BioPlex® 2200 HIV Ag-Ab Assay: Addressing Its Role and Results in the HIV Testing Algorithm

The BioPlex® 2200 HIV Ag-Ab Assay (Bio-Rad Laboratories, Inc.), an HIV-1/2 antigen/antibody immunoassay (Ag/Ab IA), received FDA approval on July 23, 2015. This assay is a multiplex flow immunoassay for use on the fully automated BioPlex® 2200 system. The intended use is for the simultaneous qualitative detection and differentiation of HIV-1 p24 antigen, HIV-1 antibodies (including groups M and O) and HIV-2 antibodies in serum and plasma. The detector is able to distinguish reactions to each of the specific dyed bead populations and the amount of captured antibody or antigen enabling fluorescence detection and differentiation.

In this document, APHL will provide clarification on reporting BioPlex® 2200 HIV Ag-Ab Assay results and what to use when determining next steps in the HIV Laboratory Diagnostic Testing Algorithm.[1,2]

How should this assay be used?

The BioPlex® 2200 HIV Ag-Ab Assay is to be used as an aid in the diagnosis of infection with HIV-1 and/or HIV-2. Per the FDA approval, it is an Ag/Ab IA that is only intended to be used as a screening test in the first step of the HIV Laboratory Diagnostic Testing Algorithm.[1,2]

How should this assay not be used?

Despite its ability to differentiate reactivity for HIV-1 antibody and HIV-2 antibody, this test does not have an indication as a supplemental antibody assay that differentiates HIV-1 and HIV-2 (HIV-1/HIV-2 antibody differentiation immunoassay) and should not be used as the second step in the HIV Laboratory Diagnostic Testing Algorithm.[1,2] Additionally, the package insert indicates that the performance of the assay has not been established for neonates and should not be used with specimens from individuals younger than 2 years of age.[3]

What are the results from the assay?

There are several different terms used in the results and reporting sections of the BioPlex® 2200 HIV Ag-Ab Assay package insert.[3] Similar to other Ag/Ab IAs, this assay combines and summarizes the results from the three analytes it detects and this result is designated as the overall HIV Ag-Ab Assay result. Unlike other Ag/Ab IAs, this assay also provides a separate result for HIV-1 Ab, HIV-2 Ab and HIV-1 p24 Ag which are referred to as the individual analytes. The package insert also uses the term “Final Interpretation” when describing how and when to report the overall assay result and the individual analyte results.

HIV Ag-Ab Overall Result

Similar to the other Ag/Ab IAs, the overall HIV Ag-Ab result can be either reactive or non-reactive. The overall HIV Ag-Ab result is reactive if at least one of the three individual analytes is reactive (index value ≥ 1.00). The overall HIV Ag-Ab result is non-reactive when all individual analytes are non-reactive. If the overall HIV Ag-Ab result is reactive, repeat testing in duplicate must be performed per the package insert.[3] If at least one replicate is reactive for at least one analyte (i.e., repeatedly reactive), the overall HIV Ag-Ab result for that sample is reactive. The overall HIV Ag-Ab result should be used to determine the next step in the HIV Laboratory Diagnostic Testing Algorithm.[1,2] For example if the specimen is repeatedly reactive, it should be followed by a supplemental HIV-1/HIV-2 antibody differentiation immunoassay regardless of which individual analytes were reactive. For more information about

reporting results and additional testing that may be required refer to the Suggested Reporting Language for the HIV Laboratory Diagnostic Testing Algorithm.[4]

Individual Analyte Results

- HIV-1 Ab: This result is based on reactivity of the specimen with two HIV-1 antigen-coated beads; one coated with HIV-1 gp160 and one coated with a HIV-1 group O antigen. The result can either be **reactive**, **non-reactive**, or **reactive-undifferentiated** (See BioPlex Results, Scenario 1).
- HIV-2 Ab: This result is based on reactivity of the specimen with a HIV-2 antigen-coated bead. The result can either be **reactive**, **non-reactive**, or **reactive-undifferentiated** (See BioPlex Results, Scenario 1).
- HIV-1 Ag: This result is based on reactivity of the specimen with a bead coated with a monoclonal antibody to HIV-1 p24 Ag. The result can be either **reactive**, **non-reactive**, or **not reportable due to high Ab level** (See BioPlex Results, Scenario 3).

How are the results reported?

The overall HIV Ag-Ab result, when reactive, must be reported along with the individual analyte results to the submitting healthcare provider and surveillance program. When the overall HIV Ag-Ab result is non-reactive, the individual analyte results will also be non-reactive and will be displayed on the instrument report, but only the non-reactive overall HIV Ag-Ab should be reported to the submitting healthcare provider or surveillance program. The assay produces an index value for each analyte and the BioPlex® 2200 software uses these values to calculate the result as reactive or non-reactive (Table 1). The overall HIV Ag-Ab index is calculated using the highest index value of the individual analytes and a final interpretation is reported based on that index. While the package insert does not specifically address reporting the index values to healthcare providers, the assay's intended use is "qualitative detection and differentiation" and only qualitative results appear in the performance characteristics section of the package insert. Therefore, APHL suggests that the index values should not be included on reports to the provider or surveillance program.

The section below explains the results derived from this assay in more detail. APHL suggests that laboratories performing this assay use the HIV Ag-Ab overall result (reactive or non-reactive) to determine the next step in the HIV Laboratory Diagnostic Testing Algorithm. Additionally, when referring to the APHL Suggested Reporting Language document (Table 1) to determine laboratory algorithm interpretation, the HIV Ag-Ab overall result is applicable to Step 1.[4]

Table 1: Summary of Results and Interpretations for BioPlex® 2200 HIV Ag-Ab (BioPlex HIV Ag-Ab Package Insert, pg 12[3])

Index (IDX)	Retest		Final Interpretation#
< 1.00 for all analytes	No	Not Applicable	Non-Reactive
≥ 1.00 for at least one analyte	Yes	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 for HIV-1 and HIV-2 Abs **

#This indicates which results (overall or overall and individual) should appear on the provider report

** Results are not reportable for HIV-1 Ag if the HIV-1 Ag index is ≥ 1.00 and the index for HIV-1 Ab or HIV-2 Ab is ≥ 1.00 (See Scenarios).*

*** If 2 of 3 results are Reactive, Undifferentiated for HIV-1 Ab and HIV-2 Ab, both results are reported as Reactive, Undifferentiated. If 2 of 3 results are specific for HIV Ab type, that specific HIV type is reported as HIV Reactive (See Scenarios).*

BioPlex® HIV Ag-Ab Results

While the overall HIV Ag-Ab result for the BioPlex® 2200 HIV Ag-Ab Assay is similar to the result produced by other Ag/Ab IAs, reporting of the individual analytes has created a new reporting structure. In all of the scenarios we describe below, the overall HIV Ag-Ab result is reactive, but we wanted to display example reports of how the overall HIV Ag-Ab result is calculated from different individual analyte results. In all scenarios, the overall HIV Ag-Ab result is reactive, and therefore, the next step would be to perform a supplemental antibody assay in accordance with the HIV Laboratory Diagnostic Testing Algorithm.[1,2]

Scenario 1: Reactive for HIV Ag-Ab, Reactive for HIV-1 Ab

This result occurs when the index value for the HIV-1 Ab is ≥ 1.00 and the index values for HIV-1 Ag and HIV-2 Ab analytes are < 1.00. In this situation, the HIV Ag-Ab overall result and the HIV-1 Ab analyte result are reported as "Reactive." Because the HIV Ag-Ab overall result is reactive, this would need to be followed with a supplemental HIV-1/HIV-2 antibody differentiation immunoassay.

Instrument Report

Analyte	Value (IDX)	Result
HIV Ag-Ab (overall result)	3.20	Reactive
HIV-1 Ab	3.20	Reactive
HIV-1 Ag	0.03	Non-reactive
HIV-2 Ab	0.12	Non-reactive

Mock Laboratory Report for Healthcare Provider

Analyte	Result
HIV Ag-Ab (overall result)	Reactive
HIV-1 Ab	Reactive
HIV-1 Ag	Non-reactive
HIV-2 Ab	Non-reactive

Scenario 2: Reactive for HIV Ag-Ab, Reactive-Undifferentiated HIV Antibody Results

This result occurs when the index value for the HIV-1 Ab and HIV-2 Ab result are ≥ 1.00 and within a fivefold difference from each other. For the HIV-1 Ab and HIV-2 Ab analytes, the result is reported as "Reactive, Undifferentiated." The HIV Ag-Ab overall result is again reactive and would need to be followed with a supplemental HIV-1/HIV-2 antibody differentiation immunoassay.

Instrument Report

Analyte	Value (IDX)	Result
HIV Ag-Ab (overall result)	2.14	Reactive
HIV-1 Ab	2.10	Reactive, Undifferentiated
HIV-1 Ag	0.03	Non-reactive
HIV-2 Ab	2.14	Reactive, Undifferentiated

Mock Laboratory Report for Provider

Analyte	Result
HIV Ag-Ab (overall result)	Reactive
HIV-1 Ab	Reactive, Undifferentiated
HIV-1 Ag	Non-reactive
HIV-2 Ab	Reactive, Undifferentiated

Scenario 3: Reactive for HIV Ag-Ab, Reactive- Unreportable HIV-1 p24 Result

This result occurs when the HIV-1 p24 antigen result is reactive with an index value 1.00 (reported as "not reportable due to high HIV Ab level") and the index values for the HIV-1 Ab or HIV-2 Ab result are ≥1.00. As stated above, since the HIV Ag-Ab overall result is reactive, a sample with this type of result would still be followed with a supplemental HIV-1/HIV-2 antibody differentiation immunoassay.

Instrument Report

Analyte	Value (IDX)	Result
HIV Ag-Ab (overall result)	133.09	Reactive
HIV-1 Ab	133.09	Reactive
HIV-1 Ag		Not reportable due to high HIV Ab level
HIV-2 Ab	0.17	Non-reactive

Mock Laboratory Report for Provider

Analyte	Result
HIV Ag-Ab (overall result)	Reactive
HIV-1 Ab	Reactive
HIV-1 Ag	Not reportable due to high HIV Ab level
HIV-2 Ab	Non-reactive

What supplemental testing is required for specimens that have a reactive result on the BioPlex® 2200 HIV Ag-Ab Assay?

A reactive overall result on this assay should be treated the same as a reactive result on any other HIV-1/2 Ag/Ab immunoassay. As recommended by the HIV Laboratory Diagnostic Testing Algorithm,[1,2] any reactive result (as specified by the package insert for the assay) should be followed by a HIV-1/HIV-2 antibody differentiation immunoassay. If the result of the supplemental assay is non-reactive or indeterminate, an HIV-1 NAT should be performed to complete the algorithm. At this time there are no special considerations or alternative algorithms when only a single individual analyte of the BioPlex® 2200 HIV Ag-Ab Assay is reactive.

Note: If your laboratory has data to support an alternative algorithm, please ensure the data are published. The recommended algorithms can only be updated based on published data (conference presentations and peer-reviewed journals).

References

1. Centers for Disease Control and Prevention and Association of Public Health Laboratories. Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations. 2014. Available at: http://stacks.cdc.gov/view/cdc/23447
2. Centers for Disease Control and Prevention. 2018 Quick reference guide: Recommended laboratory HIV testing algorithm for serum or plasma specimens. Available at: https://stacks.cdc.gov/view/cdc/50872
3. Bio-Rad Laboratories, Inc. BioPlex® 2200 System: HIV Ag-Ab: Instructions for Use. Ref. 665-3455, Reference Version Available at: https://www.aphl.org/programs/infectious_disease/Documents/665-3455_BIOPLEX_HIV_AG-AB_IFU_US_060816.pdf
4. Association of Public Health Laboratories. Suggested Reporting Language for the HIV Laboratory Diagnostic Testing Algorithm. 2019. Available at: https://www.aphl.org/aboutAPHL/publications/Documents/ID-2019Jan-HIV-Lab-Test-Suggested-Reporting-Language.pdf

Additional Tools/Resources for BioPlex® 2200 HIV Ag/Ab Assay Users

1. New York State 2018 Laboratory Guidelines. Published March 2018. Available at: https://www.health.ny.gov/diseases/aids/providers/testing/index.htm#algorithm
2. APHL HIV Diagnostic Updates. Published February 2016. Available at: https://www.aphl.org/programs/infectious_disease/Documents/2015_Informational%20Update_02_12_16_FINAL.pdf

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