MULTICENTER EVALUATION OF THE NEW ACCESS HIV AG/AB COMBO ASSAY WITH HIGHLY SENSITIVE HIV-1 P24 ANTIGEN DETECTION ON THE DXI 9000 ACCESS IMMUNOASSAY ANALYZER

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BACKGROUND

Beckman Coulter developed the fully automated Access HIV Ag/Ab combo assay for the new DxI 9000 Immunoassay analyzer*. This paramagnetic particle chemiluminescent immunoassay is dedicated to the simultaneous qualitative *in vitro* detection and differentiation of HIV-1 p24 antigen (HIV-Ag) and HIV-1/2 antibody (HIV-Ab). Assay design (Figure 1) allows for the reporting of two sub-results for antigen and antibody, provided as both a signal-to-cutoff ratio (S/CO) and as a qualitative interpretation ("reactive" or "nonreactive"), and a combined result provided as qualitative interpretation on the DxI 9000 analyzer (Figure 2). Time to first result is limited to ~30 minutes and sample volume is only 60 µL.

The aim of this study was to assess the Ag sensitivity (critical for early detection of HIV infection (Figure 3)) of the Access HIV Ag/Ab combo assay especially through the testing of seroconversion panels, HIV-1 p24 Ag WHO International Reference Reagent (NIBSC code 90/636), and HIV-1 and HIV-2 Ag from different viral strains. Furthermore, imprecision of this Access HIV Ag/Ab combo assay was evaluated as clinical specificity and sensitivity.

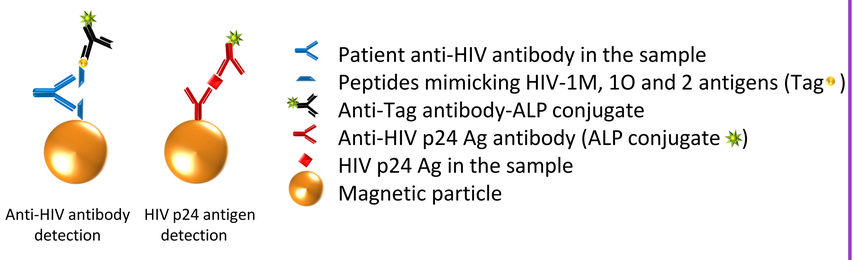


Figure 1. Access HIV Ag/Ab combo assay design based on the use of independent detection of both Ag and Ab.

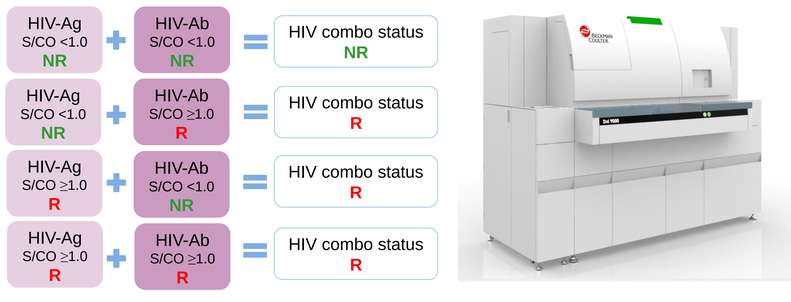


Figure 2. Access HIV Ag/Ab combo assay result interpretation (R= Reactive, NR = Nonreactive). Sub-results for HIV-Ag and HIV-Ab are also available for review.

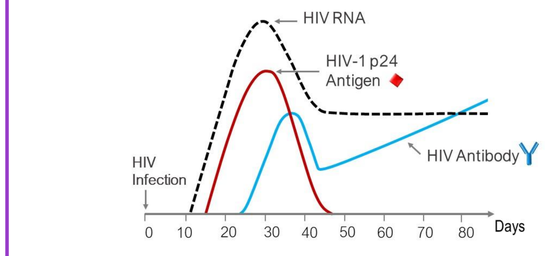


Figure 3. HIV biomarker evolution during early infection. Early detection of HIV-1 p24 antigen enables the shortening of the window period compared to HIV-1/2 antibody tests.

METHOD

- Thirty commercially available seroconversion panels were run on the Access HIV Ag/Ab combo (3 lots tested), the Abbott ARCHITECT HIV Ag/Ab Combo, and the Roche Elecsys HIV Duo assays.
- The HIV-1 p24 Ag analytical sensitivity of the Access assay was determined for three reagent pack and calibrator lots: testing of dilutions of HIV-1 p24 Ag WHO International Reference Reagent (NIBSC code 90/636) prepared in HIV negative human serum and plasma, ranging from 0.1 to 1.0 IU/mL.
- Fifty 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 (groups A and B) were spiked into HIV negative human-plasma and tested on both the Access and ARCHITECT assays. A Weighted Deming analysis was applied for each viral lysate to establish the S/CO obtained on the Access assay corresponding to 1.00 S/CO on the ARCHITECT assay.
- Imprecision of Ag module was evaluated through the testing of an eight-member panel of patient samples for HIV-1 p24 antigen, including serum (S), plasma (P), and Access HIV Ag/Ab combo QC samples (QC1= negative; QC3 = Ag positive). They were assayed in duplicate in two runs per day over 20 days. Three lots of Access HIV Ag/Ab combo reagent, calibrator and QC were tested on three DxI 9000 Immunoassay Analyzers for the study (one lot per instrument).
- Clinical sensitivity and specificity were determined for the Access assay using the ARCHITECT assay as comparator by testing samples from HIV low-risk, high-risk and HIV known positive populations at three United States clinical laboratories. An HIV 1/2 differentiation assay and HIV-1 RNA PCR were also run to confirm patient infection status.

RESULTS

Detection of seroconversion panels by the Access, ARCHITECT and Elecsys assays: the Access assay detected a higher number of bleeds (n=90) than the Elecsys (n = 80) and ARCHITECT (n = 77) assays.

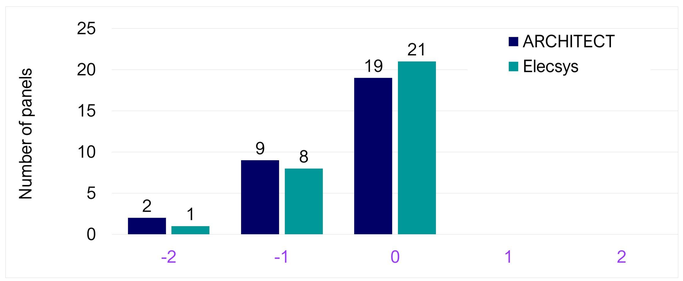


Figure 4. Difference in number of detected bleeds between the Access and ARCHITECT or Elecsys assays. The Access assay detected reactivity earlier in 11 of 30 or 9 of 30 tested panels versus ARCHITECT and Elecsys respectively, due to earlier antigen detection.

The average number of days observed for serological tests compared to the first bleed previously determined reactive with Nucleic Acid Test (NAT): Access = 6.8 days, Elecsys = 8.0 days, ARCHITECT = 8.4 days.

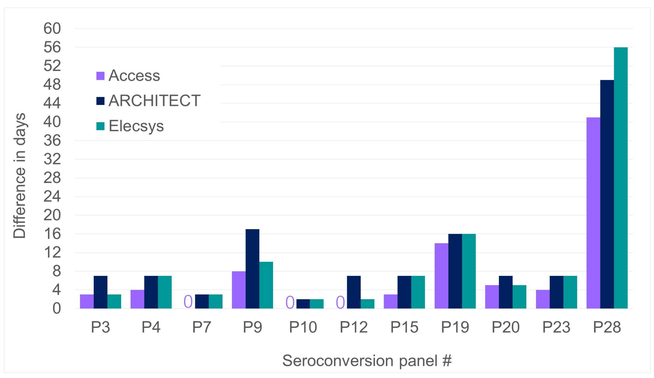
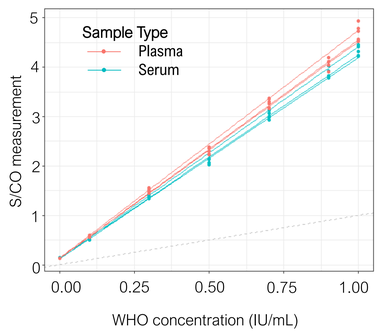


Figure 5. Difference in days between the first bleed determined reactive with serological vs NAT assay was reduced by 1.2 to 1.6 days using the Access assay (Only panels leading to difference between assays are reported; 0 is reported on the graph when no difference was measured between serological and NAT assay).

Analytical sensitivity – HIV-1 p24 WHO (90/636)



Sample Type	Reagent Lot	Intercept	Slope	Analytical sensitivity HIV-1 p24 Ag IU/mL (95% CI)
Serum	#1	0.118	4.128	0.22
	#2	0.115	4.299	0.21
	#3	0.114	4.085	0.22
Plasma	#1	0.132	4.420	0.20
	#2	0.137	4.612	0.19
	#3	0.127	4.392	0.20

Figure 6 and Table 1 describe HIV-1 p24 WHO reference reagent analytical sensitivity results of the Access HIV Ag/Ab combo assay (Passing Bablok Regressions), ranging between 0.19 and 0.22 IU/mL in plasma and serum.

HIV-1 p24 Ag sensitivity in viral lysates across multiple genotypes

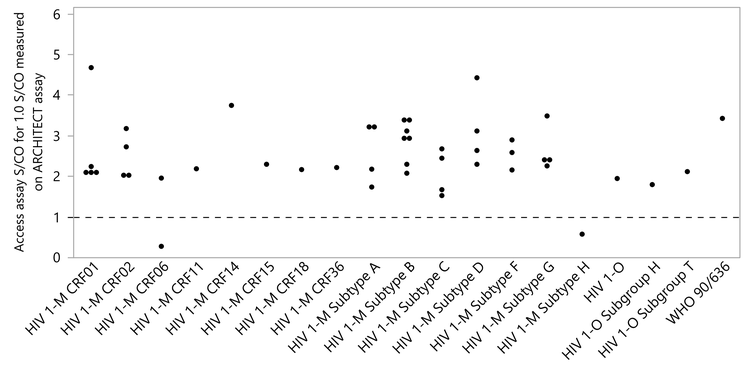


Figure 7. The Access assay demonstrated a higher sensitivity for HIV-1 p24 Ag compared to the ARCHITECT assay*: overall median Signal/Cut-Off = 2.45 S/CO for the 46 HIV-1 strains and 3.86 S/CO for the WHO 90/636 with the Access assay when the ARCHITECT assay was normalized at 1.00 S/CO.

* Note: HIV-2 strains were detected by the Access assay but not by the Architect assay. Therefore, these results were excluded from this calculation and Figure 7.

Table 2: Imprecision values of the Access HIV Ag/Ab combo assay on DxI 9000 analyzer.

Sample	N	Mean (S/CO)	Repeatability (Within-run)		Between-run		Between-day		Within-Laboratory	
			SD (S/CO)	%CV	SD (S/CO)	%CV	SD (S/CO)	%CV	SD (S/CO)	%CV
QC1 (negative)	80	0.12	0.011	9.0%	0.003	2.4%	0.002	1.4%	0.012	9.3%
QC3 (HIV-Ag)	80	3.06	0.035	1.1%	0.058	1.9%	0.050	1.6%	0.068	2.2%
S1	80	0.15	0.010	6.8%	0.004	2.9%	0.001	0.9%	0.011	7.3%
S3	80	0.79	0.011	1.3%	0.022	2.8%	0.020	2.6%	0.025	3.1%
S10	80	1.08	0.015	1.4%	0.013	1.2%	0.011	1.0%	0.020	1.9%
S11	80	3.64	0.042	1.2%	0.067	1.9%	0.059	1.6%	0.079	2.2%
P2	80	0.86	0.011	1.2%	0.017	1.9%	0.000	0.0%	0.020	2.3%
P6	80	1.18	0.015	1.3%	0.017	1.4%	0.006	0.5%	0.023	1.9%

Maximum within-Laboratory %CV were limited to 2.3% for positive samples and SD to 0.025 S/CO for negative samples

Table 3. Clinical specificity and sensitivity results of the Access and ARCHITECT assays.

	N	Access HIV Ag/Ab combo assay	ARCHITECT HIV Ag/Ab Combo assay
Specificity on low-risk pop	6,981	99.6 % (95% CI: 99.5-99.8)	98.8 % (95% CI: 98.5-99.0)
Specificity on high-risk pop	1,486	99.8 % (95% CI: 99.4-99.9)	99.2 % (95% CI: 98.6-99.5)
Sensitivity (known Ag or Ab pos)	1,787	100.0 % (95% CI: 99.8-100.0)	99.8 % (95% CI: 99.5-99.9)

Higher sensitivity and specificity were observed with the Access HIV Ag/Ab combo assay.

CONCLUSION

The new Access HIV Ag/Ab combo assay demonstrated excellent analytical performances, particularly a high HIV-1 p24 Ag sensitivity.

This new assay developed on the DxI 9000 analyzer, allows for the separate reporting of antigen and antibody results from one reagent pack.

The results reported herein indicate the new Access HIV Ag/Ab combo assay could be a powerful tool for reducing the diagnosis window and, thus, preventing a delay of treatment for an infected patient.

In addition, the assay showed clinical sensitivity and specificity performance meeting or exceeding that of two currently marketed HIV combo immunoassays.