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 assay chemistry consists of a multiplex flow detection mechanism that utilizes dyed bead populations coated with specific antibodies or antigens that are detected through dual fluorescence detection which enables differentiation and reporting of each respective captured antibody or antigen.

In this document, the Association of Public Health Laboratories (APHL) provides clarification on reporting BioPlex® 2200 HIV Ag-Ab Assay results and how results provided by BioPlex are used to determine next steps in the HIV Laboratory Diagnostic Testing Algorithm.[1,2]

Key Points

- The BioPlex® 2200 HIV Ag-Ab Assay should only be used as a screening test in the first step of the algorithm.
- The assay should not be used a supplemental antibody test and should not be used as the second step of the algorithm.
- The overall HIV Ag-Ab assay result should always be reported including when reporting results and used to determine the next step in the algorithm.

How should this assay be used?

The BioPlex® 2200 HIV Ag-Ab Assay is intended to aid in the diagnosis of infection with HIV-1 and/or HIV-2. Consistent with the FDA approval, it is an Ag/Ab IA that may 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 only has an intended use claim from the FDA for screening. The assay should not be used a supplemental antibody test and should not be used as the second step (HIV-1/HIV-2 antibody differentiation immunoassay) 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 two years of age.[3]

What are the results from the assay?

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

Overall HIV Ag-Ab Result

- Similar to the other Ag/Ab IAs, the overall HIV Ag-Ab assay result can be either **reactive** or **nonreactive**.
- The overall HIV Ag-Ab assay result is reactive if at least one of the three individual analytes is reactive (index

value ≥ 1.00).

- The overall HIV Ag-Ab assay result is nonreactive when all individual analytes are nonreactive.
- If the overall HIV Ag-Ab assay 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 assay 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** (See Scenario 2), **nonreactive**, or **reactive-undifferentiated** (See Scenario 3).
- HIV-2 Ab: This result is based on reactivity of the specimen with a HIV-2 antigen-coated bead; a peptide mimicking HIV-2 envelope protein. The result can either be **reactive**, **nonreactive**, or **reactive-undifferentiated** (See Scenario 3).
- 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** (See Scenario 1), **nonreactive**, or **not reportable due to high Ab level** (See Scenario 4).

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 public health surveillance program. When the overall HIV Ag-Ab result is nonreactive, all individual analyte results will be nonreactive and will be displayed on the instrument report, but nevertheless APHL recommends that for clarity only the nonreactive overall HIV Ag-Ab should be reported to the submitting healthcare provider or public health surveillance program. The BioPlex assay produces an index value for each analyte and the BioPlex® 2200 software uses these values to calculate the result as reactive or nonreactive (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 recommends against including the index values on reports to the submitting healthcare provider or public health surveillance program.

The section below explains the results derived from this assay in more detail. APHL suggests that laboratories performing this assay use the overall HIV Ag-Ab assay result (reactive or nonreactive) 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	Retest Result	Final Interpretation#
< 1.00 for all analytes	No	Not Applicable	Nonreactive
≥ 1.00 for at least one analyte	Yes	Both retest results have an IDX < 1.00 for all analytes	Nonreactive
		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 ≥ 100 (See Scenario 4).*

*** 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 Scenario 3).*

BioPlex® HIV Ag-Ab Results

While the overall HIV Ag-Ab assay 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 scenarios described below, the overall HIV Ag-Ab result is reactive, however, these example reports display how the overall HIV Ag-Ab result is calculated from different individual analyte results. Since the overall HIV Ag-Ab result is reactive 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 Ag

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

At the time the original HIV lab algorithm was published, an initial screening immunoassay with the ability to differentiate HIV analytes was not available, and therefore, there are currently insufficient data to support alternative algorithms for confirming the HIV-1 p24 Ag only result.

Instrument Report

Analyte	Value (IDX)	Result
HIV Ag-Ab (overall result)	1.51	**Reactive**
HIV-1 Ab	0.05	Nonreactive
HIV-1 Ag	1.51	**Reactive**
HIV-2 Ab	0.04	Nonreactive

Mock Laboratory Report for Healthcare Provider

Analyte	Result
HIV Ag-Ab (overall result)	**Reactive**
HIV-1 Ab	Nonreactive
HIV-1 Ag	**Reactive**
HIV-2 Ab	Nonreactive

Scenario 2: 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 overall HIV Ag-Ab assay result and the HIV-1 Ab analyte result are reported as "Reactive." Because the overall HIV Ag-Ab assay 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	Nonreactive
HIV-2 Ab	0.12	Nonreactive

Mock Laboratory Report for Healthcare Provider

Analyte	Result
HIV Ag-Ab (overall result)	**Reactive**
HIV-1 Ab	**Reactive**
HIV-1 Ag	Nonreactive
HIV-2 Ab	Nonreactive

Scenario 3: 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 five-fold 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	Nonreactive
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	Nonreactive
HIV-2 Ab	**Reactive, Undifferentiated**

Scenario 4: Reactive for HIV Ag-Ab, Reactive-Unreportable HIV-1 p24 Result

When HIV-1 and or HIV-2 antibody levels are very high, the antibody may interfere with HIV-1 p24 Ag results and the HIV-1 p24 Ag result is not reliable. The HIV-1 p24 Ag result "Reactive, Not reportable due to high HIV Ab level" occurs when the HIV-1 p24 antigen index value is ≥ 1.00 (which is not reported) and the index value(s) for the HIV-1 Ab or HIV-2 Ab result are ≥ 100. As stated above, since the overall HIV Ag-Ab assay 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	0.03	Not reportable due to high HIV Ab level
HIV-2 Ab	0.17	Nonreactive

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	Nonreactive

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 nonreactive 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® 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

Association of Public Health Laboratories

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