Evaluation of the BioPlex® 2200 HIV Ag-Ab assay*: A Fully Automated Screening Method Providing Discrete Detection of HIV-1 p24, HIV-1 Antibody, and HIV-2 Antibody

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Abstract

Purpose:
Evaluate the performance of an automated HIV assay with enhanced 4th generation sensitivity that can report HIV-1 antibody and antigen results individually, and differentiate HIV-1 from HIV-2 infection.

Methods:
The BioPlex* 2200 HIV Ag-Ab assay is a multiplex flow immunoassay intended for the simultaneous qualitative detection and differentiation of the individual analytes HIV-1 p24 antigen, HIV-1 (groups M and O) antibodies, and HIV-2 antibodies in a single reaction vessel using a mixture of four populations of dyed microparticles. Each population is coated with a different HIV antigen or with HIV-1 p24 antibody. It can be used to order an overall combination result for HIV with detailed results for HIV-1 p24 antigen, HIV-1 antibody, and/or HIV-2 antibody. Specimens reactive for antibody are typed as HIV-1 or HIV-2, or are reported as undifferentiated if typing is unresolved. Studies presented were performed at four external sites and at Bio-Rad Laboratories using three lots of reagents.

Results:
To assess specificity, 6395 samples from populations at low risk for HIV infection (unknown HIV status) were tested resulting in specificity of 99.86%. The analytical sensitivity for HIV-1 p24 antigen was 0.33 IU/mL when testing the WHO HIV international standard NIBSC 90/636 and 5.2 pg/mL when testing a standard approved by the Agence français de sécurité sanitaire des produits de santé (AFSSAPS). Additionally, 54 cell culture supernatants of varying subtype were tested and HIV-1 p24 was detected in all. To assess clinical sensitivity, 1483 known HIV-1 Ab specimens (including 63 group O) and 200 HIV-2 Ab specimens were tested and all were reactive. All HIV-1 samples were correctly identified as HIV-1 and 187 of 200 (93.5%) HIV-2 samples were correctly identified as HIV-2, the remaining 13 were HIV Ab reactive undifferentiated. Sensitivity was also assessed by testing 42 commercially available seroconversion panels with the BioPlex* 2200 HIV HIV Ag-Ab assay and a FDA-approved HIV Ag/Ab assay. The first reactive bleed occurred earlier with the BioPlex* 2200 HIV Ag-Ab in seven of the panels, one panel was detected one bleed earlier by the FDA-approved HIV Ag/Ab assay and both tests gave equivalent results for the remaining 34 of the 42 panels. Specimens from brain dead individuals were tested for specificity, sensitivity and reproducibility and found to be equivalent to normal donors allowing for testing of organ donors.

Conclusion:
The BioPlex* 2200 HIV Ag-Ab assay is highly sensitive and specific, and can provide detailed HIV screening results earlier that will assist in identifying specimens from acute or primary HIV-1 infection and chronic HIV-2 infection and in guiding the selection of supplemental testing.

Introduction

HIV antigen/antibody (Ag/Ab) combination or 4th generation assays represent a significant advancement in assays used for diagnosing HIV infection, based on their ability to detect acute and established infections. These combination assays provide improved sensitivity reducing the average window period of detection from 20-30 days to 15-20 days.[1-4]

Although these technological advances have improved HIV screening, current HIV Ag/Ab combination assays have limitations. HIV Ag/Ab combination assays cannot provide individual results for each analyte, distinguish between acute and established infections or differentiate between HIV-1 and HIV-2 infections.

The BioPlex* 2200 HIV Ag-Ab assay is the next generation in HIV screening. Improving on 4th generation capabilities, the BioPlex* 2200 HIV Ag-Ab 5th generation assay simultaneously detects, differentiates, and reports HIV-1 p24 antigen and antibodies to HIV-1 (groups M and O) and HIV-2 using a fully automated, random access analyzer.

The Next (5th) Generation HIV Screen:
- Has high sensitivity for HIV-1 and HIV-2 infection
- Identifies specimens from acute HIV-1 infection
- Differentiates between HIV-1 Ab and HIV-2 Ab
- Provides individual results for HIV-1 p24 Ag, HIV-1 Ab and HIV-2 Ab
- Has high specificity
- Can guide the selection of supplemental testing

Purpose

To evaluate the performance of an automated HIV assay with enhanced sensitivity that can report antibody and antigen results individually, and distinguish HIV-1 from HIV-2.

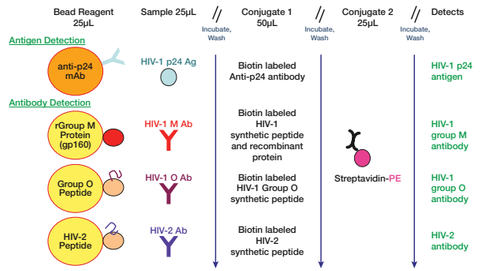
Assay Principle

The BioPlex* 2200 HIV Ag-Ab assay is a multiplex flow immunoassay intended for the simultaneous qualitative detection and differentiation of the individual analytes HIV-1 p24 antigen, HIV-1 (groups M and O) antibodies, and HIV-2 antibodies (Figure 1). It is used as a combination test that reports a single result for all the HIV analytes in addition to providing individual results for the specific HIV analytes. Each sample is analyzed using a mixture of seven bead populations:

- Monoclonal antibody against HIV-1 p24 antigen
- HIV-1 gp160 (group M) recombinant protein
- Synthetic peptide mimicking an artificial HIV-1 (group O) epitope
- Synthetic peptide mimicking the immunodominant epitope of the HIV-2 envelope protein
- Signal normalization bead (SNB) to normalize raw signal values
- Internal standard bead (ISB) to monitor detector fluctuations
- Serum verification bead (SVB) to verify the addition of serum or plasma to the reaction vessel

After incubation with serum or plasma, the beads are washed to remove unbound patient sample. Conjugate 1 is added forming a biotinylated Ag-Ab-Ag complex and/or Ab-Ag-Ab complex, and is followed by a wash to remove excess conjugate. Conjugate 2 is then added whereby phycoerythrin (PE) labeled streptavidin binds the biotinylated Ab-Ag-Ab and Ag-Ab-Ag complexes captured on the dyed beads and a third wash step follows. Bound patient HIV-1 p24 antigen, HIV-1 antibodies and HIV-2 antibodies are detected by fluorescence of the PE-bound Ab-Ag-Ab and Ag-Ab-Ag complexes.

Figure 1: The 5th generation assay design allows for the simultaneous detection and identification of multiple HIV analytes for each sample processed



Methods

Specificity was determined by analysis of 6395 samples from low risk populations including first time blood donors, normal healthy patients, military recruits, pregnant women and healthy pediatric subjects, using three lots of BioPlex* 2200 HIV Ag-Ab assay. Reactive samples were also tested by an FDA-licensed HIV-1 RNA assay and an FDA-approved HIV-1/HIV-2 differentiation assay.

The analytical sensitivity for HIV-1 p24 antigen was assessed by testing dilutions of the WHO HIV international standard NIBSC 90/636 and dilutions of a standard approved by the Agence français de sécurité sanitaire des produits de santé (AFSSAPS) and interpolating the standards' concentration at the assay cutoff. Additionally, 54 inactivated cell culture supernatants of varying subtypes were tested for HIV-1 p24 detection.

To test antibody sensitivity and the ability to differentiate HIV-1 and HIV-2, 1683 known positive samples were tested (1483 HIV-1 including 63 HIV-1 group O and 200 HIV-2). Additionally, 42 seroconversion panels were tested and results were compared to an FDA-approved HIV Ag/Ab assay.

Reproducibility testing was performed at three sites. Each of the panel members was tested in replicates of three on one run per day for five days with three lots of the BioPlex* 2200 HIV Ag-Ab assay (45 replicates per member per site). The data were analyzed for intra-assay and inter-assay reproducibility according to the principles described in the Clinical and Laboratory Standards Institute (CLSI) guidance EP15-A2 and the International Standards Organization guidance ISO/TR 22971:2005. The mean Index value, standard deviation (SD) and percent coefficient of variation (% CV) were calculated.

Organ donor studies were performed by testing 50 known HIV negative samples from brain dead donors and 50 known HIV negative normal living donor samples for specificity and then spiking them with the four HIV analytes (HIV-1 antigen, HIV-1 group M antibody, HIV-1 group O antibody, and HIV-2 antibody) at a potency near the assay's cutoff to test for sensitivity. Reproducibility was assessed by spiking 20 specimens from each population with the four HIV analytes and testing in a single replicate for six days with three lots of reagent, resulting in 18 replicates per sample and a total of 360 replicates per analyte.

BioPlex® 2200 HIV Ag-Ab Result Interpretation

Specimens were interpreted as reactive if the Index value was ≥ 1.00 and non-reactive if < 1.00. Reactive specimens were repeated in duplicate and, if at least one replicate was ≥ 1.00, the sample was deemed reactive. If the HIV-1 Ab Index was at least 5 times the HIV-2 Ab Index, the specimen was classified as HIV-1 Ab reactive. Conversely, if the HIV-2 Ab Index was at least 5 times the HIV-1 Ab Index, the specimen was classified as HIV-2 Ab reactive. If neither of these applied, dual reactive specimens were classified as HIV Ab reactive, undifferentiated.

Index (IDX)	Retest	Retest Result	Final Interpretation
< 1.00 for all analytes	No	Not Applicable	Non-Reactive
≥ 1.00 for at least one analyte	Yes Duplicate	Both retest results have an Index (IDX) < 1.00 for all analytes	Non-Reactive
		Index (IDX) of at least one retest result is ≥1.00 for the analyte(s) that was initially reactive	REACTIVE for HIV Ag-Ab with REACTIVE for HIV-1 Ag and/or REACTIVE for HIV-1 Ab and/or REACTIVE for HIV-2 Ab or REACTIVE, Undifferentiated

Results

Specificity

Of the 6395 low risk samples tested, 6367 were non-reactive in the BioPlex* 2200 HIV Ag-Ab assay, 19 were confirmed positive by supplemental testing and nine were falsely reactive by the BioPlex* 2200 HIV Ag-Ab resulting in 99.86% overall specificity.

BioPlex® 2200 HIV Ag-Ab: Specificity

Sample Status	HIV Ag-Ab (Composite of All Assays)	HIV-1 Ab	HIV-1 p24 Ag	HIV-2 Ab
True Negative	6367			
False Positive*	9	5	6	2
Confirmed Positive	19			
% Specificity	99.86 (95% CI 99.73-99.93)			

*False Positive reactivity may occur on more than one assay bead for the same sample. Therefore, FP results among the different assay beads are not additive and counted only once in the HIV Ag-Ab composite result.

HIV-1 p24 Sensitivity

Dilutions of the WHO and AFSSAPS HIV-1 p24 standards were tested using three kit lots; the average sensitivity was 0.33 IU/mL and 5.2 pg/mL, respectively.

BioPlex® 2200 HIV Ag-Ab: HIV-1 p24 Sensitivity

Standard	Standard Concentration Corresponding to Cut Off			
	Lot 1	Lot 2	Lot 3	Average
WHO (IU/mL)	0.35	0.29	0.35	0.33
AFSSAPS (pg/mL)	5.4	5.0	5.2	5.2

HIV-1 p24 sensitivity was also tested by assaying 54 (52 group M, 2 group O) cell culture supernatants of various subtypes. All were reactive in the BioPlex* 2200 HIV Ag-Ab kit by the HIV-1 p24 Ag assay.

BioPlex® 2200 HIV Ag-Ab: HIV-1 p24 Antigen Subtypes

HIV-1 p24 Subtype	# Tested	# Reactive
A	3	3
CRF01_AE	10	10
CRF01_AG	2	2
B	13	13
C	8	8
D	3	3
F	5	5
G	4	4
H	1	1
J	2	2
N	1	1

Sensitivity with Known HIV-1 and HIV-2 Positive Samples

Of the 1683 (1483 HIV-1 including 63 HIV-1 group O and 200 HIV-2) known positive samples tested, all were reactive. All 1483 HIV-1 samples were correctly identified as HIV-1, and 187 of 200 HIV-2 samples were correctly identified as HIV-2. The remaining 13 samples were classified as HIV Ab reactive, undifferentiated. These 13 samples with HIV reactive undifferentiated results were tested with an FDA-approved HIV-1/HIV-2 differentiation assay. Two were undifferentiated, and the remaining 11 were HIV-2 positive (1 was HIV-2 positive when tested undiluted, 8 were HIV-2 positive when tested at 1:10 dilution and 2 were HIV-2 positive when tested at 1:100 dilution).

BioPlex® 2200 HIV Ag-Ab: HIV-1/HIV-2 Differentiation

Population	BioPlex® 2200 HIV Ag-Ab Result			
	HIV-1 Ab Reactive	HIV-2 Ab Reactive	HIV Ab Reactive, Undifferentiated	% Differentiation Capability
HIV-1 Known Positive	1483	0	0	100
HIV-2 Known Positive	0	187	13	93.5

In addition to known HIV positive samples, 216 HIV-1 group M subtype samples were assayed and all were reactive in the BioPlex* 2200 HIV Ag-Ab kit by the HIV-1 Ab assay.

BioPlex® 2200 HIV Ag-Ab: HIV-1 group M Antibody Subtypes

HIV-1 Ab Subtype	# Tested	# Reactive	HIV-1 Ab Subtype	# Tested	# Reactive
A	29	29	CRF13	7	7
B	4	4	D	16	16
C	5	5	F	20	20
CRF01	11	11	G	16	16
CRF02	77	77	H	7	7
CRF05	1	1	J	2	2
CRF06	3	3	K	3	3
CRF07	1	1	U	1	1
CRF09	2	2	Unknown	1	1
CRF11	10	10			

Seroconversion Panels

Sensitivity was also assessed by testing 42 commercially available seroconversion panels (365 total members) with the BioPlex* 2200 HIV Ag-Ab assay and an FDA-approved HIV Ag/Ab assay. Both the BioPlex* 2200 HIV Ag-Ab assay and the FDA-approved HIV Ag/Ab assay detected reactive bleeds in 100% (42/42) of the seroconversion panels. Of these panels, the first reactive bleed occurred earlier on the BioPlex* 2200 HIV Ag-Ab in 16.7% (7/42) of the panels. A total of 81.0% (34/42) of the panels were detected at the same bleed and 1 panel (2.4%) was detected 1 bleed earlier by the FDA-approved HIV Ag/Ab assay.

BioPlex® 2200 HIV Ag-Ab: Reactivity in HIV-1 Seroconversion Panels

Seroconversion Panels	BioPlex® 2200			FDA-approved HIV Ag/Ab assay
	HIV Ag-Ab	HIV-1 Ab	HIV-1 p24 Ag	
Total Reactive Bleeds	214	140	133	205
Total Reactive Panels	42	39	39	42
# of Panels More Sensitive	7 (16.7%)			1 (2.4%)

Inter-site Reproducibility

Reproducibility testing at three sites each with three lots of reagent for five days showed very good performance with a range of 4.5 - 11.7% total CV.

BioPlex® 2200 HIV Ag-Ab: Inter-Site Reproducibility

Spiked Analyte	Sample Type	N	Mean	Total*	
				SD	%CV
HIV-1 group M Ab	Serum	135	4.957	0.234	4.7
HIV-1 group M Ab	Serum	135	2.042	0.099	4.8
HIV-1 group M Ab	EDTA Plasma	135	1.981	0.090	4.5
HIV-1 group M Ab	Serum	135	0.593	0.039	6.6
HIV-1 group O Ab	Serum	135	1.949	0.131	6.7
HIV-1 group O Ab	EDTA Plasma	135	2.053	0.148	7.2
HIV-2 Ab	Serum	135	4.972	0.394	7.9
HIV-2 Ab	Serum	135	1.958	0.181	9.2
HIV-2 Ab	EDTA Plasma	135	1.962	0.172	8.8
HIV-2 Ab	EDTA Plasma	135	0.582	0.068	11.7
HIV-1 p24 Ag	Serum	135	2.125	0.143	6.7
HIV-1 p24 Ag	EDTA Plasma	135	2.193	0.156	7.1
HIV-1 p24 Ag	Serum	135	5.207	0.304	5.8
Negative	Serum	135	0.084	0.092	N/A
Negative	EDTA Plasma	135	0.083	0.034	N/A

*Total variability includes within-run, between-day, between-lot and between-site variability.

Organ Donor Testing

Specificity, sensitivity and reproducibility studies using samples from brain dead donors and normal living donor samples indicated equivalent performance with the two populations. All samples were initially non-reactive on three lots of BioPlex* 2200 HIV Ag-Ab assay and all subsequently spiked brain dead specimens were reactive for each spiked analyte by the intended assay. Reproducibility between the two populations was equivalent.

Conclusion

The BioPlex* 2200 HIV Ag-Ab assay, with its unique assay design and reporting capability, demonstrated excellent assay performance including:

- High diagnostic sensitivity
  – 100% sensitivity in known HIV-1 and HIV-2 positive samples
- High sensitivity for HIV-1 p24 antigen
  – 0.33 IU/mL WHO standard, 5.2 pg/mL AFSSAPS standard
- High specificity of 99.86%
- Excellent HIV-1/HIV-2 Ab differentiation capability
  – Differentiation rate of 100% in HIV-1 populations and 93.5% in HIV-2 populations
- Very good reproducibility
  – Less than 12% total CV over three sites with three kit lots each
- Ability to test samples from potential organ donors
- Simultaneous detection and reporting of a composite result and three individual analyte results from a single sample aspiration
- Detailed screening results that can guide the selection of supplemental testing through the identification of specimens from acute HIV-1 infection and established HIV-2 infection
  – HIV Ag-Ab "Composite"
  – HIV-1 p24 Ag
  – HIV-1 Ab (Composite of groups M and O)
  – HIV-2 Ab

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