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Running Head: Clinical evaluation of BioPlex 2200 HIV Ag-Ab.

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Abstract

Early and accurate diagnosis is essential for optimal therapeutic outcomes in patients infected with HIV. Currently none of the commercially available fourth generation assays are able to differentiate HIV-1 and HIV-2 antibodies, and HIV-1 p24 antigen.

The aim of this study was to evaluate the performance of a novel assay, the BioPlex® 2200 HIV Ag-Ab. This assay uses a multiplex flow immunoassay design allowing the simultaneous detection and identification of antibodies to HIV-1 (groups M and O) and HIV-2, and HIV-1 p24 antigen, in addition to providing a traditional composite result.

A total of 1505 routine serum samples were prospectively tested. Results were compared with those from Architect HIV Combo assay. The sensitivity of the BioPlex 2200 was 100%. Specificity assessed on repeated false positive samples was 99.5%. In addition, 524 frozen specimens from patients known to be infected by HIV-1 or HIV-2 were tested. Of these specimens, 420 were infected by HIV-1 including 156 of known genotypes, 86 were infected by HIV-2, 7 were infected by both HIV-1 and HIV-2 (HIV-1+2), and 11 were from patients with acute HIV infection. Sensitivity was 100% for the HIV genotypes tested. The differentiation capability of the BioPlex 2200 HIV Ag-Ab assay for HIV-1, HIV-2, dual HIV-1+2 and early infections was 100%, 90.7%, 100% and 90.9% respectively.

The BioPlex 2200 is a sensitive and specific assay that can offer the advantage over conventional fourth generation assays to accurately differentiate and report HIV-1 p24 antigen, HIV-1 and HIV-2 antibodies.
Introduction

Early diagnosis is essential for optimal outcomes in patients infected with HIV because it facilitates timely initiation of appropriate care, and it decreases the rate of HIV transmission by three to fivefold (1). The importance of early detection is underlined by studies demonstrating increased life expectancy following early initiation of antiviral treatment. Moreover, several recent high profile studies have highlighted the potential for limiting viral reservoirs expansion and offering protection of innate and specific immunity from the deleterious effect of chronic immune activation by initiating antiretroviral therapy (ART) during acute HIV-1 infection (AHI) (2, 3). For 30 years, remarkable progress has been made in the development of tools for HIV diagnosis. HIV combo assays, also referred to, as fourth generation assays, detect both HIV-1 and HIV-2 antibodies (Ab) and the HIV-1 p24 antigen (Ag) which reduces, compared to third generation assays, the window period to an average of 2 weeks (4–12).

HIV viruses display extraordinary genetic diversity, subdivided into HIV-1 and -2 and among HIV-1, 4 groups (M, N, O and P) in which the pandemic group M including 9 subtypes and more than 40 Circulating Recombinant Forms (CRFs), as well as numerous Unique Recombinant Forms (URFs), due to its fantastic properties to recombine. In France, the epidemic in recent years is characterized by predominance of subtype B strains but increase of non-B subtypes (around 50%).

Although sensitivity and specificity of screening assays have improved, the genetic variability of HIV still represents a challenge, in particular for early detection of infection. For example, a correct serological diagnosis of HIV-2 infection may be missed. The use of HIV-1 Western blots assay as the sole confirmatory test in areas where the HIV-2 is not endemic may in fact lead to misclassification of HIV-2 infected individuals as HIV-1 positive. This is due to cross reactivity
between HIV-2 antibodies and envelope glycoproteins of HIV-1. The precise diagnosis of HIV-2 has
implications for the choice of antiretroviral treatment (13). Indeed, HIV-2 strains are naturally
resistant to non-nucleoside reverse transcriptase (NNRTI) and fusion inhibitors, and are less sensitive
in vitro to some protease inhibitors (14, 15).

Another challenge is posed by HIV-O strains which are highly divergent from the major group
M, leading to their designation as “outliers.” These strains also display marked intragroup genetic
diversity (16). This genetic diversity has important implications for diagnosis and monitoring of HIV-
O infection, including a risk of false negativity and viral load underestimations (17–19).

New assays allowing the detection and differentiation of HIV-1 (group M and O) and HIV-2
are necessary to improve the diagnosis of HIV infection. Currently none of the commercially
available fourth generation assays have this capability. The BioPlex® 2200 HIV Ag-Ab uses a
multiplex flow immunoassay design that permits simultaneous detection, identification, and reporting
of antibodies to HIV-1 (groups M and O), HIV-2 and the HIV-1 p24 antigen in a single reaction
vessel.

The aim of this study was to evaluate the sensitivity and specificity of the BioPlex® 2200 HIV
Ag-Ab assay, and its ability to detect and differentiate acute HIV infection (AHI), HIV-1 and HIV-2
infection.
Materials and Methods

Patient samples

Patient samples were from two populations. The first population consisted of 1505 fresh specimens obtained during a prospective study between October 2012 and February 2013 from hospitalized patients (Saint-Louis Hospital, Paris, France) for whom screening for HIV infection was requested. The second population included 524 frozen specimens from patients known to be infected by HIV-1 or HIV-2. Of these specimens, 420 were from individuals infected by HIV-1, 156 of which were of known genotype including 8 HIV-1 group O samples. There were 86 HIV-2 samples, 7 samples from individuals infected by both HIV-1 and HIV-2 (HIV-1+2), and 11 samples from patients in acute HIV-1 infection (AHI).

HIV screening assay:

(i) BioPlex 2200 HIV Ag-Ab assay

The BioPlex® 2200 HIV Ag-Ab assay uses three reagents. Bead reagent is a mixture of four populations of dyed microparticles, or “beads.” One dyed bead population is coated with monoclonal antibodies against HIV-1 p24 antigen. Three other dyed bead populations are coated, respectively, with three different antigens: (1) HIV-1 gp160 recombinant protein, (2) a synthetic peptide mimicking HIV-1 group O epitopes, and (3) a peptide mimicking the immunodominant epitope of the HIV-2 envelope protein [BioPlex® 2200 HIV Ag-Ab Reagent Kit : Reference 665-3450. Hercules, CA: Bio-Rad Laboratories; 2013].

Results for each bead population are determined from a two-point calibration plot from which Index Values (IDX) are derived. IDX values <0.90 are Non-Reactive, values >1.00 are Reactive, and values...
of 0.90 – 0.99 are Gray Zone. For every sample processed, three internal quality control beads are employed in order to check for detector fluctuations and sample integrity, and to normalize the assay signals. An HIV undifferentiated result is yielded when Bioplex® 2200 HIV-Ag-Ab is reactive for both HIV-1 and HIV-2, but with insufficient HIV-2/HIV-1 Ab ratio to differentiate HIV-1 or HIV-2.

The BioPlex 2200 HIV Ag-Ab assay was designed to have an analytical sensitivity of <12.5 pg/mL for HIV-1 p24 antigen on a panel derived from the National Agency for the Safety of Medicines and Health Products (MSNA) and <2 IU/mL on the WHO HIV international standard NIBSC 60/636. Results for antigen sensitivity are 7.02 pg/mL (range of 6.82 – 7.22 pg/mL) on the MSNA standard, and 0.637 IU/mL (range of 0.615 – 0.658 IU/mL) on the WHO standard. (Bioplex® 2200 HIV Ag-Ab. Hercules, CA: Bio-Rad Laboratories; 2013.)

(ii) Architect® HIV Combo assay

The Architect® HIV Combo assay is a chemiluminescent magnetic microparticle-based immunoassay run on an automated random access instrument. Briefly, the assay is designed to give a single, confounded result based on detection of HIV type 1 antibodies (HIV-1; groups M, O, and N), HIV-2 antibodies, and HIV-1 p24 antigen. Specimens with signal-to-cutoff (S/CO) ratios of 1.0 or greater are considered reactive.

Analysis

We compared results of Bioplex® 2200 HIV Ag-Ab assay to Architect® HIV Ag/Ab Combo assay according to the manufacturers’ instructions (Abbott Diagnostic, Rungis, France). Additional reference tests for HIV diagnosis were performed to discriminate HIV-1 and HIV-2 antibodies, such as Immunocomb® II HIV-1 & 2 BiSpot differentiation assay (Organics, Courbevoie, France), New Lav Blot I & II confirmatory assay (BioRad, Marnes la Coquette, France). Also, plasma HIV-RNA
were determined by Ampliprep/Cobas® Taqman® HIV-1 v2.0 assay (Roche Diagnostics, Meylan, France) for RNA-HIV-1 and a non-commercial RNA-HIV-2 RT-PCR (20). Sensitivity of plasma HIV-1 and HIV-2-RNA was 20 copies/mL and 100 copies/mL, respectively. The viral subtype of HIV-1 was determined with the sequence of the Pol (protease and reverse transcriptase) nucleotide region using a 16-capillary sequencer (ABI PRISM Genetic Analyzer, Applied Biosystems, Les Ulis, France), using the Los Alamos HIV sequence database (HIV BLAST, URL: http://www.hiv.lanl.gov/).

Final classification was established using predicate assays following a test algorithm (Figure 1). To calculate sensitivity and specificity, specimens were considered to be HIV-infected if they were repeatedly reactive by Architect HIV Ag/Ab Combo assay and reactive by Western blot test or HIV-1 or 2 RNA detected. They were considered to be AHI if they had indeterminate serology results (using Architect or Western blot) and detected HIV-1 or 2 RNA. Samples that were discordant on BioPlex®2200 from final classification were repeated in duplicate after centrifugation.

The BioPlex® 2200 HIV Ag-Ab assay (BioRad, Marnes-la-Coquette) was compared to Abbott Architect® HIV Combo assay and to final classification results based on additional testing. The sensitivity analysis included HIV-positive samples only, collected during both prospective and retrospective studies. The specificity analysis included only hospitalized patient samples collected during the prospective study.

Results

During the prospective study in March 2013, a total of 1505 consecutive specimens were tested using the BioPlex® 2200 assay, of which 35 were considered HIV infected, based on Architect reactivity and confirmation test results. During the screening, the BioPlex® 2200 correctly identified
these 35 positive specimens giving a sensitivity of 100% (95% confidence interval, CI: 96.7-100) and
1461 negative specimens giving a specificity of 99.4% (95% CI: 99-99.8). There were 9 false
positives on initial screening. Out of the 9 specimens, repeat testing with the BioPlex® 2200 assay
reported 1 negative result and 8 had repeatedly reactive results, leading to a specificity of 99.5% (95%
CI: 99.1-99.9%, Table 1).

In the retrospective study, a total of 420 specimens HIV-1 positive, 11 AHI specimens (HIV-1), 86
HIV-2 positive specimens and 7 specimens HIV-1+2 from co-infected patients, where tested using the
BioPlex® 2200 assay. All these specimens were reactive on the BioPlex® 2200. (Table 2)

The 420 specimens from HIV-1 infected-patients tested reactive on BioPlex® 2200 HIV Ag-Ab, with
only HIV-1 Ab reactivity. At least 28 different genotypes were represented and the sensitivity was
100% (95% CI: 99.3-100) (Table 3). All specimens were correctly classified as HIV-1 reactive by the
BioPlex® 2200, indicating a differentiation capability of 100% (95% CI: 99.3-100). The BioPlex®
2200 assay’s sensitivity was unaffected by HIV-1 group or subtype. Eight HIV-1 group O samples
were classified as HIV-1.

All of the 11 AHI HIV-1 specimens tested reactive on the BioPlex® 2200. Five of them were reactive
with both HIV-1 Ab and HIV-1 p24 Ag, two with only HIV-1 p24 Ag, two with only HIV-1 Ab, and
one with a HIV undifferentiated result.

All of the 86 specimens considered HIV-2 infected tested reactive on the BioPlex® 2200 with 78
specimens giving a HIV-2 Ab reactive result, and 8 an HIV undifferentiated result. These results gave
a sensitivity for HIV-2 positive specimens of 100% (95% CI: 96.6; 100) and a HIV-1/2 differentiation
capability of 90.7% (95% CI: 82.5-95.9).
All of the HIV-1+2 positive specimens tested reactive on BioPlex® 2200 with HIV undifferentiated results giving a sensitivity for HIV-1+2 positive specimens of 100%.

Discussion

The results of this study show that the BioPlex® 2200 is highly sensitive and specific in clinical sample populations and has performance comparable to the Architect® HIV Combo assay. In contrast to the alternative assays, the BioPlex® 2200 assay offers the advantage of calculating and reporting separate values for HIV-1 p24 Ag and HIV-1 (group M and O), and HIV-2 antibody. Currently, to our knowledge, no other commercial screening assay can differentiate HIV-1 (group M and O) and HIV-2. Individual reporting of HIV-1 group M and O results are for research use only (RUO) since the BioPlex® 2200 will not report results for the two bead populations separately.

The detection of AHI has been shown to be beneficial for the prompt initiation of appropriate antiretroviral therapy. In fact, HIV primary infections are now considered as a therapeutic emergency to limit, by early and adapted HAART, the viral reservoir constitution. In this way, the BioPlex® 2200 is an interesting test because it allows differentiation between HIV-1 p24 antigen and HIV antibody reactivity. During this study the BioPlex® 2200 showed excellent sensitivity for the detection of AHI by detecting HIV-1 p24 antigen reactivity in 7 of the 11 AHI samples.

Because of the growing number of people with HIV-1 group M non-B subtype infections, it is essential that HIV assays can detect infection independent of subtype. Studies have demonstrated that some fourth generation HIV assays are unable to detect certain HIV-1 group M, non B subtypes, yielding false negative results (8, 21). The BioPlex® 2200 HIV Ag-Ab assay had 100% sensitivity for all of the HIV-1 groups (M and O) and subtypes (n>20) tested, and for HIV-2. Various HIV-1
subtypes tested in our study were represented as major recombinant forms and very divergent strains currently observed in Africa, Europe and elsewhere.

Two samples from individuals infected by HIV-1 group O tested positive on BioPlex® 2200 with HIV-O antibody reactivity with no cross-reactivity with the group M antigen target. This shows the importance of including in BioPlex® 2200 HIV Ag-Ab beads with the HIV-1 group O specific epitope. This observation suggests that it may be possible for specialists to observe, under RUO conditions, specific binding reactivity to characterized samples suspected of being group O positive.

The BioPlex® 2200 HIV Ag-Ab assay identifies patients infected by HIV-1 or HIV-2 and has excellent specificity to discriminate between two HIV infections, allowing early initiation of adapted antiretroviral treatment, and avoiding inappropriate therapy in HIV-2 patients with natural resistance to NNRTI.

It is conventional to classify the HIV screening EIA tests according to their technological advancement. So we went past from the first generation to 4th generation according to the nature of antigens, EIA detection formats and P24 antigen detection. The shift to a test using sandwich method with detection and differentiation by the Luminex® technology allows us to consider this new tool as a new generation of test.

This study shows that the next generation BioPlex® 2200 HIV Ag-Ab assay performs well in the diagnosis of HIV infection, with excellent sensitivity and specificity. In conclusion the BioPlex® 2200 could be an alternative to fourth generation assays by allowing screening for early infection for rapid HAART initiation and a simultaneous differentiation of HIV-1 and HIV-2.

Conflict of interest
We are grateful to Marie-Charlotte Bassara for her technical assistance and Bio-Rad Laboratories for their financial support.


Figure 1: Algorithm for determining sample's final classification using additional testing.

Tables

Table 1: Specificity and sensitivity of the Bioplex® 2200 hiv ag-ab assay
No. = number

Table 2: Samples tested and summary of results
No. = number, neg = negative, undiff. = undifferentiating, sen= sensitivity, spe = specificity, diff. = differentiation

Table 3: Bioplex® 2200 hiv ag-ab assay's sensitivity for hiv-1 positive samples by group and subtype.
**Tables**

**Table 1: Specificity and sensitivity of the BioPlex® 2200 HIV Ag-Ab assay**

<table>
<thead>
<tr>
<th>Result</th>
<th>No. of samples</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIV infected (n=35)</td>
<td>HIV uninfected (n=1470)</td>
<td></td>
</tr>
<tr>
<td><strong>Initial screening</strong></td>
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<td></td>
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<tr>
<td>Positive</td>
<td>35</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>1461</td>
<td></td>
</tr>
<tr>
<td>Performance</td>
<td></td>
<td>100% (96.7-100)</td>
<td>99.4% (99.0-99.8)</td>
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<tr>
<td><strong>Retest screening</strong></td>
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<tr>
<td>Positive</td>
<td>35</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>1462</td>
<td></td>
</tr>
<tr>
<td>Performance</td>
<td></td>
<td>100% (96.7-100)</td>
<td>99.5% (99.1-99.9)</td>
</tr>
</tbody>
</table>

No. = number
Table 2: Samples tested and summary of results

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>1470</td>
<td>1462</td>
<td>1 Ag p24 HIV-1 Ab HIV-1 Ag+Ab Ab HIV-2 Undiff. Total</td>
<td>99,5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV-1 (No AHI)</td>
<td>420</td>
<td>0</td>
<td>0 Ag p24 HIV-1 Ab HIV-1 Ag+Ab Ab HIV-2 Undiff. Total</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>HIV-1 AHI</td>
<td>11</td>
<td>0</td>
<td>2 Ag p24 HIV-1 Ab HIV-1 Ag+Ab Ab HIV-2 Undiff. Total</td>
<td>11</td>
<td>100</td>
<td>90,9</td>
</tr>
<tr>
<td>HIV-2</td>
<td>86</td>
<td>0</td>
<td>0 Ag p24 HIV-1 Ab HIV-1 Ag+Ab Ab HIV-2 Undiff. Total</td>
<td>86</td>
<td>100</td>
<td>90,7</td>
</tr>
<tr>
<td>HIV-1+2</td>
<td>7</td>
<td>0</td>
<td>0 Ag p24 HIV-1 Ab HIV-1 Ag+Ab Ab HIV-2 Undiff. Total</td>
<td>7</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

No. = number, Neg = negative, Undiff. = undifferentiating, Sen = sensibility, Spe = specificity, Diff. = differentiation
Table 3: HIV-1 genotypes tested on Bioplex® 2200 HIV Ag Ab assay.

<table>
<thead>
<tr>
<th>HIV-1 classification (group/subtype)</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group M / subtype A</td>
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</tr>
<tr>
<td>Group M / subtype B</td>
<td>29</td>
</tr>
<tr>
<td>Group M / subtype C</td>
<td>4</td>
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<tr>
<td>Group M / subtype D</td>
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<tr>
<td>Group M / subtype F</td>
<td>7</td>
</tr>
<tr>
<td>Group M / subtype G</td>
<td>4</td>
</tr>
<tr>
<td>Group M / subtype H</td>
<td>5</td>
</tr>
<tr>
<td>Group M / subtype J</td>
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</tr>
<tr>
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<tr>
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<td>25</td>
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<tr>
<td>Group M / CRF 06</td>
<td>1</td>
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<td>Group M / CRF 09</td>
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</tr>
<tr>
<td>Group M / CRF 11</td>
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<tr>
<td>Group M / CRF 12</td>
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<tr>
<td>Group M / CRF 13</td>
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<tr>
<td>Group M / CRF 18</td>
<td>3</td>
</tr>
<tr>
<td>Group M / CRF 22</td>
<td>7</td>
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<tr>
<td>Group M / CRF 30</td>
<td>3</td>
</tr>
<tr>
<td>Group M / CRF 37</td>
<td>1</td>
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<tr>
<td>Group M / CRF02/CRF02-B</td>
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</tr>
<tr>
<td>Group M / recombinant complex</td>
<td>20</td>
</tr>
<tr>
<td>Group O</td>
<td>8</td>
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</table>
Figure 1: Algorithm for determining sample's final classification using additional testing.