BIO-RAD

BioPlex® 2200 System
HIV Ag-Ab

Instructions For Use

REF 665-3455

IVD

Σ 200

UNITED STATES: *Bio-Rad Laboratories, Inc. Clinical Diagnostics Group 4000 Alfred Nobel Drive; Hercules, CA 94547*

INTENDED USE

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 human serum or plasma (fresh or frozen K2 EDTA, K3 EDTA, lithium heparin, sodium heparin; fresh citrate). This assay is intended as an aid in the diagnosis of infection with HIV-1 and/or HIV-2, including acute (primary) HIV-1 infection. The assay may also be used as an aid in the diagnosis of infection with HIV-1 and/or HIV-2 in pediatric subjects as young as two years of age, and pregnant women.

The BioPlex 2200 HIV Ag-Ab assay is also intended for use in testing plasma specimens to screen organ donors when specimens are obtained while the donor's heart is still beating.

The BioPlex 2200 HIV Ag-Ab assay is not intended for use in screening blood or plasma donors, as the effectiveness of this test for use in the screening of these donors has not been established. However, in urgent situations where traditional licensed blood donor screening tests are unavailable or their use is impractical, this assay can be used as a blood donor screening assay.

The BioPlex 2200 HIV Ag-Ab assay is intended for use with the BioPlex 2200 System.

SUMMARY AND EXPLANATION

Acquired immunodeficiency syndrome (AIDS) is caused by viruses transmitted by sexual contact, exposure to blood (including sharing contaminated needles and syringes) or certain blood products, or from an infected mother to her fetus or child during the perinatal period.[1] Additionally, transmission of these viruses can occur through tissue transplantation.[2] Human Immunodeficiency Virus Type 1 (HIV-1) has been isolated from patients with AIDS and AIDS-related complex (ARC).[3-5] HIV-1 was thought to be the sole causative agent of these syndromes until 1986, when a second type of Human Immunodeficiency Virus (Human Immunodeficiency Virus Type 2 or HIV-2) was isolated and also reported to cause AIDS.[6-7] Since the initial discovery, hundreds of cases of HIV-2 infection have been documented worldwide, including cases of AIDS related to HIV-2.[8] In the United States, there have been more than 80 cases of infection with HIV-2 reported, including two blood donors.[9-16]

This second immunodeficiency virus is similar to, but distinct from, HIV-1. Both viruses have similar morphology and lymphotropism[17] and the modes of transmission appear to be identical.[8,18] The HIV-1 and HIV-2 genomes exhibit about 60% homology in conserved genes such as *gag* and *pol*, and 39-45% homology in the envelope genes.[19] Serologic studies have also shown that the core proteins of HIV-1 and HIV-2 display frequent cross-reactivity, whereas the envelope proteins are more type-specific.[20]

Within the two major HIV types, there is significant variation as well. By analyzing sequences of representative strains, HIV-1 has been divided into three groups: Group M (for major), including at least ten subtypes (A through J); Group O (for outlier); and Group N (for non-M, non-O).[21-23] Similarly, the HIV-2 strains have been classified into at least five subtypes (A through E).[24] Some HIV-1 variants share ≤ 50% homology in their envelope genes with the sequences of more common prototype strains.

Despite some degree of immunological cross-reactivity between types and subtypes of HIV, reliable detection of the more divergent strains may only be achieved by incorporating specific sequences into the assay design. In one study, detection of HIV-2 reactive samples by licensed HIV-1 antibody kits ranged from 60% to 91%, depending on the test used.[16, 25]

Detection of HIV-1 Group O samples by HIV-1 and HIV-1/HIV-2 assays varied from 0% to 100% in studies with U.S.-licensed and European test kits.[26,27]

HIV antigens and antibodies appear and are detectable at different stages of the infection.[28,29]

The BioPlex 2200 HIV Ag-Ab assay is an immunoassay that incorporates highly conserved recombinant and synthetic peptide sequences representing HIV-1 (groups M and O) and HIV-2,[30-36] as well as a monoclonal antibody specific for HIV-1 p24 antigen.

The assay's design allows results of antibody and antigen detection to be reported separately and to differentiate between HIV-1 and HIV-2 reactivity. This information can be useful in selecting additional, more specific supplemental tests to verify specimens reactive with the BioPlex 2200 HIV Ag-Ab assay. Supplemental tests may include nucleic acid amplification test (NAAT), HIV-1 / HIV-2 differentiation assays, Western Blot, or immunofluorescence procedures.

PRINCIPLE OF THE PROCEDURE

The BioPlex 2200 HIV Ag-Ab assay is a multiplex flow immunoassay that is run on the fully automated BioPlex 2200 system. The assay methodology greatly resembles traditional Enzyme Immunoassay (EIA), but permits simultaneous detection and identification of anti-HIV-1, anti-HIV-2 and HIV-1 p24 antigen in a single reaction vessel.

A mixture of four populations of dyed beads is used. One dyed bead population is coated with monoclonal antibody against HIV-1 p24 antigen. Three dyed bead populations are coated, respectively, with three different antigens: (1) HIV-1 gp160 recombinant protein, (2) a synthetic peptide mimicking a totally artificial (i.e., encoded by no existing virus) HIV-1 Group O epitope, and (3) a peptide mimicking the immunodominant epitope of the HIV-2 envelope protein. Conjugate 1 reagent contains a mixture of biotinylated HIV peptides and polyclonal antibodies to HIV-1 p24 Ag. Conjugate 2 reagent contains streptavidin conjugated to phycoerythrin, a fluorescent dye purified from marine algae.

The BioPlex 2200 System using the BioPlex 2200 HIV Ag-Ab assay combines an aliquot of the patient sample and bead reagent into a reaction vessel. After an incubation step, excess patient sample is removed by a wash step. The BioPlex 2200 System then adds Conjugate 1 reagent to the reaction vessel.

If HIV-1 p24 antigen is present in the patient sample, it binds to the monoclonal antibodies on the dyed beads and also to the biotinylated polyclonal antibodies in the Conjugate 1 reagent. A biotinylated antibody-antigen-antibody (Ab-Ag-Ab) complex is formed. If HIV antibodies are present in the patient sample, they bind to the HIV-1 (Groups M & O) and HIV-2 antigens immobilized on the dyed beads and also the respective biotin-labeled antigens in the Conjugate 1 reagent. A biotinylated antigen-antibody-antigen (Ag-Ab-Ag) complex is formed.

Excess Conjugate 1 reagent is removed by a second wash step. The BioPlex 2200 system then adds Conjugate 2 reagent to the reaction vessel. Labeled streptavidin reacts with the biotinylated Ab-Ag-Ab and Ag-Ab-Ag complexes captured on the dyed beads. After incubation, excess Conjugate 2 reagent is removed by a third wash step. The beads are then resuspended in wash buffer.

The bead mixture then passes through the detector. The identity of the dyed beads is determined by the fluorescence of the bead dyes and the amount of captured HIV-1 p24 antigen or HIV-1 and/or HIV-2 antibodies is determined by the fluorescence of the attached phycoerythrin. Raw data are calculated in relative fluorescence intensity (RFI). The results, calculated for each bead, are compared against their own respective cut-off values and are expressed as an Index. Using the results for each analyte, PC-based software calculates a final output of reactive or non-reactive for each analyte.

Three additional dyed beads are included: Signal Normalization Bead (SNB) to normalize RFI values, Internal Standard Bead (ISB) to monitor detector fluctuations, and Serum Verification Bead (SVB) to verify the addition of serum or plasma to the reaction vessel. Refer to the BioPlex 2200 Operation Manual for more information.

KIT COMPONENTS

BioPlex 2200 HIV Ag-Ab Reagent Pack (665-3455). The reagent pack is sufficient for 200 tests.

MATERIALS PROVIDED

REF	Vial	Description
665-3455	Bead Set	One (1) vial, containing 5 mL of reagent with dyed beads coated with monoclonal antibody against HIV-1 p24 antigen or purified HIV-1/ HIV-2 antigen (recombinant protein or peptides), Internal Standard Beads (ISB), Serum Verification Beads (SVB), and Signal Normalization Beads (SNB), with protein stabilizers (bovine, murine and human IgG) and ProClin 300 (≤ 0.3%), sodium benzoate (≤0.1%) and Sodium Azide (< 0.1%) as preservatives.
	Conjugate 1	One (1) vial, containing 10 mL of reagent with biotinylated peptides of HIV-1 (M & O) and HIV-2 and biotinylated polyclonal sheep antibodies to HIV-1 p24 antigen, and biotinylated Factor XIII antibody with protein stabilizers (bovine and human IgG), and ProClin 300 (≤ 0.5%) and Sodium Azide (< 0.1%) as preservatives.
	Conjugate 2	One (1) vial, containing 5 mL of reagent with streptavidin conjugated to phycoerythrin with protein stabilizers (bovine and human IgG), and ProClin 300 (≤ 0.5%) and Sodium Azide (< 0.1%) as preservatives.
660-0306		BioPlex 2200 Manual Reagent Pack Piercer

MATERIALS REQUIRED BUT NOT PROVIDED (AVAILABLE FROM BIO-RAD)

REF	Description
663-3405	BioPlex 2200 HIV Ag-Ab Calibrator Set
663-3435	BioPlex 2200 HIV Ag-Ab Control Set
660-0817	BioPlex 2200 Sheath Fluid
660-0818	BioPlex 2200 Wash Solution
660-2003	Reaction Vessels
660-0000	BioPlex 2200 System and software

PRECAUTIONS/WARNINGS

For In Vitro Diagnostic Use

Warning: FDA has approved this test for use with serum and plasma specimens only. Use of this test kit with specimens other than those specifically approved for use with this test kit may result in inaccurate test results.

This test is not intended for use in children younger than 2 years of age.

1. For professional use only.
2. WARNING: This product is preserved with ≤ 0.5% or ≤ 0.3% ProClin 300 a biocidal preservative that is irritating to eyes and skin, may be detrimental if enough is ingested, and may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.

WARNING
H317

≤ 0.5% ProClin 300
H317: May cause an allergic skin reaction.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352: IF ON SKIN: Wash with plenty of soap and water.
P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention.
P501: Dispose of contents and container in accordance to local, regional, national and international regulations.

3. This product contains human blood components. No test method can offer complete assurance that infectious agents are absent. In accordance with Good Laboratory Practice (GLP), all human source material should be considered potentially infectious for Hepatitis B (HBV), Hepatitis C (HCV), HIV-1, HIV-2, and all other infectious agents; therefore, handle the BioPlex 2200 HIV Ag-Ab kit (including reagent packs and calibrator and control sets) with the same precautions used with patient specimens. All human blood derivatives, reagents and human specimens should be handled as if capable of transmitting infectious disease, following recommended *Universal Precautions* for bloodborne pathogens as defined by OSHA[37] Biosafety Level 2 guidelines from the current CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories*[38], WHO *Laboratory Biosafety Manual*[39] and/or local, regional, and national regulations.[40]. The following human blood derivatives are found in this kit:

   a. Each unit of human plasma used in the manufacture of the BioPlex 2200 HIV Ag-Ab (including calibrator and control sets) was tested by FDA accepted methods and found nonreactive for Hepatitis B surface antigen (HBsAg) and antibodies to Hepatitis C virus (HCV Ab).
   b. The Human source material used in the preparation of the Negative Control was also tested by FDA accepted methods and found nonreactive for human immunodeficiency virus (HIV-1 and HIV-2).
   c. Human source material, containing HIV-1 and HIV-2 human antibody has been heat-treated.
   d. The HIV-1 p24 Antigen Calibrator and Positive Control have been inactivated using a chaotropic agent.
4. Consider any materials of human origin as infectious and handle them using typical universal biosafety procedures and Universal Precautions according to 29 CFR 1910.1030.
5. Do not smoke, eat, or drink in areas where patient samples and kit reagents are handled.
6. This test kit should be handled only by qualified personnel trained in laboratory procedures and familiar with their potential hazards. Wear appropriate personal protective equipment, including lab coat, eye/face protection, and disposable gloves (synthetic, non-latex gloves are recommended) while handling all reagents and samples and while operating the BioPlex 2200 System. Wash hands thoroughly after performing the test.
7. Dispose of all wastes in accordance with applicable national, and/or local regulations.
8. Waste material containing patient samples or biological products should be considered biohazardous when disposing or treating.

9. Chemical reagents should be handled in accordance with Good Laboratory Practices.
10. Clean up all spills immediately and thoroughly. Decontaminate the area for any spills involving biohazardous materials with an effective disinfectant. Dispose of all contaminated materials appropriately.
11. Refer to the kit and additional required component Safety Data Sheets (SDS) for more safety information and warnings about chemical and biological hazards. The Safety Data Sheets are available at www.bio-rad.com and on request.
12. Do not use tests beyond their expiration date. The date is printed on all boxes.
13. Do not interchange vial or bottle caps and stoppers; this will lead to cross-contamination of calibrators or controls and may compromise the performance of the product.
14. Adherence to the protocol specified herein is necessary to ensure proper performance of this product. If aberrant results are obtained, contact Bio-Rad Technical Service.
15. Never mix the contents from different bottles of the same reagent. Doing so may lead to reagent contamination and compromise the performance of the product.
16. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.

SPECIMEN COLLECTION AND HANDLING

Specimen Collection Precaution

Consider any materials of human origin as infectious and handle them using standard biosafety procedures.

Specimen Type

Serum or plasma (K2 EDTA, K3 EDTA, lithium heparin, sodium heparin) specimens, either fresh or frozen, may be used in the test. Fresh samples that have been collected in sodium citrate may also be used. Do not use samples collected in sodium citrate that have been previously frozen, as false positive results may occur. Avoid hemolysis. Samples collected into anticoagulant tubes should be processed according to the manufacturer's direction for centrifugation and removal of the plasma from the cells. False positive results may occur if the plasma remains with the cells for >24 hours.

The substances listed below have been tested with the BioPlex 2200 HIV Ag-Ab assay and resulted in no significant effect on the clinical interpretation of results:

Bilirubin: 20 mg/dL
Triglycerides: 1250 mg/dL
Protein: 12 g/dL
Hemoglobin: 500 mg/dL

In studies of potential organ donors (brain dead individuals whose heart is still beating), no interference with assay performance was observed when specimens contained heparin, dopamine, norepinephrine, triiodothyronine, thyroxine, or insulin.

Specimen Storage

Samples may be stored for no longer than 4 days at room temperature or 7 days at 2-8°C, including the time that samples are in transit. For longer storage of samples, keep at -20°C or colder.

Specimen Preparation

Ensure specimens are thoroughly mixed and homogenous. Centrifuge specimens to remove bubbles, foam, or gross particulate matter.

For frozen samples perform the following steps:

- Thaw samples completely
- Mix thoroughly by inverting 10 times or by vortexing. Continue to mix until samples are visibly homogeneous.
- Centrifuge at >10,000 RCF for 10 minutes.
- Avoid multiple freeze/thaw cycles (up to 4 cycles is acceptable).

Specimen Shipping

If specimens are to be shipped, they should be packed in compliance with applicable local, regional and international regulations covering the transportation of etiologic agents. Studies have demonstrated that specimens may be shipped at 2-8°C or frozen (e.g., dry ice). For shipments that are in transit for more than 7 days, specimens should be kept frozen at -20°C or lower.

Preparation and Storage of Reagents

- Do not freeze the BioPlex 2200 HIV Ag-Ab reagent pack.
- Reagents in the BioPlex 2200 HIV Ag-Ab reagent pack are ready to use. After initial use, the reagents are stable for 2 months, or until the date of expiration when stored unopened on the instrument, or refrigerated at 2-8°C.
- Do not use reagent packs beyond expiration dates.
- Calibrators can be used on the BioPlex 2200 for 5 calibration events over 30 days after opening, with an on-board (open vial) time of 3 hours at room temperature. Controls may be used on the BioPlex 2200 for 32 control events over 60 days, with an on-board (open vial) time of 3 hours at room temperature.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

Store all reagents at the labeled temperature and do not use past their expiration dates. Do not use any reagents which have any indications of discoloration, cloudiness, or precipitation. Reagents that show any signs of leakage should not be used. Do not exceed the storage time and temperature limitations listed above.

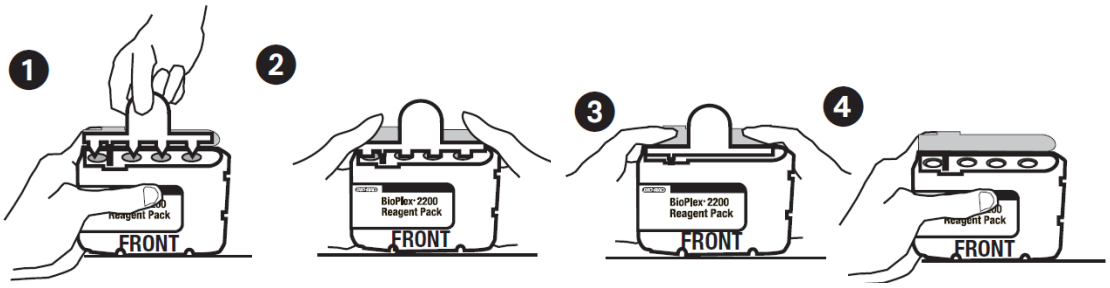
PROCEDURE

NOTE: When testing with the BioPlex 2200 HIV Ag-Ab assay, all of the individual HIV analytes (HIV-1 Ab, HIV-2 Ab, and HIV-1 p24 Ag) must be ordered and reported.

In order to obtain reliable and consistent results, strictly adhere to the directions in these Instructions for Use. Do not modify the handling and storage conditions for kit reagents or patient samples.

Use the Manual Reagent Pack Piercer to pierce the reagent pack. Follow the directions below:

1. Place the reagent pack on a solid, flat surface. Hold reagent pack firmly with one hand while piercing with your other hand ❶.
2. Push down firmly until the top of vials touches the base of the Manual Reagent Pack Piercer as indicated below ❷ ❸.
3. Ensure that the reagent pack is sufficiently pierced as indicated below ❹ to prevent interference with the reagent probe when loaded on the BioPlex 2200 System. Remove the piercer and place it on a clean surface (e.g. on a Kimwipe) to avoid contamination.
   NOTE: After piercing, foils should be flush against the inside wall of the reagent vials. If foil is protruding outside the vial(s), perform a partial re-pierce as illustrated in ❶ below, only to push the foil inside.
4. Discard the piercer after single use. Do not reuse.



CAUTIONS:

1. For use with BioPlex 2200 HIV Ag-Ab reagent pack only.
2. Use of any device other than the Manual Reagent Pack Piercer may result in reagent contamination.
3. Contents of an open reagent pack can be spilled if the pack is tipped. Use caution when loading/unloading open reagent packs to prevent spillage.

Operating instructions, including calibration, quality control, and maintenance for the BioPlex 2200 System are further described in the BioPlex 2200 System Operation Manual. Prior to using the BioPlex 2200 HIV Ag-Ab assay, ensure that the BioPlex 2200 System is powered on, loaded with reagent packs and bulk solutions, and that all required maintenance has been performed. Refer to the BioPlex 2200 System Operation Manual for more information regarding these activities.

The BioPlex 2200 System Sheath Fluid and BioPlex 2200 System Wash Solution are not lot specific and can be interchanged.

A) Calibration

The BioPlex 2200 HIV Ag-Ab Calibrator Set should be loaded and assayed in triplicate (3) every 30 days and with each new Reagent Pack lot, whichever is first. The Calibrator Set is used to assign the relative fluorescence intensity (RFI) corresponding to the cutoff value. Each assay in the multiplex is calibrated separately using a 2-point plot. The BioPlex 2200 HIV-1/HIV-2 Antibody Calibrator contains antibodies specific to HIV 1 group M, HIV 1 group O, and HIV 2, and each population of antibodies has an Index based on reference calibrators. The fluorescence values of the bead populations that detect these antibodies are plot-

ted as y and the value assignment is plotted as x. The BioPlex 2200 HIV Antigen Calibrator contains no HIV antibodies and is the negative calibrator for the antibody detection bead populations.

Similarly, the BioPlex 2200 HIV Antigen Calibrator contains HIV-1 p24 antigen and has an Index based on reference material. The fluorescence of the bead population involved with antigen detection is plotted as y and the value assignment is plotted as x. The BioPlex 2200 HIV Antibody Calibrator contains no detectable HIV-1 p24 antigen and is the negative calibrator for the antigen detection bead population. For all plots, the cutoff is the fluorescence corresponding to an index value of 1.00.

Refer to the BioPlex 2200 System Operation Manual for more information.

B) Pack Validation

Pack Validation using the BioPlex 2200 HIV Ag-Ab Control Set must be run on each BioPlex 2200 HIV Ag-Ab reagent pack to verify its performance prior to running patient samples. Refer to the BioPlex 2200 System Operation Manual for instructions on performing Pack Validation.

C) Quality Control

The BioPlex 2200 HIV Ag-Ab Control Set must be run at least once every 24 hours, and after each calibration. Refer to the BioPlex 2200 System Operation Manual for instructions on processing quality controls.

The BioPlex 2200 HIV Ag-Ab Control Set includes a Negative Control and two Positive Controls in plasma or synthetic matrix containing HIV-1 and HIV-2 antibodies or antigen for HIV-1.

The Positive Controls are manufactured to give reactive results (i.e., values above the cut-off for each specific bead). The Negative Control is manufactured to give nonreactive results (i.e., values below the cut-off for all beads). The Negative Control must have a nonreactive result, and the Positive Controls must have reactive results for the HIV-1 p24 antigen, HIV-1 antibody (Groups M and O), and HIV-2 antibody, as appropriate.

Values for a given lot of Controls are loaded into the BioPlex 2200 System database via the provided media or by manual input. After identifying the control via the barcoded vial, the BioPlex 2200 System compares the control results to the expected lot-specific control values stored in the BioPlex 2200 System database. Failure to obtain the appropriate values for controls will invalidate the assay and indicates procedural error, improper sample handling, or deterioration of reagents. Additional controls may be tested in accordance with the laboratory's quality control policy. If a control result is out of its specified range, any test results generated since the last acceptable control results must be evaluated to determine if test results may have been affected adversely. Results that have been affected adversely are invalid and these samples must be retested.

D) Load/Process Samples

Load samples into the racks provided with the BioPlex 2200 System as indicated in the BioPlex 2200 System Operation Manual. Sample processing on the BioPlex 2200 System is fully automated. Refer to the BioPlex 2200 System Operation Manual for appropriate software setup.

INTERPRETATION OF RESULTS

Calculation

All calculations necessary to interpret the results are performed automatically by the BioPlex 2200 System software.

Data Analysis

NOTE: When testing with the BioPlex 2200 HIV Ag-Ab assay, results for all of the individual HIV analytes (HIV-1 Ab, HIV-2 Ab, and HIV-1 p24 Ag) must be reported.

Patient results are reported if the QC testing meets the required acceptance criteria. Results are expressed by the BioPlex 2200 System as Indices derived from the calibrators, and specimens are interpreted as Non-Reactive or REACTIVE.

Results from patient samples that are accompanied by the Warning message *SVB Too Low* are not valid, and the sample must be tested again. If repeat testing also has this Warning message a new sample should be collected, or the sample should be tested by another method. Samples that have been diluted (i.e. proficiency or survey samples) may produce this Warning message due to lack of serum in the sample.

Interpretation

The BioPlex 2200 HIV Ag-Ab assay generates an Index for each of the individual HIV analytes. It outputs the Index for each analyte along with the interpretation based on that Index, and it also outputs an overall result using the highest Index and an overall interpretation based on that Index.

- A specimen is REACTIVE if at least one analyte has an Index of ≥ 1.00. Repeat testing in duplicate is required for all initially reactive samples.
- A specimen is Non-Reactive if all analytes have Indices < 1.00. No repeat testing is required.

The table below summarizes the interpretation of results for each HIV analyte.

Interpretation of Results

Index (IDX)	Interpretation
< 1.00	Non-Reactive
≥ 1.00	REACTIVE

HIV-1 p24 Ag Results:

When HIV-1 and/or HIV-2 antibody levels are very high the antibody may interfere with HIV-1 p24 Ag results. Therefore the output of the BioPlex 2200 will not contain HIV-1 p24 Ag results when:

- the Index for HIV-1 or HIV-2 Ab is at least 100, AND
- the HIV-1 p24 Ag Index is at least 1.00.

The BioPlex 2200 System output for the HIV-1 p24 Ag results for these specimens will be "Not reportable due to high HIV Ab level", without Indices.

HIV-1 and HIV-2 Ab Results:

Some specimens that are reactive for HIV-1 antibody can cross-react with HIV-2 antigens, causing results on the HIV-2 beads to be reactive. Likewise, some specimens reactive for HIV-2 antibody can cross-react with HIV-1 antigens causing results on the HIV-1 beads to be reactive. Therefore, the BioPlex 2200 System output for specimens with Indices of at least 1.00 for both HIV-1 Ab and HIV-2 Ab is as follows:

- If the HIV-1 Ab Index is at least 5-fold the HIV-2 Ab Index, the BioPlex 2200 System output for HIV-1 Ab is REACTIVE with its Index. The HIV-2 Ab result output is Non-Reactive without an Index.

- If the HIV-2 Ab Index is at least 5-fold the HIV-1 Ab Index, the BioPlex 2200 System output for HIV-2 Ab is REACTIVE with its Index. The HIV-1 Ab result output is Non-Reactive without an Index.
- If the HIV-1 and HIV-2 Ab Indices have less than a 5-fold difference, the BioPlex 2200 System output for both HIV-1 and HIV-2 Ab is REACTIVE, Undifferentiated, with Indices.

Initially reactive specimens must be retested in duplicate. If they are repeatedly reactive, they must be investigated by additional, more specific, or supplemental tests. Refer to CDC guidelines for the current recommended HIV testing algorithm.

Reporting of Results

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	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 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.
**If 2 of 3 results are REACTIVE, Undifferentiated for HIV-1 Ab and HIV-2 Ab, the final result is REACTIVE, Undifferentiated. If 2 of 3 results are specific for an HIV Ab type, that specific HIV type is reported as HIV REACTIVE.

LIMITATIONS OF THE PROCEDURE

1. For In Vitro Diagnostic (IVD) Use.
2. The BioPlex 2200 HIV Ag-Ab assay procedure and the Interpretation of Results must be followed closely when testing for the presence of HIV-1 p24 antigen or antibodies to HIV-1 and/or HIV-2 in plasma or serum specimens. The user of this kit is advised to read the Instructions for Use carefully prior to testing. Testing of other body specimens, pooled blood, processed plasma, or products made from such pools, is not recommended.
3. HIV-1 p24 antigen results are not reliable in high-antibody-titer specimens. HIV-1 p24 antigen is not reported in specimens with HIV-1 or HIV-2 antibody Indices ≥100.
4. The BioPlex 2200 HIV Ag-Ab assay detects circulating antibodies to HIV-1 (groups M and O) and HIV-2 and HIV-1 p24 antigen, and thus is useful in evaluating patients with signs or symptoms of HIV infection and in establishing prior infection with HIV-1 or HIV-2. Clinical studies continue to clarify and refine the interpretation and medical significance of the presence of antibodies to HIV-1 or HIV-2.[41] Reactive specimens must be investigated by additional, more specific supplemental tests. Recommendations for appropriate use of such additional tests may be issued periodically by the United States Public Health Service. For individuals who are confirmed reactive for antigen or antibodies, appropriate counseling and medical evaluation should be offered. Both confirmation of the test result on a freshly drawn sample and counseling should be considered an important part of testing for HIV-1 p24 antigen and antibodies to HIV-1 and HIV-2.
5. AIDS and AIDS-related conditions are clinical syndromes and their diagnosis can only be established clinically. Testing alone cannot be used to diagnose AIDS, even if the recommended investigation of reactive specimens suggests a high probability that antibody to HIV-1 or HIV-2, or HIV-1 p24 antigen, is present.
6. A Non-Reactive test result at any point in the investigation of individual subjects does not preclude the possibility of exposure to or infection with HIV-1 and/or HIV-2.
7. Non-Reactive results can occur if the quantity of marker present in the sample is too low for the detection limits of the assay.
8. Repeat testing should be considered where there is clinical suspicion of infection or procedural error.
9. The performance of this assay has not been established for neonates and the assay should not be used in individuals younger than 2 years of age.[42]

10. A person who has antibodies to HIV is presumed to be infected with the virus, although a person who has participated in an HIV vaccine study may develop antibodies to the vaccine and may or may not be infected with HIV. Clinical assessment is indicated with appropriate counseling, medical evaluation, and possibly additional testing to decide whether a diagnosis of HIV infection is accurate.
11. Bacterially contaminated, icteric, lipemic, hemolyzed or heat-inactivated samples may cause erroneous results and should be avoided.
12. Specimens containing HIV-2 p26 antigen can cross-react with HIV-1 p24 antigen detection beads, generating reactive results for HIV-1 p24 antigen.
13. The calculated Index Values for anti-HIV antibodies and/or HIV-1 p24 antigen in a given specimen as determined by the BioPlex 2200 HIV Ag-Ab assay cannot be correlated to an endpoint titer or viral load.

PERFORMANCE CHARACTERISTICS

Testing to determine the performance characteristics of the BioPlex 2200 HIV Ag-Ab assay was performed on the BioPlex 2200 System and an FDA-approved HIV Ag/Ab assay. Supplemental testing of reactive specimens was performed with an FDA-approved HIV-1/HIV-2 differentiation assay and a licensed HIV-1 RNA test.

Specificity

Low Risk Populations

The BioPlex 2200 HIV Ag-Ab assay was tested with 6395 samples from populations at low risk for HIV infection (unknown HIV status), including 4358 serum and 2037 plasma samples. The reactive samples were also tested by an FDA-licensed HIV-1 RNA assay and an FDA-approved HIV-1/HIV-2 differentiation assay. Results for the BioPlex 2200 HIV Ag-Ab with serum and plasma samples are presented in Tables 1a-c.

Table 1a: Reactivity in Low Risk Populations - Serum

Low Risk Population	N	BioPlex 2200 HIV Ag-Ab Assay											Supplemental Results for HIV Repeatedly Reactive Specimens		
		NR	HIV Ag-Ab		HIV-1 Ab		HIV-2 Ab		HIV Ab Undifferentiated		HIV-1 p24 Ag		HIV-1 RNA Assay Pos	HIV-1/HIV-2 Differentiation Assay	
			IR	RR	IR	RR	IR	RR	IR	RR	IR	RR		HIV-1 Pos	HIV-2 Pos
First Time Blood Donors	1346	1344	2	2	1	0	0	0	0	0	2	2	NT	0	0
Normal Healthy Individuals	991	988	3	3	3	3	0	0	0	0	0	0	2	2	0
Military Recruits – Fresh	500	499	1	1	1	1	0	0	0	0	0	0	NT	1	0
Military Recruits – Frozen	499	498	1	1	1	1	0	0	0	0	0	0	0	0	0
Pregnant Women	922	918	4	4	2	2	0	0	1	1	2	2	NT	1	0
Healthy Pediatric Subjects	100	100	0	0	0	0	0	0	0	0	0	0	NA	NA	NA
Total	4358	4347	11	11	8	7	0	0	1	1	4	4[1]	2	4	0

NR = Non-Reactive IR = Initially Reactive RR = Repeatedly Reactive

NT = Not Tested. The repeatedly reactive samples had insufficient volume for testing on the HIV-1 RNA assay.

NA = Not applicable because none of the samples were reactive on the BioPlex 2200 HIV Ag-Ab assay

[1] Three of the 4 samples were non- reactive for HIV-1 antibody. The fourth sample was HIV Ab undifferentiated on initial and repeat testing.

Table 1b: Reactivity in Low Risk Populations - Plasma

Low Risk Population	N	BioPlex 2200 HIV Ag-Ab Assay											Supplemental Results for HIV Repeatedly Reactive Specimens		
		NR	HIV Ag-Ab		HIV-1 Ab		HIV-2 Ab		HIV Ab Undifferentiated		HIV-1 p24 Ag[1]		HIV-1 RNA Assay Pos	HIV-1/HIV-2 Differentiation Assay	
			IR	RR	IR	RR	IR	RR	IR	RR	IR	RR		HIV-1 Pos	HIV-2 Pos
First Time Blood Donors	653	653	0	0	0	0	0	0	0	0	0	0	NA	NA	NA
Normal Healthy Individuals	1306	1289	17	17	15	15	0	0	1	1	2	2	14	14	0
Pregnant Women	78	78	0	0	0	0	0	0	0	0	0	0	NA	NA	NA
Total	2037	2020	17	17	15	15	0	0	1	1	2	2	14	14	0

NR = Non-Reactive IR = Initially Reactive RR = Repeatedly Reactive

NT = Not Tested. The repeatedly reactive samples had insufficient volume for testing on the HIV-1 RNA assay.

NA = Not applicable because none of the samples were reactive on the BioPlex 2200 HIV Ag-Ab assay

[1] One of the samples from the normal healthy individuals population was not reportable for HIV p24 Ag due to high Ab level on initial and repeat testing.

Table 1c: Reactivity in Low Risk Populations – Summary of Serum and Plasma

Low Risk Population	N	BioPlex 2200 HIV Ag-Ab Assay											Supplemental Results for HIV Repeatedly Reactive Specimens		
		NR	HIV Ag-Ab		HIV-1 Ab		HIV-2 Ab		HIV Ab Undifferentiated		HIV-1 p24 Ag[1]		HIV-1 RNA Assay Pos	HIV-1/HIV-2 Differentiation Assay	
			IR	RR	IR	RR	IR	RR	IR	RR	IR	RR		HIV-1 Pos	HIV-2 Pos
Total Serum and Plasma	6395	6367	28	28	23	22	0	0	2	2	6	6	16	18	0

NR = Non-Reactive IR = Initially Reactive RR = Repeatedly Reactive

[1] One of the samples in the low risk population was not reportable for HIV p24 Ag due to high Ab level on initial and repeat testing.

As shown in Table 1c, 99.56% (6367/6395) of the samples from the low risk populations were non-reactive for HIV and 0.44% (28/6395) were repeatedly reactive for HIV with the BioPlex 2200 HIV Ag-Ab assay. Of the 28 repeatedly reactive samples, 19 were confirmed positive by supplemental testing. Fifteen (15) were positive for HIV-1 with both the FDA-approved HIV-1/HIV-2 Ab differentiation test and the licensed HIV-1 RNA test, 1 was positive for HIV-1 antibody on the differentiation test only, 2 were positive for HIV-1 antibody on the differentiation test and QNS for testing with the HIV-1 RNA test, and 1 was positive on the HIV-1 RNA test only. The 19 confirmed positive samples were removed from the specificity calculations. The specificity in this population was 99.86% (6367/6376) with a 95% confidence interval (CI) of 99.73 – 99.93%.

With the BioPlex 2200 HIV Ag-Ab assay, 99.62% (6371/6395) of the samples from the low risk populations were non-reactive and 0.34% (22/6395) were repeatedly reactive for HIV-1 Ab. Two (2) of the samples (0.03%) were repeatedly reactive undifferentiated for HIV-1 and HIV-2 Ab. The 19 confirmed HIV-1 Ab positive samples were removed from the specificity calculations. The specificity for HIV-1 Ab in this population was 99.92% (6371/6376) with a 95% CI of 99.82 – 99.97%.

In the low risk populations, 99.97% (6393/6395) of the samples were non-reactive for HIV-2 Ab and 0.03% (2/6395) were reactive for HIV Ab, undifferentiated, with the BioPlex 2200 HIV Ag-Ab assay. The specificity for HIV-2 Ab in this population was 99.97% (6393/6395) with a 95% CI of 99.89 – 99.99%.

For the detection of HIV-1 p24 Ag in low risk populations, 99.89% (6388/6395) of the samples were non-reactive, 0.09% (6/6395) were reactive, and 0.02% (1/6395) was not reportable for HIV-1 p24 Ag due to high Ab level. Of the 6 repeatedly reactive samples, none were confirmed reactive in supplemental testing. The sample that was not reportable for HIV-1 p24 Ag due to high Ab level was removed from the specificity calculations. The specificity for HIV-1 p24 Ag in this population was 99.91% (6388/6394) with a 95% CI of 99.80 – 99.96%.

Table 2 shows the combined results for the samples from low risk populations that were tested with the BioPlex 2200 HIV Ag-Ab assay in comparison to results of testing the same samples with an FDA-approved HIV Ag/Ab assay. Samples that were reactive on one or both of these assays were also tested with an HIV-1/HIV-2 differentiation assay and an HIV-1 RNA assay.

Table 2: Combined Low Risk Populations – Assay Comparison Summary

BioPlex 2200 HIV Ag-Ab Result	FDA-Approved HIV Ag/Ab assay	N	HIV-1 RNA Assay Reactive	HIV-1/HIV-2 Differentiation Assay		
				HIV-1 Positive	HIV-2 Positive	HIV Positive Undifferentiated
Repeatedly Reactive	Repeatedly Reactive	19[a,b]	15[c]	18	0	0
Repeatedly Reactive	Non-Reactive	9[d]	1	0	0	0
Non-Reactive	Repeatedly Reactive	3[e]	0	0	0	0
Non-Reactive	Non-Reactive	6364	NA	NA	NA	NA
Total		6395	16	18	0	0

NA = Not applicable because none of the samples were reactive on the BioPlex 2200 HIV Ag-Ab assay or FDA-approved HIV Ag/Ab assay.

[a] 3 samples had insufficient volume for testing with the HIV-1 RNA assay; 1 of these was tested and negative with the HIV-1 Ag EIA.

[b] 1 sample was negative with both the HIV-1/HIV-2 differentiation assay and an HIV-1 Ag EIA.

[c] All 15 samples that were reactive on the HIV-1 RNA assay were also reactive for HIV-1 on the HIV-1/HIV-2 differentiation assay.

[d] 4 samples had insufficient volume for testing with the HIV-1 RNA assay and were negative with an HIV-1 Ag EIA.

[e] 1 sample had insufficient volume for testing with the HIV-1 RNA assay and was negative with an HIV-1 Ag EIA.

Reactivity in Repeat Blood Donors

The BioPlex 2200 HIV Ag-Ab was tested with samples collected from 1340 repeat blood donors. The reactive samples were also tested by an FDA-licensed HIV-1 RNA assay and an FDA-approved HIV-1/HIV-2 differentiation assay. Results for the Bio-Plex 2200 HIV Ag-Ab are presented in Table 3.

Table 3: Reactivity in Repeat Blood Donors

Population	N	BioPlex 2200 HIV Ag-Ab Assay											Supplemental Results for HIV Repeatedly Reactive Specimens		
		NR	HIV Ag-Ab		HIV-1 Ab		HIV-2 Ab		HIV Ab Undifferentiated		HIV-1 p24 Ag		HIV-1 RNA	HIV-1/HIV-2 Differentiation Assay	
			IR	RR	IR	RR	IR	RR	IR	RR	IR	RR	Reactive	HIV-1 Pos	HIV-2 Pos
Repeat Blood Donors	1340	1339	4	1	0	0	0	0	1	0	4	1	0	0	0

NR = Non-Reactive IR = Initially Reactive RR = Repeatedly Reactive

A total of 99.70% (1336/1340) of the repeat blood donors were initially non-reactive, 0.30% (4/1340) were initially reactive, and 0.075% (1/1340) was repeatedly reactive for HIV with the BioPlex 2200 HIV Ag-Ab assay. Of the 4 initially reactive specimens, 3 were reactive for HIV-1 p24 Ag and 1 was reactive for both HIV-1 p24 Ag and for HIV Ab undifferentiated. The 1 repeatedly reactive sample was reactive for HIV-1 p24 Ag and it was not confirmed positive on the licensed HIV-1 RNA assay or reactive on the FDA-approved HIV-1/HIV-2 differentiation assay. For the BioPlex 2200 HIV Ag-Ab assay the specificity of the repeat blood donors was 99.93% (1339/1340) with a 95% CI of 99.58 – 99.99%.

Reactivity in Individuals with Unrelated Medical Conditions

The BioPlex 2200 HIV Ag-Ab assay was evaluated with samples collected from individuals that had unrelated medical conditions in order to assess potential cross-reactivity. Performance was evaluated with unspiked samples as well as aliquots of each specimen that were spiked with antibody to HIV-1 and HIV-2 and another aliquot of each specimen that was spiked with HIV-1 antigen. Results are shown in Table 4.

Table 4: Unrelated Medical Conditions

Cross-Reactivity Disease Category	N	Unspiked Samples	HIV Ab Spiked Samples		HIV Ag Spiked Samples	
		Non-Reactive	Ab Reactive	Ag Reactive	Ab Reactive	Ag Reactive
Autoimmune Disease (Lupus)	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Chlamydia	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Common Cold	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Cord Blood (Neonates)	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Crohn's Disease	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Cytomegalovirus Ab	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Dialysis Patients	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Hypergammaglobulinemia IgG	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Hypergammaglobulinemia IgM	20	20/20 (100%)	19/20 (95%)	0/20 (0%)	0/20 (0%)	20/20 (100%)
IgM Monoclonal Gammopathy	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Multiparous Pregnancies	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Multiply Transfused Patients	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
P. aeruginosa	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
E. coli	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Epstein-Barr Virus Ab positive	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Fungal Infection	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Graves' Disease	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Hemophilia	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Hepatitis A Virus Ab positive	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Hepatitis B Virus Ab positive	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Hepatitis C Virus Ab positive	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Herpes Simplex Virus Ab positive	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Human Anti-Mouse Antibodies (HAMA) pos	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Human T-Lymphotropic Virus (HTLV)	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Pregnancy First Trimester	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Pregnancy Second Trimester	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Pregnancy Third Trimester	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Pre-Influenza Vaccine Recipient	20	20/20 (100%)	20/20 (100%)	0/20 (0%)	0/20 (0%)	19/20 (95%)
Post-Influenza Vaccine Recipient	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Rheumatoid Factor positive	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
S. aureus	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Smallpox Vaccine Recipient	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Syphilis (Positive Serology)	18 [a]	18/18 (100%)	18/18 (100%)	0/18 (0%)	0/18 (0%)	18/18 (100%)
Varicella Zoster Virus Ab Positive	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Viral Diarrheal Illness	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Yeast Reactive	10	10/10 (100%)	10/10 (100%)	0/10 (0%)	0/10 (0%)	10/10 (100%)
TOTAL	388	388/388 (100%)	387/388 (99.74%)	0/388 (0%)	0/388 (0%)	387/388 (99.74%)
Specificity[b]		388/388 (100%)				

[a] 2 of the initial 10 samples in the category of syphilis serology positive were reactive on the BioPlex 2200 HIV Ag-Ab assay and were also reactive on an approved HIV Ag/Ab assay. Since they were reactive on initial testing, they were not spiked with HIV Ab or Ag for subsequent testing. Ten (10) additional samples were tested, and they were nonreactive on initial testing with the BioPlex assay and reactive for the applicable HIV analyte after spiking.

[b] Samples that were confirmed reactive by an FDA-approved HIV Ag/Ab assay were excluded from the specificity calculation.

In the 390 samples from individuals with medical conditions unrelated to HIV, 388 were nonreactive on the BioPlex 2200 HIV Ag-Ab assay. Two (2) samples, from individuals serologically positive for syphilis, were reactive for HIV-1 antibody and were also reactive for HIV by an approved HIV-1/HIV-2 Ag/Ab assay, and identified as true positive samples. Therefore, the specificity of the BioPlex 2200 HIV Ag-Ab in these specimens was 100% (388/388). Of the 388 samples spiked with HIV-1 and HIV-2 antibody, 387/388 (99.74%) were reactive for both HIV-1 and HIV-2 antibody and nonreactive for HIV-1 antigen; 1 sample spiked with HIV-1 group O antibody was nonreactive. Of the same samples that were spiked with HIV-1 antigen, 387/388 (99.74%) were reactive for HIV-1 antigen and nonreactive for both HIV-1 and HIV-2 antibody; 1 sample spiked with HIV-1 antigen was nonreactive.

HIV-2 p26 Antigen Cross-Reactivity in Culture Supernatants

HIV-2 p26 antigen cross-reactivity was evaluated by testing four culture supernatants (strains CBL-20, CBL-21, CBL-23 and Cam-2), two HIV-2 viral lysates (strains ROD and NIH-Z) and a recombinant HIV-2 gag p26 (ROD strain) with the BioPlex 2200 HIV Ag-Ab assay. Of the four HIV-2 culture supernatant samples tested, three were reactive on the BioPlex 2200 HIV Ag-Ab assay and with an FDA-approved HIV Ag/Ab assay. The non-reactive supernatant (CBL-21) was also non-reactive with an FDA-approved HIV Ag/Ab assay. The two HIV-2 lysates and the HIV-2 recombinant protein were reactive with the BioPlex 2200 HIV Ag-Ab assay and with an FDA-approved HIV Ag/Ab assay. An HIV-2 p26 cross-reactivity of 0.06% was obtained with the BioPlex 2200 HIV Ag-Ab assay when testing the recombinant HIV-2 p26; 11ng/mL p26 versus 6pg/mL HIV-1 p24 antigen were required to obtain a reactive result.

Sensitivity

HIV-1 p24 Antigen Analytical Sensitivity

The analytical sensitivity of the BioPlex 2200 HIV Ag-Ab assay for HIV-1 p24 antigen was assessed by testing a standard approved by the Agence Nationale de Sécurité du Médicament et des Produits de Santé and by testing the WHO HIV international standard NIBSC 90/636. The results from an internal study demonstrated an antigen sensitivity of 5.2 pg/mL (range of 5.0 – 5.4 pg/mL) with the ANSM standard, and an antigen sensitivity of 0.33 IU/mL (range of 0.29 – 0.35 IU/mL) with the WHO standard.

HIV-1 Antigen Detection in Culture Supernatants

HIV-1 antigen subtypes were evaluated in the testing of 54 culture supernatants with the BioPlex 2200 HIV Ag-Ab assay. These supernatants were from HIV-1 Group M subtypes A (n = 3), CRF01_AE (n = 10), CRF01_AG (n = 2), B (n = 13), C (n = 8), D (n = 3), F (n = 5), G (n = 4), H (n = 1), J (n = 2) and N (n=1). Two (2) HIV-1 Group O supernatants were also tested. Of the 54 HIV-1 culture supernatant samples tested with the BioPlex 2200 HIV Ag-Ab assay, 100% (54/54) were reactive.

Known HIV-1 Ag Positive

Four known HIV-1 Ag positive samples were tested with the BioPlex 2200 HIV Ag-Ab. The samples were also tested by an FDA-approved HIV Ag/Ab assay and results are presented in Table 5.

Table 5: Reactivity in Known HIV-1 Ag Positive Samples

Population	N	BioPlex 2200 HIV Ag-Ab Assay					FDA-Approved HIV Ag/Ab Assay Repeatedly Reactive
		Non-Reactive	Repeatedly Reactive				
			HIV Ag-Ab	HIV-1 Ab	HIV-2 Ab	HIV-1 p24 Ag	
Known HIV-1 Antigen Positive	4	0	4	1	0	4	4

As shown above in Table 5, all 4 known HIV-1 Ag positive samples were repeatedly reactive for HIV-1 p24 Ag on the BioPlex 2200 HIV Ag-Ab assay, as well as with an FDA-approved HIV Ag/Ab assay. One (1) of the 4 samples was also repeatedly reactive for HIV-1 Ab.

HIV-1 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. A summary of the results is presented in Table 6.

Table 6: Reactivity in HIV-1 Seroconversion Panels

Panel #	# of Panel Members Tested	Number of Reactive Panel Members				Days to First Reactive Result				Difference in Days to 1st Reactive FDA-approved HIV Ag/Ab assay Result (Based on Bleed Date)
		BioPlex 2200 HIV Ag-Ab Assay			FDA-approved HIV Ag/Ab assay	BioPlex 2200 HIV Ag-Ab Assay			FDA-approved HIV Ag/Ab assay	
		HIV Ag-Ab	HIV-1 Ab	HIV-1 p24 Ag		HIV Ag-Ab	HIV-1 Ab	HIV-1 p24 Ag		
1	10	7	3	7	5	21	30	21	23	2
2	10	4	2	4	4	25	32	25	25	0
3	9	4	0	4	4	21	NA	21	21	0
4	7	2	0	2	2	18	NA	18	18	0
5	11	2	1	2	2	36	38	36	36	0
6	8	5	3	5	4	0	0	0	0	0
7	7	6	5	1	6	0	10	0	0	0
8	8	2	1	2	2	33	38	33	33	0
9	11	4	1	4	3	25	35	25	28	3
10	3	1	1	0	1	8	8	NA	8	0
11	9	3	1	3	3	23	32	23	23	0
12	7	2	0	2	2	53	NA	53	53	0
13	14	7	5	2	5	22	36	22	36	14
14	6	4	3	3	4	22	32	22	22	0
15	10	3	2	3	3	66	69	66	66	0
16	27	16	14	5	16	45	52	45	45	0
17	25	17	13	6	17	40	55	40	40	0
18	4	3	2	3	3	24	26	24	24	0
19	9	6	4	3	6	53	60	53	53	0
20	13	6	4	5	5	23	33	23	28	5
21	6	3	2	1	3	15	30	15	15	0
22[a]	5	4	2	4	5	53	65	53	0	-53
23	8	4	3	4	4	26	33	26	26	0
24	6	2	2	2	2	44	44	44	44	0
25	6	4	2	3	4	7	27	7	7	0
26	9	5	4	2	5	15	28	15	15	0
27	9	4	1	3	4	16	103	16	16	0
28	8	8	6	4	7	0	11	0	7	7
29	7	5	2	5	5	7	19	7	7	0
30	6	5	2	5	4	2	14	2	7	5
31	6	4	2	4	3	7	15	7	13	6
32	4	3	3	2	3	9	9	9	9	0
33	6	4	1	4	4	8	19	8	8	0
34	6	4	2	2	4	10	17	10	10	0
35	4	3	1	3	3	3	10	3	3	0
36	7	2	1	2	2	17	21	17	17	0
37	7	2	1	2	2	23	28	23	23	0
38	6	4	2[b]	4	4	7	15[b]	7	7	0
39	7	7	5	7	7	0	9	0	0	0
40	6	5	3	4	5	5	12	5	5	0
41	14	14	14	0	14	0	NA	0	0	0
42	14	14	14	0	14	0	NA	0	0	0
Total	365									
Total Reactive Bleeds		214	140	133	205					
Total Reactive Panels		42	39	39	42					

[a] Bleed #2 in Panel #22 is 53 days after bleed #1. The C of A for this panel indicates that no EIA or RNA results were positive from tube 1. The difference between the historic results and the BioPlex 2200 HIV Ag-Ab Assay is 1 tube.

[b] One replicate of Panel #38 was reactive and one was non-reactive for HIV-1 Ab on the BioPlex 2200 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 that were reactive by both BioPlex 2200 HIV Ag-Ab and the FDA-approved HIV Ag/Ab assay, 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.

Reactivity in Known HIV-1 Antibody Positive Samples

A study was performed with 1363 known HIV-1 antibody positive samples (348 sera and 1015 plasma) that were tested on the BioPlex 2200 HIV Ag-Ab assay and an FDA-approved HIV Ag/Ab assay. Results were compared to an FDA-approved HIV Ag/Ab combination assay. Within this group of samples there were 997 retrospective HIV-1 antibody positive samples, 100 samples from AIDS patients with CDC staging, 50 samples from HIV-1 infected pediatric subjects, 60 samples from HIV-1 positive pregnant women, and156 samples of known HIV-1 antibody subtype. Table 7 summarizes the reactivity with the BioPlex 2200 HIV Ag-Ab assay in these known antibody positive samples.

Table 7: Reactivity in Known HIV-1 Antibody Positive Samples

Population	N	BioPlex 2200 HIV Ag-Ab Assay					FDA-Approved HIV Ag/Ab Assay Repeatedly Reactive
		Non-Reactive	Repeatedly Reactive				
			HIV Ag-Ab	HIV-1 Ab	HIV-2 Ab	HIV-1 p24 Ag	
HIV-1 Antibody Positive	997	0	997	997	0	0[b]	997
AIDS with CDC Stage	100	0	100	100	0	0[c]	100
HIV-1 Positive (Pediatric Subjects)	50	0	50	50	0	0	50
HIV-1 Positive (Pregnant Women)	60	0	60	60	0	0[d]	60
HIV-1 Antibody Subtypes[a]	156	0	156	156	0	0[e]	156
Total	1363	0	1363	1363	0	0[f]	1363

[a] The HIV-1 antibody subtype samples includes 3 known HIV-1 Group O positive samples
[b] 221 samples were not reportable for HIV p24 Ag due to high Ab level.
[c] 16 samples were not reportable for HIV p24 Ag due to high Ab level.
[d] 1 sample was not reportable for HIV p24 Ag due to high Ab level.
[e] 8 samples were not reportable for HIV p24 Ag due to high Ab level.
[f] 246 samples were not reportable for HIV p24 Ag due to high Ab level.

In the known HIV-1 antibody positive populations that were tested with the BioPlex 2200 HIV Ag-Ab, all specimens were repeatedly reactive for HIV-1 Ab for a sensitivity of 100% (1363/1363) with a 95% CI of 99.72 – 100%. All specimens were non-reactive for HIV-2 Ab. There were 246 samples that were not reportable for HIV-1 p24 Ag due to high Ab level and the remaining samples were non-reactive for HIV-1 p24 Ag.

Reactivity in Known HIV-2 Antibody Positive Samples

The BioPlex 2200 HIV Ag-Ab assay was tested with 200 known HIV-2 antibody positive samples obtained from individuals in different locations and results were compared with an FDA-approved HIV Ag/Ab assay. A summary of the results is shown in Table 8.

Table 8: Reactivity in Known HIV-2 Antibody Positive Samples

Population	N	BioPlex 2200 HIV Ag-Ab Assay						FDA-Approved HIV Ag/Ab Assay Repeatedly Reactive
		Non-Reactive	Repeatedly Reactive					
			HIV Ag-Ab	HIV-1 Ab	HIV-2 Ab	HIV Ab Undifferentiated	HIV-1 p24 Ag	
HIV-2 Antibody Positive	200	0	200	0	188	12	0[a]	200

[a] 4 samples were not reportable for HIV p24 Ag due to high Ab level.

As shown in the table above, the sensitivity of the BioPlex 2200 HIV Ag-Ab assay in the known HIV-2 antibody positive population was 100% (200/200) with a 95% CI of 98.12 - 100%. Twelve (12) of the 200 samples were repeatedly reactive for both HIV-1 Ab and HIV-2 Ab and were interpreted as HIV Ab undifferentiated.

HIV-1 Group O Antibody Positive Samples

The performance of the BioPlex 2200 HIV Ag-Ab assay was evaluated with 63 known HIV-1 Group O antibody positive samples. All 63/63 samples (100%) were reactive for HIV with the BioPlex 2200 HIV Ag-Ab assay.

HIV-1 Subtype Samples

In studies of HIV-1 Group M subtypes, 216 specimens were tested with the BioPlex 2200 HIV Ag-Ab assay. These subtype specimens were reactive in 100% (216/216) of the members on the BioPlex 2200 HIV Ag-Ab assay. The following subtypes were tested:

Subtype	# tested	# reactive
A	29	29
B	4	4
C	5	5
CRF01	11	11
CRF02	77	77
CRF05	1	1
CRF06	3	3
CRF07	1	1
CRF09	2	2
Unknown	1	1

Subtype	# tested	# reactive
CRF11	10	10
CRF13	7	7
D	16	16
F	20	20
G	16	16
H	7	7
J	2	2
K	3	3
U	1	1

Low Titer Panel

Sensitivity of the BioPlex 2200 HIV Ag-Ab assay was assessed with a commercially available HIV-1 Low Titer Panel, consisting of 10 plasma members with HIV-1 antibody responses near the sensitivity limit of anti-HIV screening tests. Reactivity was compared to an FDA-approved HIV Ag/Ab assay. The BioPlex 2200 HIV Ag-Ab assay and the reference HIV Ag/Ab assay were equivalent in 100% (9/9) of the reactive HIV-1 Low Titer Panel samples (one member was a negative control).

HIV-1 Incidence/Prevalence Panel

The BioPlex 2200 HIV Ag-Ab assay was also tested with a commercially available HIV-1 Incidence/Prevalence Panel, which is a plasma panel consisting of 7 incident members and 8 prevalent members from different donors. The panel was tested on the BioPlex 2200 HIV Ag-Ab assay and an FDA-approved HIV Ag/Ab assay, and compared to the HIV-1 Western blot historical data described in the Certificate of Analysis. The BioPlex 2200 HIV Ag-Ab assay, the FDA-approved HIV Ag/Ab assay, and the HIV-1 Western blot were equivalent in 100% (15/15) of the HIV-1 Incidence/Prevalence Panel samples.

AIDS with CDC Stage Classification

A total of 100 CDC AIDS stage classification samples were tested with the BioPlex 2200 HIV Ag-Ab assay and an FDA-approved HIV Ag/Ab assay. Results are summarized in Table 9.

Table 9: AIDS with CDC Stage Classification

Known HIV Antibody Status	N	BioPlex 2200 HIV Ag-Ab Assay					FDA-Approved HIV Ag/Ab Assay Repeatedly Reactive
		Non-Reactive	Repeatedly Reactive				
			HIV Ag-Ab	HIV-1 Ab	HIV-2 Ab	HIV-1 p24 Ag[a]	
CDC AIDS stage A3	31	0	31	31	0	0	31
CDC AIDS stage B3	10	0	10	10	0	0	10
CDC AIDS stage C1	12	0	12	12	0	0	12
CDC AIDS stage C2	26	0	26	26	0	0	26
CDC AIDS stage C3	21	0	21	21	0	0	21
Total	100	0	100	100	0	0	100

[a] 16 samples were not reportable for HIV-1 p24 Ag due to high Ab level.

Individuals at High Risk for HIV Infection

In a study of individuals at high risk for HIV infection, 1729 serum and 137 plasma samples were tested with the BioPlex 2200 HIV Ag-Ab assay and an FDA-approved HIV Ag/Ab assay. The individuals were identified as high risk for HIV infection due to the following factors: lifestyle, behavior, or known exposure event. All samples that were reactive on the BioPlex 2200 HIV Ag-Ab assay or repeatedly reactive on the FDA-approved HIV Ag/Ab assay were further tested by a licensed HIV-1 RNA assay and an FDA-approved HIV-1/HIV-2 differentiation assay. Results are presented in Table 10.

Table 10: Reactivity in High Risk Populations

High Risk Population	N	BioPlex 2200 HIV Ag-Ab Assay									Supplemental Results for HIV Repeatedly Reactive Specimens			
		NR	HIV Ag-Ab		HIV-1 Ab		HIV-2 Ab		HIV-1 p24 Ag[a]		HIV-1 RNA Assay Pos	HIV-1/HIV-2 Differentiation Assay		
			IR	RR	IR	RR	IR	RR	IR	RR		HIV-1 Pos	HIV-2 Pos	HIV Indeterm-inate
Individuals at High Risk for HIV Infection	999	935	67	64[b]	67	63	0	0	1	1	15	45	0	1
Pregnant Women at High Risk for HIV Infection	383	371	12	12	11	11	1	1	0	0	10	10	1	0
Pediatric Subjects at High Risk for HIV Infection	484	483	1	1	1	1	0	0	0	0	0	0	0	0
Total	1866	1789	80	77[b]	79	75[c]	1	1	1	1	25	55	1	1

NR = Non-Reactive IR = Initially Reactive RR = Repeatedly Reactive

[a] Three (3) of the samples in the high risk populations were not reportable for HIV p24 Ag due to high Ab level

[b] Four (4) samples that were reactive on the BioPlex HIV Ag-Ab assay had insufficient volume to test on the HIV-1/HIV-2 differentiation test and the HIV-1 RNA assay. All four samples were repeatedly reactive with the FDA-approved HIV Ag/Ab assay.

[c] Of the 75 specimens in the high risk population that were BioPlex HIV-1 Ab reactive, 55 were confirmed positive for HIV-1 by the combination of supplemental testing performed, 1 was confirmed positive for HIV-2, 15 were non-reactive on supplemental testing, and 4 had insufficient test data to categorize the samples. Of these 19 samples not confirmed by supplemental testing, 12 had an indeterminate status with supplemental HIV-1 Western blot testing.

As shown in Table 11, 95.87% (1789/1866) of the individuals at high risk of HIV infection were non-reactive and 4.13% (77/1866) were repeatedly reactive for HIV with the BioPlex 2200 HIV Ag-Ab assay. Of 77 specimens that were repeatedly reactive, 57 were confirmed positive by the supplemental testing performed; 55 were positive for HIV-1 antibody, 1 was HIV-1 Ab indeterminate, and 1 was positive for HIV-2 antibody on the HIV-1/HIV-2 differentiation test.

Of the individuals at high risk of HIV infection, 95.98% (1791/1866) were non-reactive and 4.02% (75/1866) were repeatedly reactive for HIV-1 Ab. The BioPlex 2200 HIV Ag-Ab assay was repeatedly reactive for HIV-1 Ab in 100% (55/55) of the samples in the high risk population that were identified as HIV-1 Ab positive by supplemental testing.

The BioPlex 2200 HIV Ag-Ab was non-reactive for HIV-2 Ab in 99.95% (1865/1866) and repeatedly reactive in 0.05% (1/1866) of the samples from the high risk populations. The repeatedly reactive sample was also reactive for HIV-2 on the HIV-1/HIV-2 differentiation test.

In the high risk population, 1862/1866 (99.79%) were non-reactive for HIV-1 p24 Ag, 1/1866 (0.05%) was repeatedly reactive, and 0.16% (3/1866) were not reportable for HIV-1 p24 Ag due to high Ab level. The one HIV-1 p24 Ag repeatedly reactive sample was non-reactive on supplemental HIV-1 RNA testing, but was reactive with a CE marked assay for HIV-1 p24 antigen.

Comparison to an FDA-approved HIV Ag/Ab Assay in a High Risk Population

The 1866 samples from high risk populations that were reactive on the BioPlex 2200 HIV Ag-Ab assay are presented in Table 11 in comparison to results of testing the same samples with an FDA-approved HIV Ag/Ab assay.

Table 11: Reactivity in High Risk Populations – Comparison to an Approved HIV Ag/Ab Assay

High Risk Population		FDA-Approved HIV Ag/Ab Assay		
		Repeatedly Reactive	Non-Reactive	Total
BioPlex 2200 HIV Ag-Ab Assay	Repeatedly Reactive	74	3[a]	77
	Non-Reactive	6[b]	1783	1789
	Total	80	1786	1866

[a] All 3 samples were negative for HIV antibody with the FDA-approved HIV-1/HIV-2 differentiation test. Two (2) samples were non-reactive on the licensed HIV-1 RNA assay and one of these was reactive on an HIV-1 Ag assay. The remaining sample was nonreactive on the HIV-1 Ag assay and not tested on the HIV-1 RNA assay.

[b] Five (5) of the 6 samples were negative for HIV antibody and 1 sample was HIV-1 indeterminate with the FDA-approved HIV-1/HIV-2 differentiation test. Four (4) of these 6 samples were non-reactive on the licensed HIV-1 RNA assay; of the remaining 2, 1 was negative with an HIV-1 Ag assay and 1 had insufficient volume for HIV-1 RNA or HIV-1 Ag testing.

Individuals from an HIV-2 Endemic Region

The study of individuals from an HIV-2 endemic region included a total of 490 samples that were tested with the BioPlex 2200 HIV Ag-Ab assay and with an FDA-approved HIV Ag/Ab assay. Samples that were repeatedly reactive were also tested with a licensed HIV-1 RNA test and an FDA-approved HIV-1/HIV-2 differentiation test. A summary of results is shown in Tables 12 and 13.

Table 12: Reactivity in HIV-2 Endemic Region

Population	N	BioPlex 2200 HIV Ag-Ab Assay						Supplemental Results for HIV Repeatedly Reactive Specimens			
		Non-Reactive	Repeatedly Reactive					HIV-1 RNA Reactive	HIV-1/HIV-2 Differentiation Assay		
			HIV Ag-Ab	HIV-1 Ab	HIV-2 Ab	HIV Ab Undifferentiated	HIV-1 p24 Ag [a]		HIV-1 Pos	HIV-2 Pos	HIV Undifferentiated
HIV-2 Endemic Region	490	182	308	236	47	25	0	129	224	43	24

[a] 55 samples were not reportable for HIV p24 Ag due to high Ab level.

As shown in the table above, 62.86% (308/490) of the samples from an HIV-2 endemic region were repeatedly reactive for HIV and 37.14% (182/490) were non-reactive with the BioPlex 2200 HIV Ag-Ab assay. Of the 308 repeatedly reactive specimens, 129 of the 190 tested with the licensed HIV-1 RNA assay were reactive. With the FDA-approved HIV-1/HIV-2 differentiation test, 224 were positive for HIV-1 antibody, 43 were positive for HIV-2 antibody, and 24 were HIV antibody undifferentiated. Of the 24 samples that were HIV antibody undifferentiated on the HIV-1/HIV-2 differentiation test, 13 were reactive, 6 were non-reactive, and 5 were not tested on the HIV-1 RNA assay.

In the testing of samples from an HIV-2 endemic region, 48.16% (236/490) were repeatedly reactive for HIV-1 Ab, 5.10% (25/490) were repeatedly reactive for HIV Ab undifferentiated, and 46.73% (229/490) were non-reactive for HIV-1 Ab with the BioPlex 2200 HIV Ag-Ab assay. The BioPlex 2200 HIV Ag-Ab assay was repeatedly reactive for HIV-1 Ab in 99.11% (222/224) of the samples in the HIV-2 endemic population that were identified as HIV-1 Ab positive by supplemental testing and reactive undifferentiated in the remaining 0.89% (2/224) samples.

As shown in the table above, 9.59% (47/490) of the samples were repeatedly reactive for HIV-2 Ab, 5.10% (25/490) were repeatedly reactive for HIV Ab undifferentiated, and 85.31% (418/490) were non-reactive for HIV-2 Ab with the BioPlex 2200 HIV Ag-Ab assay. The BioPlex 2200 HIV Ag-Ab assay was repeatedly reactive for HIV-2 Ab in 97.67% (42/43) of the samples in the high risk population that were identified as HIV-2 Ab positive by supplemental testing, and reactive undifferentiated in the remaining 2.33% (1/43) sample.

There were 25 samples that were repeatedly undifferentiated with the BioPlex 2200 HIV Ag-Ab assay. Of these, 21 were undifferentiated by supplemental testing, 2 were HIV-1 Ab reactive, 1 was HIV-2 Ab reactive, and 1 was nonreactive.

Of the samples from an HIV-2 endemic region that were tested, 88.78% (435/490) were non-reactive for HIV-1 p24 Ag and 11.22% (55/490) were not reportable for HIV-1 p24 Ag due to high Ab level.

Table 13:Reactivity in Individuals from HIV-2 Endemic Region – Assay Comparison Summary

BioPlex 2200 HIV Ag-Ab Assay	FDA-Approved HIV Ag/Ab Assay		
	Repeatedly Reactive	Non-Reactive	Total
Repeatedly Reactive	302	6[a]	308
Non-Reactive	7[b]	175	182
Total	309	181	490

[a] All 6 samples were negative for HIV antibody with the FDA-approved HIV-1/HIV-2 differentiation test; 1 of the 6 samples was non-reactive and 5 were QNS for testing on the licensed HIV-1 RNA assay but were negative with an HIV-1 Ag assay.
[b] All 7 samples were negative for HIV antibody with the FDA-approved HIV-1/HIV-2 differentiation test; 3 of the 7 samples were non-reactive and 4 were QNS for testing on the licensed HIV-1 RNA assay but were negative with an HIV-1 Ag assay.

Pediatric Populations (2 – 21 years)

Specificity in Low and High Risk Pediatric Populations

The specificity of the BioPlex 2200 HIV Ag-Ab assay in pediatric populations was determined using 100 serum samples from healthy pediatric subjects (age 2-21 years). The samples were also tested with an FDA-approved HIV Ag/Ab assay. Results are summarized in Table 14.

Table 14: Low Risk Pediatric Population

Age Range in Years	Gender	N	BioPlex 2200 HIV Ag-Ab Assay					FDA-Approved HIV Ag/Ab Assay	
			Non-Reactive	Repeatedly Reactive				Non-Reactive	Repeatedly Reactive
				HIV Ag-Ab	HIV-1 Ab	HIV-2 Ab	HIV-1 p24 Ag		
2 - 5	Female	8	8	0	0	0	0	8	0
	Male	16	16	0	0	0	0	16	0
6 - 10	Female	12	12	0	0	0	0	12	0
	Male	13	13	0	0	0	0	13	0
11 - 15	Female	13	13	0	0	0	0	13	0
	Male	13	13	0	0	0	0	13	0
16 - 21	Female	13	13	0	0	0	0	13	0
	Male	12	12	0	0	0	0	12	0
Total		100	100	0	0	0	0	100	0

In the low risk pediatric population, the specificity of the BioPlex 2200 HIV Ag-Ab assay was 100% (100/100) with a 95% CI of 96.30 - 100%.

A total of 573 samples from high risk pediatric subjects were evaluated with the BioPlex 2200 HIV Ag-Ab assay. Results are summarized in Tables 15.

Table 15: High Risk Pediatric Population

Age Range in Years	Gender	N	BioPlex 2200 HIV Ag-Ab Assay					FDA-Approved HIV Ag/Ab Assay		Number of Confirmed HIV Positive Specimens
			Non-Reactive	Repeatedly Reactive				Non-Reactive	Repeatedly Reactive	
				HIV Ag-Ab	HIV-1 Ab	HIV-2 Ab	HIV-1 p24 Ag			
2 - 5	Female	14	14	0	0	0	0	14	0	NA
	Male	9	9	0	0	0	0	9	0	NA
6 - 10	Female	1	1	0	0	0	0	1	0	NA
	Male	21	21	0	0	0	0	21	0	NA
11 - 15	Female	89	89	0	0	0	0	89	0	NA
	Male	24	24	0	0	0	0	24	0	NA
16 - 21	Not Reported	5	4	1	1	0	0	4	1	1
	Female	342	340	2[a]	1[a]	0	1[b]	341	1[b]	0
	Male	68	66	2	2	0	0	66	2	2
Total		573	568	5	4	0	1	569	4	3

NA = Not applicable because no results were reactive on the BioPlex 2200 HIV Ag-Ab assay or the FDA-approved HIV Ag/Ab assay.
[a] The sample(s) were negative for HIV antibody on the FDA-approved HIV-1/HIV-2 differentiation test and non-reactive on the licensed HIV-1 RNA assay.
[b] This sample was negative for HIV antibody on the FDA-approved HIV-1/HIV-2 differentiation test, had insufficient volume for testing on the licensed HIV-1 RNA assay, and was negative with an HIV-1 Ag EIA assay.

In the high risk pediatric population 0.87% (5/573) of the samples were repeatedly reactive with the BioPlex 2200 HIV Ag-Ab assay and 99.13% (568/573) were non-reactive. Of these samples, 0.7% (4/573) were repeatedly reactive for HIV-1 Ab, none (0/ 573) were reactive for HIV-2 Ab, and 0.17% (1/573) was repeatedly reactive for HIV-1 p24 Ag. Four (4) were repeatedly reactive with the FDA-approved HIV Ag/Ab assay. Three (3) of the samples were confirmed positive for HIV-1 antibody on the FDA-approved HIV-1/HIV-2 differentiation test and 1 of these 3 samples was reactive on the licensed HIV-1 RNA assay.

Reactivity of the BioPlex 2200 HIV Ag-Ab Assay in Known Positive Pediatric Subjects

The sensitivity of the BioPlex 2200 HIV Ag-Ab assay was determined for pediatric subjects (ranging in age from 2-21 years) that were known positive for HIV. In this study 50 retrospective HIV-1 antibody positive pediatric samples were tested with the BioPlex 2200 HIV Ag-Ab assay and with an FDA-approved HIV Ag/Ab assay. Results are summarized in Table 16.

Table 16:HIV-1 Antibody Positive Pediatric Samples

Age Range in Years	Gender	N	BioPlex 2200 HIV Ag-Ab Assay					FDA-Approved HIV Ag/Ab Assay	
			Non-Reactive	Repeatedly Reactive				Non-Reactive	Repeatedly Reactive
				HIV Ag-Ab	HIV-1 Ab	HIV-2 Ab	HIV-1 p24 Ag		
2 - 5	Female	5	0	5	5	0	0	0	5
	Male	2	0	2	2	0	0	0	2
6 - 10	Female	5	0	5	5	0	0	0	5
	Male	7	0	7	7	0	0	0	7
11 - 15	Female	1	0	1	1	0	0	0	1
	Male	11	0	11	11	0	0	0	11
16 - 21	Female	8	0	8	8	0	0	0	8
	Male	11	0	11	11	0	0	0	11
Total		50	0	50	50	0	0	0	50

In this study, 100% (50/50) of the known HIV-1 antibody positive pediatric samples were repeatedly reactive for HIV-1 Ab with the BioPlex 2200 HIV Ag-Ab, for a sensitivity of 100% (95% CI of 92.87 - 100%). All 50 samples were non-reactive for HIV-2 Ab assay and HIV-1 p24 Ag. The samples were all repeatedly reactive with the FDA-approved HIV Ag/Ab assay.

Pregnant Women Populations

Specificity in Low and High Risk Pregnant Women Populations

The specificity of the BioPlex 2200 HIV Ag-Ab assay in pregnant females was determined using 1383 samples from a pregnant women population at low and high risk for HIV infection. These samples included 1280 sera and 103 plasma. The samples were also tested with an FDA-approved HIV Ag/Ab assay. Reactive samples were tested by a licensed HIV-1 RNA test and an FDA-approved HIV-1/HIV-2 differentiation test. Results are summarized in Tables 17 and 18.

Table 17: Reactivity in Healthy Pregnant Women

Trimester	N	BioPlex 2200 HIV Ag-Ab Assay						FDA-Approved HIV Ag/Ab Assay		Number of Confirmed HIV Positive Specimens
		Non-Reactive	Repeatedly Reactive							
			HIV Ag-Ab	HIV-1 Ab	HIV-2 Ab	HIV Ab Undifferentiated	HIV-1 p24 Ag	Non-Reactive	Repeatedly Reactive	
1	335	333	2[a]	0	0	1	2[a]	335	0	0
2	334	332	2[b]	2[b]	0	0	0	332	2[b]	1
3	331	331	0	0	0	0	0	331	0	NA
Total	1000	996	4	2	0	1	2	998	2	1

NA = Not applicable because no results were reactive on the BioPlex 2200 HIV Ag-Ab assay or the FDA-approved HIV Ag/Ab test.
[a] These samples were negative for HIV antibody on the FDA-approved HIV-1/HIV-2 differentiation test and had insufficient volume for testing on the licensed HIV-1 RNA assay.
[b] One (1) sample was positive for HIV-1 antibody and one sample was negative for HIV antibody on the FDA-approved HIV-1/HIV-2 differentiation test. These samples had insufficient volume for testing on the licensed HIV-1 RNA assay.

In the low risk pregnant women population, the specificity of the BioPlex 2200 HIV Ag-Ab assay was 99.70% (996/999) with a 95% CI of 99.12 - 99.90%. The specificity for HIV-1 Ab was 99.80% (997/999) with a 95% CI of 99.27 -99.95%, the specificity for HIV-2 Ab was 99.90% (999/1000) with a 95% CI of 99.43 – 99.98%, and the specificity for HIV-1 p24 Ag was 99.80% (998/1000) with a 95% CI of 99.27 - 99.95%.

The reactivity of the BioPlex 2200 HIV Ag-Ab assay was evaluated using 383 samples from high risk pregnant women subjects, as shown in Tables 18. The samples were also tested with an FDA-approved HIV Ag/Ab assay. Samples with reactive results on the BioPlex 2200 HIV Ag-Ab assay were tested with a licensed HIV-1 RNA test and an FDA-approved HIV-1/HIV-2 differentiation test.

Table 18: Reactivity in High Risk Pregnant Women

Trimester	N	BioPlex 2200 HIV Ag-Ab Assay					FDA-Approved HIV Ag/Ab Assay		Number of Confirmed HIV Positive Specimens
		Non-Reactive	Repeatedly Reactive						
			HIV Ag-Ab	HIV-1 Ab	HIV-2 Ab	HIV-1 p24 Ag	Non-Reactive	Repeatedly Reactive	
1	118	117	1	1	0	0	116	2	0
2	132	127	5	4[a]	1[b]	0	127	5[a, b]	5
3	133	127	6	6[c]	0	0	127	6[c]	6
Total	383	371	12	11	1	0	370	13	11

[a] Four samples were confirmed positive for HIV-1 Ab on the FDA-approved HIV-1/HIV-2 differentiation test.
[b] One sample was confirmed positive for HIV-2 Ab on the FDA-approved HIV-1/HIV-2 differentiation test.
[c] All 6 samples were confirmed positive for HIV-1 Ab on the FDA-approved HIV-1/HIV-2 differentiation test.

In the high risk pregnant women population, 96.87% (371/383) of the samples were non-reactive for HIV with the BioPlex 2200 HIV Ag-Ab assay and 3.13% (12/383) of the samples were repeatedly reactive. For HIV-1 Ab, 97.13% (372/383) of the samples were non-reactive and 11 samples were repeatedly reactive. Of the 11 HIV-1 Ab repeatedly reactive samples, 10 were confirmed reactive by supplemental testing. For HIV-2 Ab, 99.74% (382/383) of the samples were non-reactive and 1 sample was repeatedly reactive. This sample was positive for HIV-2 antibody on the HIV-1/HIV-2 differentiation test. For HIV-1 p24 Ag, 100% (383/383) of the samples were non-reactive.

Reactivity of the BioPlex 2200 HIV Ag-Ab Assay in Known Positive Pregnant Women Subjects

The sensitivity of the BioPlex 2200 HIV Ag-Ab assay was determined for pregnant women subjects that were known positive for HIV. In this study, 60 frozen retrospective HIV-1 antibody positive samples from pregnant women (34 sera and 26 plasma) were tested with the BioPlex 2200 HIV Ag-Ab assay and with an FDA-approved HIV Ag/Ab assay. Samples that were repeatedly reactive on either of these assays were also tested with a licensed HIV-1 RNA test and an FDA-approved HIV-1/HIV-2 differentiation assay. Results are summarized in Table 19.

Table 19: HIV-1 Antibody Positive Pregnant Women Samples

Trimester	N	BioPlex 2200 HIV Ag-Ab Assay					FDA-Approved HIV Ag/Ab Assay	
		Non-Reactive	Repeatedly Reactive					
			HIV Ag-Ab	HIV-1 Ab	HIV-2 Ab	HIV-1 p24 Ag[a]	Non-Reactive	Repeatedly Reactive
1	23	0	23	23	0	0	0	23
2	20	0	20	20	0	0[a]	0	20
3	17	0	17	17	0	0	0	17
Total	60	0	60	60	0	0	0	60

[a] One sample was not reportable for HIV-1 p24 Ag due to high Ab level

As shown in Table 19, 100% (60/60) of the known HIV-1 antibody positive samples from pregnant women were repeatedly reactive with the BioPlex 2200 HIV Ag-Ab assay for HIV-1 Ab. None of the samples were reactive for HIV-2 Ab assay or HIV-1 p24 Ag. The sensitivity of the BioPlex 2200 HIV Ag-Ab assay was 100% (60/60) with a 95% CI of 93.98 - 100.00%.

HIV-1 and HIV-2 Differentiation

The BioPlex 2200 HIV Ag-Ab assay detects and differentiates HIV-1 and HIV-2 antibodies. The ability to differentiate HIV-1 and HIV-2 was determined by evaluation of BioPlex 2200 HIV-1 Ab assay results and BioPlex 2200 HIV-2 Ab assay results for known HIV-1 antibody positive samples (N = 1363) and for known HIV-2 antibody positive samples (N = 200). These results are summarized in Table 20 below.

Table 20: Differentiation of HIV Antibody in Known Antibody Positive Samples

Population	BioPlex 2200 HIV Ag-Ab Assay			
	HIV-1 Ab Reactive	HIV-2 Ab Reactive	Undifferentiated	% Differentiation Capability
HIV-1 Known Positive	1363	0	0	100 (1363/1363)
HIV-2 Known Positive	0	188	12*	94.0 (188/200)

* The 12 samples that were HIV Ab reactive undifferentiated with the BioPlex 2200 HIV Ag-Ab were tested with an HIV-1/HIV-2 differentiation assay. Two (2) were undifferentiated, and the remaining 10 were identified as HIV-2 positive.

HIV-1:

In the known HIV-1 positive population there were 1363 samples that were HIV-1 positive. The BioPlex 2200 HIV-1 Ab assay identified 1363/1363 (100%) as HIV-1 Reactive (95% CI of 99.71 - 100%).

HIV-2:

In the known HIV-2 positive population there were 200 samples that were HIV-2 positive. The BioPlex 2200 HIV-2 Ab assay identified 188/200 (94.0%) as HIV-2 Reactive (95% CI of 89.80 - 96.54%)

Reproducibility and Precision Testing

A panel of 18 specimens was used for determining the reproducibility and precision of the BioPlex 2200 HIV Ag-Ab assay. The 18-member reproducibility panel included 9 serum members (8 positive and 1 negative), 6 plasma (EDTA) members (5 positive and 1 negative) and 3 BioPlex 2200 HIV Ag-Ab kit controls. The composition of the panel was as follows:

#	Panel Member Composition	#	Panel Member Composition
1	HIV-1 Group M Ab Positive (Serum)	10	HIV-2 Ab High Negative (EDTA)
2	HIV-1 Group M Ab Low Positive (Serum)	11	HIV-1 Ag Low Positive (Serum)
3	HIV-1 Group M Ab Low Positive (EDTA)	12	HIV-1 Ag Low Positive (EDTA)
4	HIV-1 Group M Ab High Negative (Serum)	13	HIV-1 Ag Positive (Serum)
5	HIV-1 Group O Ab Low Positive (Serum)	14	Negative (Serum)
6	HIV-1 Group O Ab Low Positive (EDTA)	15	Negative (EDTA)
7	HIV-2 Ab Positive (Serum)	16	HIV Ab Positive Control
8	HIV-2 Ab Low Positive (Serum)	17	HIV Ag Positive Control
9	HIV-2 Ab Low Positive (EDTA)	18	Negative Control

Reproducibility

Reproducibility testing was performed at three clinical trial sites. Each of the panel members and positive and negative controls described above was tested in replicates of 3 on 1 run per day for 5 days on 3 lots of the BioPlex 2200 HIV Ag-Ab assay [3 replicates x 1 run x 5 days x 3 lots = 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. Results can be found in Tables 21a-d-.

Table 21a: Reproducibility Results – HIV Ag-Ab

Panel Member	N	Mean	Within Run[1]		Between Day[2]		Between Lot[3]		Between Site[4]		Total [5]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	135	4.957	0.164	3.3	0.135	2.7	0.097	2.0	0.000*	0.0	0.234	4.7
2	135	2.042	0.076	3.7	0.022	1.1	0.059	2.9	0.000*	0.0	0.099	4.8
3	135	1.981	0.077	3.9	0.018	0.9	0.042	2.1	0.000*	0.0	0.090	4.5
4	135	0.593	0.031	5.2	0.000*	0.0	0.024	4.0	0.000*	0.0	0.039	6.6
5	135	1.949	0.092	4.7	0.035	1.8	0.087	4.5	0.000*	0.0	0.131	6.7
6	135	2.053	0.076	3.7	0.032	1.6	0.123	6.0	0.000*	0.0	0.148	7.2
7	135	4.972	0.170	3.4	0.131	2.6	0.331	6.6	0.000*	0.0	0.394	7.9
8	135	1.958	0.083	4.2	0.041	2.1	0.155	7.9	0.000*	0.0	0.181	9.2
9	135	1.962	0.080	4.1	0.045	2.3	0.145	7.4	0.000*	0.0	0.172	8.8
10	135	0.582	0.037	6.4	0.012	2.1	0.056	9.5	0.000*	0.0	0.068	11.7
11	135	2.125	0.094	4.4	0.046	2.2	0.098	4.6	0.000*	0.0	0.143	6.7
12	135	2.193	0.099	4.5	0.043	2.0	0.113	5.1	0.000*	0.0	0.156	7.1
13	135	5.207	0.149	2.9	0.159	3.0	0.213	4.1	0.000*	0.0	0.304	5.8
14	135	0.084	0.090	N/A	0.004	N/A	0.000*	N/A	0.022	N/A	0.092	N/A
15	135	0.083	0.019	N/A	0.008	N/A	0.017	N/A	0.022	N/A	0.034	N/A
16	135	3.792	0.152	4.0	0.086	2.3	0.216	5.7	0.000*	0.0	0.278	7.3
17	135	4.075	0.148	3.6	0.137	3.4	0.079	1.9	0.000*	0.0	0.216	5.3
18	135	0.051	0.019	N/A	0.007	N/A	0.008	N/A	0.007	N/A	0.023	N/A

* Negative variances were set to zero, per statistical convention.
[1] Within-Run: Variability of the assay performance from replicate to replicate.
[2] Between-Day: Variability of the assay performance from day to day.
[3] Between-Lot: Variability of the assay performance from lot to lot.
[4] Between-Site: Variability of the assay performance from site to site which includes instrument to instrument variability.
[5] Total: Total variability of the assay performance includes within-run, between-day, between-lot and between-site variability.

Table 21b: Reproducibility Results – HIV-1 Ab

Panel Member	N	Mean	Within Run[1]		Between Day[2]		Between Lot[3]		Between Site[4]		Total [5]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	135	4.957	0.164	3.3	0.135	2.7	0.097	2.0	0.000*	0.0	0.234	4.7
2	135	2.042	0.076	3.7	0.022	1.1	0.059	2.9	0.000*	0.0	0.099	4.8
3	135	1.981	0.077	3.9	0.018	0.9	0.042	2.1	0.000*	0.0	0.090	4.5
4	135	0.593	0.031	5.2	0.000*	0.0	0.024	4.0	0.000*	0.0	0.039	6.6
5	135	1.949	0.092	4.7	0.035	1.8	0.087	4.5	0.000*	0.0	0.131	6.7
6	135	2.053	0.076	3.7	0.032	1.6	0.123	6.0	0.000*	0.0	0.148	7.2
14	135	0.055	0.089	N/A	0.025	N/A	0.000*	N/A	0.016	N/A	0.094	N/A
15	135	0.047	0.016	N/A	0.006	N/A	0.014	N/A	0.017	N/A	0.028	N/A
16	135	3.789	0.149	3.9	0.083	2.2	0.220	5.8	0.000*	0.0	0.278	7.3
18	135	0.025	0.018	N/A	0.000*	N/A	0.015	N/A	0.008	N/A	0.025	N/A

* Negative variances were set to zero, per statistical convention.
[1] Within-Run: Variability of the assay performance from replicate to replicate.
[2] Between-Day: Variability of the assay performance from day to day.
[3] Between-Lot: Variability of the assay performance from lot to lot.
[4] Between-Site: Variability of the assay performance from site to site which includes instrument to instrument variability.
[5] Total: Total variability of the assay performance includes within-run, between-day, between-lot and between-site variability.

Table 21c: Reproducibility Results – HIV-2 Ab

Panel Member	N	Mean	Within Run[1]		Between Day[2]		Between Lot[3]		Between Site[4]		Total [5]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
7	135	4.972	0.170	3.4	0.131	2.6	0.331	6.6	0.000*	0.0	0.394	7.9
8	135	1.958	0.083	4.2	0.041	2.1	0.155	7.9	0.000*	0.0	0.181	9.2
9	135	1.962	0.080	4.1	0.045	2.3	0.145	7.4	0.000*	0.0	0.172	8.8
10	135	0.582	0.037	6.4	0.012	2.1	0.056	9.5	0.000*	0.0	0.068	11.7
14	135	0.048	0.018	N/A	0.011	N/A	0.015	N/A	0.021	N/A	0.033	N/A
15	135	0.052	0.019	N/A	0.003	N/A	0.008	N/A	0.024	N/A	0.032	N/A
16	135	2.934	0.179	6.1	0.126	4.3	0.238	8.1	0.000*	0.0	0.323	11.0
18	135	0.027	0.016	N/A	0.010	N/A	0.006	N/A	0.009	N/A	0.022	N/A

* Negative variances were set to zero, per statistical convention.

[1] Within-Run: Variability of the assay performance from replicate to replicate.

[2] Between-Day: Variability of the assay performance from day to day.

[3] Between-Lot: Variability of the assay performance from lot to lot.

[4] Between-Site: Variability of the assay performance from site to site which includes instrument to instrument variability.

[5] Total: Total variability of the assay performance includes within-run, between-day, between-lot and between-site variability.

Table 21d: Reproducibility Results – HIV-1 p24 Ag

Panel Member	N	Mean	Within Run[1]		Between Day[2]		Between Lot[3]		Between Site[4]		Total [5]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
11	135	2.125	0.094	4.4	0.046	2.2	0.098	4.6	0.000*	0.0	0.143	6.7
12	135	2.193	0.099	4.5	0.043	2.0	0.113	5.1	0.000*	0.0	0.156	7.1
13	135	5.207	0.149	2.9	0.159	3.0	0.213	4.1	0.000*	0.0	0.304	5.8
14	135	0.061	0.029	N/A	0.006	N/A	0.018	N/A	0.006	N/A	0.035	N/A
15	135	0.072	0.024	N/A	0.014	N/A	0.022	N/A	0.000*	N/A	0.036	N/A
17	135	4.075	0.148	3.6	0.137	3.4	0.079	1.9	0.000*	0.0	0.216	5.3
18	135	0.038	0.022	N/A	0.010	N/A	0.011	N/A	0.000*	N/A	0.026	N/A

* Negative variances were set to zero, per statistical convention.

[1] Within-Run: Variability of the assay performance from replicate to replicate.

[2] Between-Day: Variability of the assay performance from day to day.

[3] Between-Lot: Variability of the assay performance from lot to lot.

[4] Between-Site: Variability of the assay performance from site to site which includes instrument to instrument variability

[5] Total: Total variability of the assay performance includes within-run, between-day, between-lot and between-site variability.

Precision

A precision study was performed with the BioPlex 2200 HIV Ag-Ab assay using a panel of 18 samples that were tested in duplicate, twice a day, for 20 days. Results were analyzed for within-run, between-run, between-day, and total variability using the principles described in the Clinical and Laboratory Standards Institute (CLSI) guidance EP5-A2. The standard deviation (SD) and percent coefficient of variation (%CV) were analyzed for each panel member. The results are summarized in Tables 22a-d.

Table 22a: Precision Results – HIV Ag-Ab

Panel Member	Mean	Within Run		Between Run		Between Day		Total[1]	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	5.01	0.142	2.8	0.060	1.2	1	5.01	0.184	3.7
2	2.06	0.068	3.3	0.032	1.5	2	2.06	0.075	3.7
3	2.04	0.069	3.4	0.021	1.0	3	2.04	0.074	3.6
4	0.60	0.027	4.5	0.009	1.6	4	0.60	0.030	5.0
5	1.97	0.058	3.0	0.037	1.9	5	1.97	0.081	4.1
6	2.13	0.077	3.6	0.000[2]	0.0	6	2.13	0.092	4.3
7	4.77	0.133	2.8	0.000[2]	0.0	7	4.77	0.155	3.3
8	1.92	0.081	4.2	0.000[2]	0.0	8	1.92	0.094	4.9
9	1.93	0.067	3.5	0.059	3.0	9	1.93	0.095	4.9
10	0.60	0.030	5.0	0.005	0.9	10	0.60	0.037	6.2
11	2.25	0.070	3.1	0.034	1.5	11	2.25	0.096	4.3
12	2.38	0.087	3.7	0.000	0.0	12	2.38	0.104	4.4
13	5.41	0.157	2.9	0.091	1.7	13	5.41	0.198	3.7
14	0.05	0.013	NA	0.007	NA	14	0.05	0.015	NA
15	0.07	0.017	NA	0.000[2]	NA	15	0.07	0.017	NA
16	3.39	0.107	3.2	0.000[2]	0.0	16	3.39	0.137	4.0
17	4.15	0.186	4.5	0.000[2]	0.0	17	4.15	0.186	4.5
18	0.02	0.014	NA	0.000[2]	NA	18	0.02	0.014	NA

[1] Total: Total variability of the assay performance includes within run, between run and between day.
[2] Negative variances were rounded to zero, per statistical convention.

Table 22b: Precision Results – HIV-1 Ab

Panel Member	Mean	Within Run		Between Run		Between Day		Total[1]	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	5.01	0.142	2.8	0.060	1.2	0.100	2.0	0.184	3.7
2	2.06	0.068	3.3	0.032	1.5	0.000	0.0	0.075	3.7
3	2.04	0.069	3.4	0.021	1.0	0.017	0.8	0.074	3.6
4	0.60	0.027	4.5	0.009	1.6	0.008	1.4	0.030	5.0
5	1.97	0.058	3.0	0.037	1.9	0.041	2.1	0.081	4.1
6	2.13	0.077	3.6	0.000[2]	0.0	0.052	2.4	0.092	4.3
14	0.03	0.014	NA	0.000[2]	NA	0.003	NA	0.014	NA
15	0.04	0.018	NA	0.000[2]	NA	0.004	NA	0.018	NA
16	3.39	0.107	3.2	0.000[2]	0.0	0.085	2.5	0.137	4.0
18	0.00	0.008	NA	0.000[2]	NA	0.000[2]	NA	0.008	NA

[1] Total: Total variability of the assay performance includes within run, between run and between day.
[2] Negative variances were rounded to zero, per statistical convention.

Table 22c: Precision Results – HIV-2 Ab

Panel Member	Mean	Within Run		Between Run		Between Day		Total[1]	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
7	4.77	0.133	2.8	0.000[2]	0.0	0.081	1.7	0.155	3.3
8	1.92	0.081	4.2	0.000[2]	0.0	0.047	2.4	0.094	4.9
9	1.93	0.068	3.5	0.058	3.0	0.034	1.8	0.095	5.0
10	0.60	0.030	5.1	0.003	0.5	0.021	3.5	0.037	6.2
14	0.05	0.014	NA	0.009	NA	0.000	NA	0.017	NA
15	0.07	0.017	NA	0.004	NA	0.005	NA	0.018	NA
16	2.55	0.095	3.7	0.000[2]	0.0	0.048	1.9	0.106	4.2
18	0.01	0.014	NA	0.003	NA	0.000	NA	0.014	NA

[1] Total: Total variability of the assay performance includes within run, between run and between day.
[2] Negative variances were rounded to zero, per statistical convention.

Table 22d: Precision Results – HIV-1 p24 Ag

Panel Member	Mean	Within Run		Between Run		Between Day		Total[1]	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
11	2.25	0.070	3.1	0.034	1.5	0.056	2.5	0.096	4.3
12	2.38	0.088	3.7	0.000[2]	0.0	0.056	2.4	0.104	4.4
13	5.41	0.157	2.9	0.091	1.7	0.079	1.5	0.198	3.7
14	0.02	0.020	NA	0.000[2]	NA	0.000[2]	NA	0.019	NA
15	0.04	0.029	NA	0.000[2]	NA	0.010	NA	0.031	NA
17	4.15	0.186	4.5	0.000[2]	0.0	0.000[2]	0.0	0.186	4.5
18	0.00	0.005	NA	0.000[2]	NA	0.000[2]	NA	0.005	NA

[1] Total: Total variability of the assay performance includes within run, between run and between day.
[2] Negative variances were rounded to zero, per statistical convention.

PERFORMANCE CHARACTERISTICS OF ORGAN DONOR SPECIMEN TESTING

Specificity

Specificity studies were performed for the BioPlex 2200 HIV Ag-Ab assay with organ donor specimens. In these studies, fifty (50) samples from brain dead individuals and fifty (50) normal living donor samples were tested concurrently with three lots of the BioPlex 2200 HIV Ag-Ab assay. All 100 specimens were known to be negative for HIV by FDA approved / licensed assays. Results of the testing are summarized in Tables 23a-b below.

Table 23a: Specificity Testing of Brain Dead Donors

BioPlex 2200 HIV Ag-Ab Result	Lot 1		Lot 2		Lot 3	
	N	% Agreement (95% CI)	N	% Agreement (95% CI)	N	% Agreement (95% CI)
Reactive	0	50/50	0	50/50	0	50/50
Non-Reactive	50	100% (92.8 - 100%)	50	100% (92.8 - 100%)	50	100% (92.8 - 100%)

Table 23b: Specificity Testing of Normal Living Donors

BioPlex 2200 HIV Ag-Ab Result	Lot 1		Lot 2		Lot 3	
	N	% Agreement (95% CI)	N	% Agreement (95% CI)	N	% Agreement (95% CI)
Reactive	0	50/50	0	50/50	0	50/50
Non-Reactive	50	100% (92.8 - 100%)	50	100% (92.8 - 100%)	50	100% (92.8 - 100%)

None of the specimens from brain dead individuals or normal living donors were reactive with the BioPlex 2200 HIV Ag-Ab assay, giving an estimated specificity of 100% (95% CI of 97.55 - 100%) for both populations. The mean index for the 50 samples from brain dead individuals (3 replicates each) for the HIV-1 p24 Ag, HIV-1 Ab, and HIV-2 Ab assays were 0.225, 0.214, and 0.230, respectively; the mean index values for the 50 normal living donor samples (3 replicates each) were 0.294, 0.267, and 0.280, respectively.

Sensitivity

Fifty (50) specimens from brain dead individuals and fifty (50) normal living donor samples were pre-screened for HIV-1 and HIV-2 and found to be nonreactive. To compare detection of HIV analytes in the normal living and brain-dead populations, both types of specimens were spiked in 4 separate aliquots with HIV-1 antigen, HIV-1 group M antibody, HIV-1 group O antibody, and HIV-2 antibody at potency near the assay's cutoff. The spiked samples from brain dead individuals were tested concurrently with the spiked normal living donor specimens with three lots of the BioPlex 2200 HIV Ag-Ab assay. Results are presented in Tables 24a-b.

Table 24a: Sensitivity Testing with Brain Dead Donors

Analyte	BioPlex 2200 HIV Ag-Ab Result	Lot 1		Lot 2		Lot 3	
		N	% Agreement (95% CI)	N	% Agreement (95% CI)	N	% Agreement (95% CI)
HIV-1 p24 Antigen	Reactive	50	50/50 100% (92.8 - 100%)	50	50/50 100% (92.8 - 100%)	50	50/50 100% (92.8 - 100%)
	Non-Reactive	0		0		0	
HIV-1 Group M Antibody	Reactive	50	50/50 100% (92.8 - 100%)	50	50/50 100% (92.8 - 100%)	50	50/50 100% (92.8 - 100%)
	Non-Reactive	0		0		0	
HIV-1 Group O Antibody	Reactive	50	50/50 100% (92.8 - 100%)	50	50/50 100% (92.8 - 100%)	50	50/50 100% (92.8 - 100%)
	Non-Reactive	0		0		0	
HIV-2 Antibody	Reactive	50	50/50 100% (92.8 - 100%)	50	50/50 100% (92.8 - 100%)	50	50/50 100% (92.8 - 100%)
	Non-Reactive	0		0		0	

Table 24b: Sensitivity Testing with Normal Living Donors

Analyte	BioPlex 2200 HIV Ag-Ab Result	Lot 1		Lot 2		Lot 3	
		N	% Agreement (95% CI)	N	% Agreement (95% CI)	N	% Agreement (95% CI)
HIV-1 p24 Antigen	Reactive	50	50/50 100% (92.8 - 100%)	50	50/50 100% (92.8 - 100%)	50	50/50 100% (92.8 - 100%)
	Non-Reactive	0		0		0	
HIV-1 Group M Antibody	Reactive	50	50/50 100% (92.8 - 100%)	50	50/50 100% (92.8 - 100%)	50	50/50 100% (92.8 - 100%)
	Non-Reactive	0		0		0	
HIV-1 Group O Antibody	Reactive	50	50/50 100% (92.8 - 100%)	48	48/50 96% (86.3 – 99.4%)	49	49/50 98% (89.3 – 99.7%)
	Non-Reactive	0		2		1	
HIV-2 Antibody	Reactive	50	50/50 100% (92.8 - 100%)	50	50/50 100% (92.8 - 100%)	50	50/50 100% (92.8 - 100%)
	Non-Reactive	0		0		0	

There was 100% agreement between the samples from normal living donors and brain dead donors that were spiked with HIV-1 Ag, HIV-1 group M Ab, and HIV-2 Ab. For HIV-1 group O Ab, 147/150 results were positive with the normal living donor samples and 150/150 results were positive with the brain dead donor samples. These samples were very close to the cutoff. The mean index of the 50 samples from brain dead individuals (3 replicates each) spiked with HIV-1 antigen, HIV-1 group M antibody, HIV-1 group O antibody, and HIV-2 antibody were 1.677, 1.607, 1.584, and 1.601 respectively; the mean index values for the 50 normal living donor samples (3 replicates each) were 1.730, 1.634, 1.568, and 1.616, respectively. These results demonstrate that the BioPlex 2200 HIV Ag-Ab assay can be used for the detection of HIV-1 p24 antigen, HIV-1 antibody (M and O), and HIV-2 antibody in specimens from potential organ donors.

Reproducibility

To determine the reproducibility of the BioPlex 2200 HIV Ag-Ab assay with organ donor specimens, a panel of specimens from brain dead individuals and from normal living donors was spiked to give reactivity near the cutoff. The panel consisted of 20 specimens of each population that were spiked with HIV-1 p24 antigen, and 20 specimens of each population that were spiked with HIV-1 antibody (groups M and O) and HIV-2 antibody. Each of the panel members was tested 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. Results of the testing are summarized in Table 25.

Table 25: Reproducibility of Organ Donor Samples

Spiked Analyte	N	Brain Dead Donors			Normal Living Donors		
		Mean	SD	%CV	Mean	SD	%CV
HIV-1 p24 Antigen	360	1.584	0.227	14.3	1.610	0.238	14.8
HIV-1 Group M Antibody	360	1.422	0.093	6.5	1.439	0.104	7.2
HIV-1 Group O Antibody	360	1.748	0.212	12.1	1.712	0.255	14.9
HIV-2 Antibody	360	1.623	0.294	18.1	1.625	0.278	17.1

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SYMBOLS LEXICON

	Lot Number		Number of Tests
	Catalogue Number		Temperature Limitation
	Consult Instructions for Use		Use by (YYYY-MM-DD)
	For Investigational Use only		Version
	Warning		Bead Set
	Conjugate		Caution, consult accompanying documents
	Manufacturer		Authorized Representative in the European Community
	For use with		

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