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Short communication

BioRad BioPlex® HIV Ag-Ab assay: Incidence of false positivity in a low-prevalence population and its effects on the current HIV testing algorithm

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ABSTRACT

Background: The BioPlex® HIV Ag-Ab assay, unlike other HIV 1/2 antigen/antibody immunoassays, is capable of differentiating positive HIV-1 antibodies (Groups M and O) from HIV-2 antibodies and/or HIV-1 p24 antigen in a single test.
Objective: The Alaska State Virology Laboratory (ASVL) adopted the BioPlex® HIV Ag-Ab assay early 2017 and can report on its performance in terms of false positivity in a low-prevalence population and its effects on the current HIV testing algorithm recommended by the Centers for Disease Control and Prevention (CDC).
Study Design: Specimens received between March 2017 and August 2018 were screened using the BioPlex® HIV Ag-Ab assay. Specimens screening positive for HIV antibodies or antigen were further confirmed using the Geenius™ HIV 1/2 Supplemental Assay and/or HIV RNA testing.
Results: Of the 12,338 sera screened by the BioPlex assay for HIV, 35 specimens were positive. Only 22 of the specimens were confirmed by supplemental testing and were considered to be truly positive (PPV, 62.9%). RNA was not detected in these cases suggesting initial false positivity on the BioPlex® HIV Ag-Ab assay. True positive results had index values (IDX) of > 180 whereas false positive IDX's were between 1 and 4, with the exception of one specimen.
Conclusions: We suggest that specimens demonstrating positivity with low IDX values ≤ 4 on the BioPlex® HIV Ag-Ab assay proceed directly to RNA testing, essentially bypassing supplemental antibody confirmation tests, to reduce turnaround time and cost of HIV confirmation.

1. Background

Reducing the detection window while improving HIV assay sensitivity has made a positive impact on HIV patient management and treatment. However, improved sensitivity in combination with other biological factors and technical issues can lead to false positive HIV results [1–4]. The HIV 1/2 antigen/antibody immunoassays have been shown to produce false positive results, especially in populations with low HIV prevalence [5,6]. False positives are identified as specimens with low reactivity by initial HIV1/2 antigen/antibody immunoassays, which are unable to be confirmed by subsequent testing using varied methodologies, such as the Bio-Rad Geenius™ HIV-1/2 Supplemental Assay and PCR [4,7–10].

BioRad's BioPlex® HIV Ag-Ab combination assay is a multiplex flow immunoassay that can simultaneously detect and differentiate HIV-1 p24 antigen, HIV-1 (groups M and O) antibodies, and HIV-2 antibodies in human serum or plasma. It reportedly produces the best analytical sensitivity of HIV-1 p24 antigen on the market (limit of detection, 0.33 IU/mL and 5.2 pg/mL) as well as high specificity in low risk population (99.86%) [11]. The assay has been shown to be effective in identifying early cases of HIV when compared to other automated platforms [12]. The BioPlex® HIV Ag-Ab assay is not widely used in public health settings at this time but offers a clear advantage in terms of reducing labor while improving diagnostics by separating antigen-antibody combination results into individual measurements in a fully automated manner. In this study, we look at the false positivity rate and positive predictive value when using the BioPlex® HIV Ag-Ab combination assay in a low HIV prevalence population and provide insight on its effects on the recommended HIV testing algorithm.

Abbreviations: positive predictive value, (PPV); antigen, (Ag); antibody, (Ab); index value, (IDX)

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2. Study design

A total of 12,338 sera were collected from patients ranging from age 2 to 92 (median age = 29, interquartile range = 23 to 38 years old) between March 2017 and August 2018 from various regions across Alaska and sent to the ASVL for surveillance purposes.

Specimens were screened using the BioPlex® HIV Ag-Ab assay on the BioPlex® 2200 instrument. Results are expressed as an index value (IDX) describing the measured RFI (relative fluorescent intensity unit) as a ratio to the cut-off value for each particular bead type. Four separate results were generated for each multiplexed test, where IDX values of $\geq$ 1 were considered reactive: 1) HIV Ag/Ab combo, undifferentiated, 2) HIV-1 antibody, 3) HIV-1 antigen, and 4) HIV-2 antibody. Specimens that exhibited any level of reactivity were confirmed using the Geenius™ Supplemental HIV 1/2 Antibody assay. RNA testing had to be referred to a different laboratory and therefore this process was reserved for specimens testing positive for HIV p24 antigen without evidence of antibody presence as well as settling any discrepancies between the BioPlex® HIV Ag-Ab assay and the Geenius™ Supplemental HIV 1/2 Antibody assay.

3. Results

The majority of specimens tested belonged to patients aged 19–45 years old (75.6%). Representative of typical low HIV prevalence in Alaska, only 35 specimens demonstrated presence of HIV antibodies and/or p24 antigen (0.28%) during routine HIV screening. Of these 35 positive specimens, 22 (0.18% of total) were confirmed using the Geenius™ assay. The remaining 13 specimens that could not be confirmed were referred to Wadsworth Center (Albany, NY) for nucleic acid testing (NAT) (Supplemental table), with the exception of 3 specimens which did not have enough serum volume left at this stage of the algorithm. Patients were contacted in these cases and specimens were redrawn to provide additional serum to complete the algorithm. One specimen demonstrated positivity for HIV-1 and HIV-2 antibodies, but could only be tested for HIV-1 RNA due to lack of specimen volume. An additional specimen was redrawn in this case and retested to provide serum for HIV-2 RNA NAT. The positive predictive value in the Alaska population tested was 62.9% (TP/(TP + FP), 22/(22 + 13)). Of the 22 true positives, 21 specimens (95.5%) were positive for HIV-1 antibody only. The other true positive specimen demonstrated positivity for both HIV-1 antibody and HIV-1 p24 antigen. Of the 13 false positive reactions, 5 (38.5%) were positive for HIV-1 antibody only, 5 (38.5%) were positive for HIV-1 p24 antigen only, and 3 (23.1%) were positive for all targets (HIV-1 and HIV-2 antibodies as well as HIV-1 p24 antigen).

Table 1 describes average IDX values produced by the BioPlex® HIV Ag-Ab assay for each targeted analyte by result type. Most false positive reactions demonstrated an IDX measurement $\leq$ 4 with the exception of one specimen which measured between 11–12 IDX for HIV-1 antibody. On average, true positive reactions measured 10-fold higher (> 180 IDX) when compared to false positive IDX values.

4. Discussion

Low prevalence of HIV infection in a population can lend itself to increased false positivity on diagnostic tests. For instance, BioRad Laboratories tested 6395 patients in a low-risk population and found that 28 were repeatedly reactive for HIV using the BioPlex® HIV assay (specificity of 99% and sensitivity of 100%). Only 19 of these positives could be confirmed with the Geenius™ Supplemental HIV 1/2 assay and/or NAT testing (19/(19 + 9) = 67.9% PPV) [11], which is similar to this study (62.9%). This demonstrates that the BioPlex assay performed as intended in a low-prevalence setting. Although this isn't optimal, other HIV 1/2 antigen/antibody immunoassays have reported even lower PPVs, such as the Abbott Architect HIV antigen/antibody combination assay which demonstrated a PPV of 31.2% with significant PPV differences when testing sera from males (49.9%) vs. females (2.5%) [6].

Algorithm adjustments have shown to decrease the likelihood of false positives in various populations. In one study, PPV was improved by using two separate HIV 1/2 antigen/antibody immunoassays during the screening process, in this case the Abbott Architect used in conjunction with the Vidas HIV Duo Ultra (97% PPV) [13]. A PPV of 83% was demonstrated on the Abbot Architect by considering significantly lower signal-to-cutoff ratios in a study focused pregnant women, a group that sometimes experiences higher rates of false positive HIV test results [14].

In our study, all false positive results demonstrated low IDX values, with the exception of one specimen. Based on our results, we suggest that caution be applied to any low positive BioPlex® HIV Ag-Ab assay IDX value ($\leq$ 4) when reporting preliminary HIV results. Also, supplemental antibody testing provided by the Geenius™ assay did not enhance HIV test interpretation. Sensitive RNA testing, however, helped rule out the presence of detectable virus, which allowed for the final conclusions of each false positive test result. Adjustments to the testing algorithm to bypass the supplemental antibody assay if the BioPlex® HIV Ag-Ab assay generates low positive IDX values may be helpful to reduce cost, labor, and turnaround time by following through directly to NAT testing (Fig. 1).

The BioPlex® HIV Ag-Ab assay will likely become more common among laboratories conducting HIV surveillance based on its technical ability to further characterize HIV positive specimens as well as reduce labor. In order to address the issue of false positivity in low-prevalence populations, laboratories choosing to adopt the BioPlex® HIV Ag-Ab assay may want to consider bypassing the supplemental confirmation assay for specimens exhibiting low BioPlex® IDX values and proceed to RNA testing. Similar strategies have been considered for BioPlex® HIV-1 p24 Ag positive-only results [15]. This will ensure that weaker reactions obtained during HIV Ag-Ab combination screening are followed up to test for the presence of viral RNA indicating true HIV infection.

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Table 1
Comparison of BioPlex® HIV Ag-Ab assay IDX values by result type.

Result Type	# specimens	HIV Ag Ab	HIV-1 Ab	HIV-1 Ag	HIV-2 Ab
True Positive	22	187.49 ± 35.45	187.49 ± 35.45	0.52 ± 0.87	0.17 ± 0.08
False Positive	13	2.58 ± 2.90	2.06 ± 3.03	1.09 ± 1.11	0.50 ± 0.60
Negative	12,338	< 1.00	< 1.00	< 1.00	< 1.00

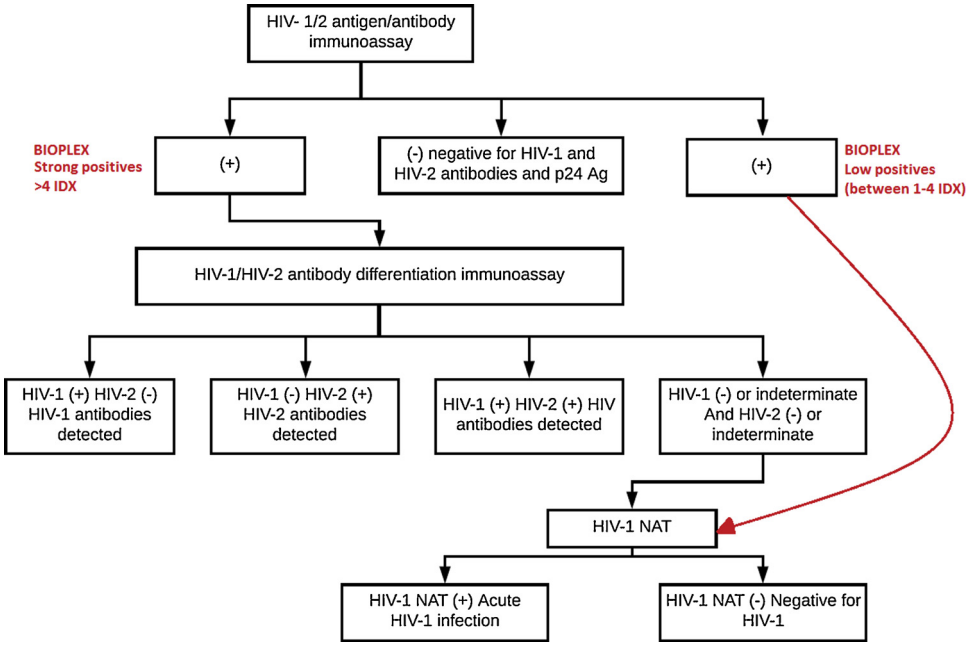

Fig. 1. Current recommended HIV testing algorithm showing suggested modifications in red (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Ethical approval

This research project was reviewed and exempted by the University of Alaska Fairbanks Institutional Review Board (IRB) (Approval letter No. 1219176-1).

CRediT author statement

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Competing interests

None declared.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcv.2019.04.002.

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