A. Bailly, J. Izopet, Analytical sensitivity of four HIV combined antigen/antibody assays using the p24 WHO standard, J. Clin. Virol. 50 (1) (2011) 57–60, https://doi.org/10.1016/j.jcv.2010.09.003. PMID: 20926337.

[18] X. Qiu, L. Sokoll, P. Yip, D.J. Elliott, R. Dua, P. Mohr, X.Y. Wang, M. Spencer, P. Swanson, G.J. Dawson, J. Hackett Jr., Comparative evaluation of three FDA-approved HIV Ag/Ab combination tests using a genetically diverse HIV panel and diagnostic specimens, J. Clin. Virol. 92 (2017) 62–68, https://doi.org/10.1016/j.jcv.2017.05.005. Epub 2017 May 13. PMID: 28535437.