SUMMARY OF SAFETY AND EFFECTIVENESS

Product:

I. General Information

Device Generic Name: Human Immunodeficiency Virus (HIV) p24 antigen and antibodies to HIV Type 1 (HIV-1 group M and group O) and/or Type 2

Device Trade Name: BioPlex® 2200 HIV Ag-Ab

Product Code: MZF

Applicant's Name and Address: Bio-Rad Laboratories
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Manufacturer: Bio-Rad Laboratories
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Premarket Approval Application (PMA) Number: BP140111

Date of Panel Recommendation: Not Applicable

Office's Signatory Authority: Jay S. Epstein, M.D.
Director, OBRR/CBER

□ **I concur with the summary review.**

□ **I concur with the summary review and include a separate review to add further analysis.**

□ **I do not concur with the summary review and include a separate review.**

Date of Notice of Approval to the Applicant:

Material Reviewed/Consulted: The PMA, amendments to the PMA, and other specific documentation used in developing the Summary of Safety and Effectiveness (SSE)

Review memos from the following reviewers were used in developing the SSE:

Discipline Reviewed	Reviewer Names
Clinical Non-clinical/Analytical Organ donor screening claim	German Anez-Gutierrez Pawan K. Jain Mohan Haleyurgirisetty Xue Wang Babita Mahajan Michelle McClure Diane Gubernot
Product Design	Krishnakumar Devadas
CMC (Chemistry, Manufacturing, and Controls)	Mohan Haleyurgirisetty Krishnakumar Devadas
Statistical	Paul Hshieh
Facility and GMP	Jennifer Schmidt Deborah Trout
Bioresearch Monitoring	Bhanu Kannan
Labeling	Krishnakumar Devadas Dana Martin
Policy	Pradip Akolkar Robin Biswas Indira Hewlett J. Peyton Hobson Sayah Nedjar

II. 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.

CLIA Complexity: High

III. Description of the BioPlex 2200 HIV Ag-Ab assay

A. Device Description

The BioPlex 2200 HIV Ag-Ab Assay is a multiplex flow immunoassay based on the principle of the sandwich technique for the detection of HIV p24 antigen and the various antibodies associated with HIV-1 and HIV-2 infection in human serum or plasma. The BioPlex 2200 uses a multiplex flow immunoassay format that permits simultaneous detection and identification of antigen and antibodies in a single tube.

The BioPlex 2200 HIV Ag-Ab is intended for use with the BioPlex 2200 Instrument, a fully automated, self-contained, multiplex immunoassay analyzer. Reactions occur on the surface of fluoromagnetic beads and all reagents needed are contained within panel-specific reagent packs that are stored onboard with refrigeration. A series of control beads are evaluated in each reaction to ensure reliable results for each sample. The analyzer incorporates a dedicated software package for instrument control, data collection, results analysis, calibration and quality control.

The Reagent Pack includes three reagents: Bead Reagent, Conjugate 1 Reagent, and Conjugate 2 Reagent. The Bead Reagent consists of a mixture of eight sets of magnetic (b) (4) beads. Each bead set is defined by having a unique ratio of two fluorescent dyes and is identified individually by the detector in the BioPlex 2200. Four of the eight bead sets are coated with monoclonal antibody or antigen used to bind HIV antigen or antibody, respectively, in serum or plasma samples. Each of these four bead sets functions as a separate immunoassay for detecting one HIV analyte, and all of the immunoassays occur

simultaneously in the same reaction vessel (RV). Three bead sets are used for internal quality control processes. [redacted].

During the first step of the assay, the sample and Bead Reagent are added to the RV and incubated at 37°C. Antibodies and/or antigens in the sample bind to the corresponding binding site on the beads. The binding reaction ends when the beads are removed magnetically from suspension and then washed.

Conjugate 1 Reagent, a mixture of biotin-labeled antigens and antibodies, is added. The reaction mixture is incubated to allow labeled antigens in the mixture to bind antibodies bound to the solid phase antigens, and to allow labeled anti-p24 antibody to bind p24 antigen bound to antibody coated beads. The beads are again removed magnetically and washed to remove excess, unbound biotin-labeled antigens and antibodies.

Conjugate 2 Reagent, streptavidin-phycoerythrin (SA-PE) in buffer, is added. The streptavidin conjugate binds the biotin component of Conjugate 1. The beads are washed after incubation and then sent to the detector.

The beads flow through the detector, single file, in a fluid sheath. A [redacted] laser determines each bead's signature (and therefore the analyte detected) and a [redacted] laser measures the level of fluorescence from the phycoerythrin (PE) bound to the bead. The median fluorescence value for each bead signature is used to determine its index value based on a two-point calibration plot. Assay beads with index values of 1.00 or greater are reactive. Using the results for each analyte, PC-based software calculates a final output of reactive or non-reactive for each analyte.

Three QC bead populations are used to monitor the reaction. The Internal Standard Bead (ISB) is coated with [redacted] in order to detect fluctuations in detector performance. The Signal Normalization Bead (SNB) is coated with (b) (4) [redacted]. Results from this bead are used to normalize relative fluorescence intensity (RFI) values from the four assay beads. The Serum Verification Bead (SVB) is coated with (b) (4) [redacted], confirming that serum or plasma and subsequent reagents were delivered to the reaction vessel. The ISB, SNB, and SVB are analyzed by the detector in the same manner as the assay beads.

Table 1: BioPlex 2200 HIV Ag-Ab Assay HIV Biologicals

Analyte Detected	Biological on Beads	Biological for Biotin-Conjugate
HIV-1, group M antibody	gp160	(b) (4) [redacted]
HIV-1, group O antibody	(b) (4) [redacted]	(b) (4) [redacted]
HIV-2 antibody	(b) (4) [redacted]	(b) (4) [redacted]

Analyte Detected	Biological on Beads	Biological for Biotin-Conjugate
HIV-1 p24 antigen	(b) (4)	Biotin-labeled polyclonal sheep antibodies to HIV-1 p24 Ag

Table 2: BioPlex 2200 HIV Ag-Ab Assay QC Beads

Designation	Failure Detected	Coating on Bead	Comments
Internal Standard Bead (ISB)	Detector fluctuation	(b) (4)	Provides constant level of (b) (4) as control
Signal Normalization Bead (SNB)	Detector fluctuations, reduction in SA-PE activity, residual wash buffer after wash, variation in incubation time.	(b) (4)	Signal is used to normalize the signal of the four assay beads. The normalization reduces variability and improves assay precision.
Serum Verification Bead (SVB)	Sample or Reagent not delivered to reaction vessel	(b) (4)	SVB is part of a "sandwich" assay for , a serum protein that is present at fairly constant levels.

B. Components of BioPlex 2200 HIV Ag-Ab Assay

Materials Provided

Reagent Packs:

- Bead set: Contains 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 the preservatives ProClin 300 (≤ 0.3%), sodium benzoate (≤0.1%) and sodium azide (< 0.1%).
- Conjugate 1: 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 the preservatives ProClin 300 (≤ 0.5%) and sodium azide (< 0.1%).
- Conjugate 2: streptavidin conjugated to phycoerythrin with protein stabilizers (bovine and human IgG), and the preservatives ProClin 300 (≤ 0.5%) and sodium azide (< 0.1%).
- BioPlex 2200 Manual Reagent Pack Piercer.

Materials required but not provided (available from Bio-Rad as accessories to the Kit)

Control Sets:

The BioPlex 2200 HIV Ag-Ab Control Set is intended to monitor the performance of the BioPlex 2200 Instrument and BioPlex 2200 HIV Ag-Ab assay in the clinical laboratory.

- Negative Control: Human plasma with the preservatives ProClin 300 (≤0.3%), sodium benzoate (≤0.1%), sodium azide (<0.1%), ProClin 950 (≤0.16%), and gentamicin sulfate (≤0.005%).
- HIV-1/HIV-2 Antibody Positive Control: Human antibody to HIV-1 Group M and HIV-2; purified rabbit antibody to HIV-1 Group O; processed human plasma; and the preservatives ProClin 300 (≤0.3%), sodium benzoate (≤0.1%), sodium azide (<0.1%), ProClin 950 (≤0.16%), and gentamicin sulfate (≤0.005%).
- HIV-1 Antigen Positive Control: Purified HIV-1 antigen (from viral lysate inactivated with a chaotropic agent) in Tris Base; protein stabilizer (bovine); and the preservatives ProClin 300 (≤0.3%), sodium benzoate (≤0.1%) and sodium azide (<0.1%).

Calibrator Sets:

The BioPlex 2200 HIV Ag-Ab Calibrator Set is intended for the calibration of the BioPlex 2200 HIV Ag-Ab assay.

- HIV-1/HIV-2 Antibody Calibrator: Human antibodies to HIV-1 Group M and HIV-2; purified rabbit antibody to HIV-1 Group O in Tris buffer; protein stabilizer (bovine); and the preservatives ProClin 300 (≤0.3%), sodium benzoate (≤0.1%) and sodium azide (<0.1%).
- HIV Antigen Calibrator: Purified HIV-1 p24 antigen (from viral lysate inactivated with a chaotropic agent) in Tris Buffer; protein stabilizer (bovine); and the preservatives ProClin 300 (≤0.3%), sodium benzoate (≤0.1%) and sodium azide (<0.1%).

Ancillary Components:

- Sheath Fluid: Sodium chloride and sodium phosphate solution with the preservatives ProClin 300 (≤0.3%) and sodium azide (<0.1%).
- Wash Solution: Sodium chloride, sodium phosphate, and Tween 20 solution with the preservatives ProClin 300 (≤0.3%) and sodium azide (<0.1%).
- The BioPlex 2200 System Sheath Fluid and BioPlex 2200 System Wash Solution are not lot specific and can be interchanged.

BioPlex 2200 Instrument:

The BioPlex 2200 HIV Ag-Ab assay is intended for use with the BioPlex 2200 Instrument, a fully automated, self-contained, immunoassay multiplex analyzer. The BioPlex 2200 Instrument incorporates a dedicated software package for instrument control, data collection, results analysis, calibration, quality control, and service software.

IV. Test Procedure

A. Specimen Collection, Preparation, and Storage

- The BioPlex 2200 HIV Ag-Ab Assay can be performed on serum or plasma, including plasma specimens collected while the donor's heart is still beating.
- Serum or plasma specimens, either fresh or frozen, may be used in the test.

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

B. Assay Procedure

- The Manual Reagent Pack Piercer is used to pierce the reagent pack.
- The samples are loaded into the racks provided with the BioPlex 2200 Instrument.
- Sample processing on the BioPlex 2200 Instrument is fully automated.

C. Calibration

- The BioPlex 2200 HIV Ag-Ab Calibrator Set should be loaded and assayed in triplicate every 30 days and with each new Reagent Pack lot.
- 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 BioPlex 2200 HIV Antigen Calibrator contains no HIV antibodies and is the negative calibrator for the antibody detection bead sets.
- The BioPlex 2200 HIV Antigen Calibrator contains HIV-1 p24 antigen and has an Index based on reference material. The BioPlex 2200 HIV Antibody Calibrator contains no detectable HIV-1 p24 antigen and is the negative calibrator for the antigen detection bead sets.
- For all plots, the cutoff is the fluorescence corresponding to an index value of 1.00.

D. 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.

E. Quality Control

- The BioPlex 2200 HIV Ag-Ab Control Set must be run at least once every 24 hours, and after each calibration.
- The BioPlex 2200 HIV Ag-Ab Control Set includes a Negative Control and Ab Positive Control in plasma; the Ab Positive Control contains HIV-1 and HIV-2 antibodies; the Ag Positive Control contains HIV-1 p24 antigen in synthetic matrix.
- 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.

V. Interpretation of Test Results by Laboratory Technician

The BioPlex 2200 HIV Ag-Ab assay generates an Index for each of the individual HIV analytes. A specimen is interpreted as REACTIVE if at least one analyte has an Index of ≥ 1.00. Repeat testing in duplicate is required for all initially reactive samples. If confirmed reactive, the highest Index result is used for interpretation of results. A specimen is Non-Reactive if all analytes have Indices < 1.00. No repeat testing is required for Non-Reactive results. The Index for each analyte, together with an overall test result based on the Index of all analytes, is provided.

Table 3: Interpretation of Test Results

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

Table 3 summarizes the interpretation of results for each HIV analyte.

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.

Analysis of 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 BioPlex 2200 output 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 HIV-1 p24 Ag results for these specimens will be reported as “Not reportable due to high HIV Ab level”, without Indices.

Analysis of HIV-1 and HIV-2 Ab Results
Specimens reactive for HIV-1 antibody can cross-react with HIV-2 antigens, causing results on the HIV-2 beads to be reactive. In addition, specimens reactive for HIV-2 antibody can cross-react with HIV-1 antigens causing results on the HIV-1 beads to be reactive. Therefore,

specimens with Indices of at least 1.00 for both HIV-1 Ab and HIV-2 Ab are reported as follows:

- If the HIV-1 Ab Index is at least 5-fold the HIV-2 Ab Index, the HIV-1 Ab result is reported as Reactive with its Index. The HIV-2 Ab result is reported as Non-Reactive without an Index.
- If the HIV-2 Ab Index is at least 5-fold the HIV-1 Ab Index, the HIV-2 Ab result is reported as Reactive with its Index. The HIV-1 Ab result is reported as Non-Reactive without an Index.
- If the HIV-1 and HIV-2 Ab Indices have less than a 5-fold difference, both HIV-1 and HIV-2 Ab results are reported as Reactive, Undifferentiated, with Indices.

Table 4 summarizes the interpretation of results.

Table 4: 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 REACTIVE for HIV-1 Ab or REACTIVE for HIV-2 Ab, as appropriate.

Initially reactive specimens must be retested in duplicate. If they are repeatedly reactive, they must be investigated by additional, more specific, or supplemental tests.

VI. Limitations of the Test

1. The BioPlex 2200 HIV Ag-Ab is for In Vitro Diagnostic use only.
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 package insert carefully prior to conducting the test. Testing of other body specimens, pooled blood or plasma, 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 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. Reactive specimens must be investigated by additional, more specific supplemental tests.

5. Specimens containing HIV-2 p26 antigen can cross-react with HIV-1 p24 detection beads, generating reactive results for HIV-1 p24.
6. 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 of HIV-1 or HIV-2 infection.
7. 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.
8. Non-Reactive results can occur if the quantity of marker present in the sample is below the detection limit of the assay.
9. Repeat testing of a Non-Reactive result should be considered where there is clinical suspicion of infection or if procedural error is suspected.
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.
12. Samples contaminated with bacteria, samples that are icteric, lipemic, or hemolyzed, and heat-inactivated samples may cause erroneous results and should be avoided.
13. The calculated Index Values for anti-HIV antibodies and/or p24 antigen in a given specimen as determined by the BioPlex 2200 Ag-Ab assay cannot be correlated to an endpoint titer or viral load.

VII. Marketing History

The BioPlex 2200 HIV Ag-Ab is not currently being marketed in any country.

VIII. Potential Adverse Effects of the Device on Health

Potential adverse effects of BioPlex 2200 HIV Ag-Ab relate to the risk of false positive and false negative results. While performance studies indicate that this risk is likely to be very small, the potential for inaccurate results exists. The risk of incorrect results is minimized by following the procedures and instructions outlined in the Package Insert.

IX. Summary of Preclinical Studies:

HIV-1 p24 Analytical Sensitivity

The analytical sensitivity of the BioPlex 2200 HIV Ag-Ab assay for HIV-1 p24 antigen was assessed by testing a standard obtained from the Agence nationale de

sécurité du médicament et des produits de santé (ANSM), and by testing the WHO HIV international standard NIBSC 90/636.

Results: The results 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 in normal human serum.

HIV-1 p24 Antigen Detection in Culture Supernatants
Fifty-four (54) HIV-1 culture supernatants containing HIV-1 p24 antigen in RPMI media, representing HIV-1 Group M antigen 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) and HIV-1 Group O antigens (n=2) were tested with the BioPlex 2200 HIV Ag-Ab assay.

Results: Of the 54 HIV-1 culture supernatant samples tested, 100% (54/54) were reactive.

HIV-2 p26 Antigen Detection in Culture Supernatants
To test the ability of the monoclonal anti-p24 antibody that is coated on the BioPlex beads to detect HIV-2 antigen, a recombinant HIV-2 gag p26 (>98% purity), two purified HIV-2 viral lysate preparations and four heat inactivated HIV-2 cell culture supernatants were diluted to different levels in human serum, and tested using the BioPlex 2200 Ag-Ab assay. A total of 7 HIV-2 p26 antigen containing samples were tested. The samples were also tested with an FDA approved HIV Ag/Ab assay.

Results: Samples containing HIV-2 p26 antigen were reactive (6/7) on the BioPlex 2200 HIV Ag-Ab assay, with the exception of one of the supernatants (ARP123), indicating that the solid phase monoclonal antibody had bound HIV-2 p26, and that the polyclonal anti-p24 antibody was also able to bind the same antigen to form the expected Ab-Ag-Ab complex. An FDA approved HIV Ag/Ab assay was also non-reactive with ARP123 and demonstrated a comparable response with the cell supernatants and the purified HIV-2 viral lysates.

Seroconversion Panels
Forty-two (42) commercially available HIV-1 seroconversion panels (365 total members) were tested with the BioPlex 2200 HIV Ag-Ab assay and an FDA approved HIV Ag/Ab assay.

Results: Both the BioPlex 2200 HIV Ag-Ab assay and the FDA approved HIV Ag/Ab assay detected some reactive bleeds in 100% (42/42) of the seroconversion panels. Of these panels, 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. A summary of the results is presented in Table 5.

Table 5: BioPlex 2200 HIV Ag-Ab 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-2 Ab	HIV-1 p24 Ag		HIV Ag-Ab	HIV-1 Ab	HIV-2 Ab	HIV-1 p24 Ag		
1	10	7	3	0	7	5	21	30	NA	21	23	2
2	10	4	2	0	4	4	25	32	NA	25	25	0
3	9	4	0	0	4	4	21	NA	NA	21	21	0
4	7	2	0	0	2	2	18	NA	NA	18	18	0
5	11	2	1	0	2	2	36	38	NA	36	36	0
6	8	5	3	0	5	4	0	0	NA	0	0	0
7	7	6	5	0	1	6	0	10	NA	0	0	0
8	8	2	1	0	2	2	33	38	NA	33	33	0
9	11	4	1	0	4	3	25	35	NA	25	28	3
10	3	1	1	0	0	1	8	8	NA	NA	8	0
11	9	3	1	0	3	3	23	32	NA	23	23	0
12	7	2	0	0	2	2	53	NA	NA	53	53	0
13	14	7	5	0	2	5	22	36	NA	22	36	14
14	6	4	3	0	3	4	22	32	NA	22	22	0
15	10	3	2	0	3	3	66	69	NA	66	66	0
16	27	16	14	0	5	16	45	52	NA	45	45	0
17	25	17	13	0	6	17	40	55	NA	40	40	0
18	4	3	2	0	3	3	24	26	NA	24	24	0
19	9	6	4	0	3	6	53	60	NA	53	53	0
20	13	6	4	0	5	5	23	33	NA	23	28	5
21	6	3	2	0	1	3	15	30	NA	15	15	0
22[a]	5	4	2	0	4	5	53	65	NA	53	0	-53
23	8	4	3	0	4	4	26	33	NA	26	26	0
24	6	2	2	0	2	2	44	44	NA	44	44	0
25	6	4	2	0	3	4	7	27	NA	7	7	0
26	9	5	4	0	2	5	15	28	NA	15	15	0
27	9	4	1	0	3	4	16	103	NA	16	16	0
28	8	8	6	0	4	7	0	11	NA	0	7	7
29	7	5	2	0	5	5	7	19	NA	7	7	0
30	6	5	2	0	5	4	2	14	NA	2	7	5
31	6	4	2	0	4	3	7	15	NA	7	13	6
32	4	3	3	0	2	3	9	9	NA	9	9	0
33	6	4	1	0	4	4	8	19	NA	8	8	0
34	6	4	2	0	2	4	10	17	NA	10	10	0
35	4	3	1	0	3	3	3	10	NA	3	3	0
36	7	2	1	0	2	2	17	21	NA	17	17	0
37	7	2	1	0	2	2	23	28	NA	23	23	0
38	6	4	2[b]	0	4	4	7	15[b]	NA	7	7	0
39	7	7	5	0	7	7	0	9	NA	0	0	0
40	6	5	3	0	4	5	5	12	NA	5	5	0
41	14	14	14	0	0	14	0	0	NA	NA	0	0
42	14	14	14	0	0	14	0	0	NA	NA	0	0
Total	**365**											
Total Reactive Bleeds		**214**	**140**	**0**	**133**	**205**						
Total Reactive Panels		**42**	**39**	**0**	**39**	**42**						

[a] Bleed #2 in Panel #22 is 53 days after bleed #1. The Certificate of Analysis for this panel indicates that no EIA or RNA results were positive from bleed # 1.

[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

Table 5 summary: The first reactive bleed occurred earlier on the BioPlex 2200 HIV Ag-Ab in 7 of the 42 panels tested, and earlier on the FDA approved HIV Ag-Ab assay in one of the 42 panels. Evaluation of the seroconversion panels demonstrated acceptable performance of the BioPlex 2200 HIV Ag-Ab assay.

HIV-1 Subtype Antibody Panel

In a study of HIV-1 Group M subtypes, 216 specimens were tested with the BioPlex 2200 HIV Ag-Ab assay.

Results: This subtype panel was reactive in 100% (216/216) of the members on the BioPlex 2200 HIV Ag-Ab assay, as shown in Table 6.

Table 6: HIV-1 Subtype Antibody Panel

Subtype	Number per Subtype	BioPlex 2200 HIV Ag-Ab Reactive			
		HIV Ag-Ab Assay	HIV-1 Ab Assay	HIV-2 Ab Assay	HIV-1 p24 Ag Assay
A	29	29	29	0	0[a]
B	4	4	4	0	0[b]
C	5	5	5	0	0
CRF01	11	11	11	0	0[b]
CRF02	77	77	77	0	0[c]
CRF05	1	1	1	0	0
CRF06	3	3	3	0	0
CRF07	1	1	1	0	0
CRF09	2	2	2	0	0
CRF11	10	10	10	0	0
CRF13	7	7	7	0	0[b]
D	16	16	16	0	0[b]
F	20	20	20	0	0[d]
G	16	16	16	0	0
H	7	7	7	0	0
J	2	2	2	0	0
K	3	3	3	0	0
U	1	1	1	0	0
Unknown	1	1	1	0	0
TOTAL	**216**	**216**	**216**	**0**	**0**

[a] Three results were not reportable for HIV-1 Ag due to high HIV-1 Ab levels
[b] One result was not reportable for HIV-1 Ag due to high HIV-1 Ab levels
[c] Four results were not reportable for HIV-1 Ag due to high HIV-1 Ab levels
[d] Two results were not reportable for HIV-1 Ag due to high HIV-1 Ab levels

Table 6 summary: All 216 members of the subtype panel were reactive on the BioPlex 2200 HIV Ag-Ab assay. Ten HIV-1 Ag results were non-reportable due to high HIV-Ab levels. The BioPlex 2200 HIV Ag-Ab assay demonstrated acceptable performance with the ability to detect all HIV-1 subtypes evaluated.

Reactivity with Low Titer Panel

Analytical Sensitivity of the BioPlex 2200 HIV Ag-Ab assay was assessed with a commercially available HIV-1 Low Titer Panel, consisting of 9 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 combination assay.

Results: The BioPlex 2200 HIV Ag-Ab and the FDA approved HIV Ag/Ab combination assay demonstrated acceptable performance resulting in 100% (9/9) detection of the reactive HIV-1 Low Titer Panel samples. The negative control was non-reactive in both assays.

HIV-1 Incidence/Prevalence Panel
The BioPlex 2200 HIV Ag-Ab assay was 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.

Results: The BioPlex 2200 HIV Ag-Ab assay, the FDA approved HIV Ag/Ab assay and the HIV-1 Western blot were equivalent in identifying 100% (15/15) of the HIV-1 Incidence/Prevalence Panel samples.

Anti-HIV-1/-2 Performance Panel
Reactivity of the BioPlex 2200 HIV Ag-Ab assay with a commercially available Anti-HIV-1/-2 Performance Panel was tested. The BioPlex 2200 HIV Ag-Ab assay results were compared to an FDA approved HIV Ag/Ab assay and to the FDA approved HIV-1/HIV-2 differentiation test.

Results: The BioPlex 2200 HIV Ag-Ab assay and the FDA approved HIV Ag/Ab assay were equivalent in detecting 100% (14/14) of the reactive Anti-HIV-1/-2 Performance Panel samples. In addition, the BioPlex 2200 HIV Ag-Ab individual results for the HIV-1 Ab and HIV-2 Ab were equivalent to the results obtained with the FDA approved HIV-1/HIV-2 differentiation test.

Effect of Potentially Interfering Substances
To assess the impact of potentially interfering substances on the performance of the BioPlex 2200 HIV Ag-Ab assay, HIV negative samples as well as those spiked with both low and high levels of HIV-1 antigen, HIV-1 (group M and group 0) antibody, and HIV-2 antibody samples were evaluated. The following interfering substances were tested: Bilirubin (20 mg/dL), Triglycericdes (1250 mg/dL), Protein (12 g/dL), and Hemoglobin (500 mg/dL). Twenty matched sample pairs (interfering substance spiked and unspiked control), made from the four interfering substances and the five types of HIV samples (HIV-1 Group M, HIV-1 Group O, and HIV-2 antibody positive; HIV p24 antigen positive; HIV negative). The presence of abnormal concentrations of interfering substances did not produce a change in the index for HIV-1 Group M, HIV-1 Group O and HIV-2 antibodies and HIV-1 p24 antigen.

Results: The presence of abnormal concentrations of potentially interfering substances demonstrates no significant effect on the detection of HIV-1 Group M, HIV-1 Group O,

and HIV-2 antibodies, and HIV-1 antigen using the BioPlex 2200 HIV-Ag/Ab assay, and no significant effect on background signal in negative specimens.

Effect of Anticoagulants
The impact of anticoagulants (K2 EDTA, K3 EDTA, lithium heparin, sodium citrate, sodium heparin) on the performance of the BioPlex 2200 HIV Ag-Ab assay was evaluated using 10 matched sets of serum and plasma samples of all specified types, spiked and unspiked, with HIV-1 antigen, HIV-1 antibody, and HIV-2 antibody.

Results: The results indicate that all unspiked samples were non-reactive, with the exception of thawed samples collected in sodium citrate, and all spiked samples were reactive in all anticoagulants and tube types. The average recoveries for spiked HIV analytes in various anticoagulants and tube types were within acceptable ranges. Therefore, serum or plasma (K2 EDTA, K3 EDTA, lithium heparin, sodium heparin) specimens, either fresh or frozen, may be used in the test. Fresh samples collected in sodium citrate may also be used. A high rate of false positive results was observed when frozen plasma samples collected in sodium citrate were used. Therefore, samples collected in sodium citrate that have been previously frozen should not be used.

Effect of Unrelated Medical Conditions
To assess the impact of unrelated medical conditions on the analytical sensitivity and specificity of BioPlex 2200 HIV Ag-Ab assay, samples collected from individuals that had unrelated medical conditions were tested. Performance was evaluated on unspiked samples, and two aliquots of each specimen spiked with antibody to HIV-1 and HIV-2, or HIV-1 p24 antigen.

Results: 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 FDA approved HIV-1/HIV-2 Ag/Ab assay, and identified as true positive samples (these samples were removed from the calculation). Therefore, the BioPlex HIV Ag-Ab assay was nonreactive in 388 of 388 samples from individuals with medical conditions unrelated to HIV. Of the 388 samples spiked with HIV-1 and HIV-2 antibody, 387/388 were reactive for both HIV-1 and HIV-2 antibody and nonreactive for HIV-1 antigen; 1 sample (Hypergammaglobulinemia IgM sample) spiked with HIV-1 group O antibody was HIV-1 antibody nonreactive. Of the same samples that were spiked with HIV-1 antigen, 387/388 were reactive for HIV-1 antigen and nonreactive for both HIV-1 and HIV-2 antibody; 1 sample (Pre-influenza vaccine recipient sample) spiked with HIV-1 antigen was nonreactive. A summary of the results is presented in Table 7.

Table 7: Effect of Unrelated Medical Conditions

Cross-Reactivity Disease Category	N	Unspiked samples	HIV-1/2 Ab spiked samples		HIV-1 Ag spiked samples	
		Nonreactive	Ab Reactive	Ag Reactive	Ab Reactive	Ag Reactive
Autoimmune Disease (Lupus)	10	10/10	10/10	0/10	0/10	10/10
Chlamydia	10	10/10	10/10	0/10	0/10	10/10
Common Cold	10	10/10	10/10	0/10	0/10	10/10
Cord Blood (Neonates)	10	10/10	10/10	0/10	0/10	10/10

Cross-Reactivity Disease Category	N	Unspiked samples	HIV-1/2 Ab spiked samples		HIV-1 Ag spiked samples	
		Nonreactive	Ab Reactive	Ag Reactive	Ab Reactive	Ag Reactive
Crohn's Disease	10	10/10	10/10	0/10	0/10	10/10
Cytomegalovirus Ab	10	10/10	10/10	0/10	0/10	10/10
Dialysis Patients	10	10/10	10/10	0/10	0/10	10/10
Hypergammaglobulinemia IgG	10	10/10	10/10	0/10	0/10	10/10
Hypergammaglobulinemia IgM	20	20/20	19/20	0/20	0/20	20/20
IgM Monoclonal Gammopathy	10	10/10	10/10	0/10	0/10	10/10
Multiparous Pregnancies	10	10/10	10/10	0/10	0/10	10/10
Multiply Transfused Patients	10	10/10	10/10	0/10	0/10	10/10
P. aeruginosa	10	10/10	10/10	0/10	0/10	10/10
E. coli	10	10/10	10/10	0/10	0/10	10/10
Epstein-Barr Virus Ab positive	10	10/10	10/10	0/10	0/10	10/10
Fungal Infection	10	10/10	10/10	0/10	0/10	10/10
Graves' Disease	10	10/10	10/10	0/10	0/10	10/10
Hemophilia	10	10/10	10/10	0/10	0/10	10/10
Hepatitis A Virus Ab positive	10	10/10	10/10	0/10	0/10	10/10
Hepatitis B Virus Ab positive	10	10/10	10/10	0/10	0/10	10/10
Hepatitis C Virus Ab positive	10	10/10	10/10	0/10	0/10	10/10
Herpes Simplex Virus Ab positive	10	10/10	10/10	0/10	0/10	10/10
Human Anti-Mouse Antibodies (HAMA) pos	10	10/10	10/10	0/10	0/10	10/10
Human T-Lymphotropic Virus (HTLV)	10	10/10	10/10	0/10	0/10	10/10
Pregnancy First Trimester	10	10/10	10/10	0/10	0/10	10/10
Pregnancy Second Trimester	10	10/10	10/10	0/10	0/10	10/10
Pregnancy Third Trimester	10	10/10	10/10	0/10	0/10	10/10
Post-Influenza Vaccine Recipient	10	10/10	10/10	0/10	0/10	10/10
Pre-Influenza Vaccine Recipient	20	20/20	20/20	0/20	0/20	19/20
Rheumatoid Factor positive	10	10/10	10/10	0/10	0/10	10/10
S. aureus	10	10/10	10/10	0/10	0/10	10/10
Smallpox Vaccine Recipient	10	10/10	10/10	0/10	0/10	10/10
Syphilis (Positive Serology)	18[a]	18/18	18/18	0/18	0/18	18/18
Varicella Zoster Virus Ab Positive	10	10/10	10/10	0/10	0/10	10/10
Viral Diarrheal Illness	10	10/10	10/10	0/10	0/10	10/10
Yeast Reactive	10	10/10	10/10	0/10	0/10	10/10
Total	**388**	**388/388**	**387/388**	**0/388**	**0/388**	**387/388**

[a] 2 of the initial 10 samples in the category of syphilis serology positive were reactive on the BioPlex 2200 HIV Ag-Ab assay. They were also reactive on an approved HIV Ag/Ab assay, and deemed to be true positive results. 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.

Results: The data demonstrate that the BioPlex 2200 HIV Ag-Ab assay does not exhibit cross-reactivity in potentially cross-reacting specimens associated with unrelated clinical conditions tested. However, when matched samples were spiked with HIV antibody or HIV antigen a slight impact on performance was observed. In IgM monoclonal gammopathy, of samples spiked with HIV-1/2 Ab, 1/20 was nonreactive for antibody. In Pre-Influenza vaccine recipient specimens spiked with HIV-1-Ag, 1/20 was nonreactive for antigen.

Dose Effect

A study was conducted to determine if very high levels of analyte produce unacceptably low values due to high dose hook effect interference. Twenty (20) HIV-1 Group M, five (5) HIV-2, and one HIV-1 Group O antibody positive samples and a concentrated stock solution (300 ng/ml) of HIV-1 p24 antigen were used in the test panel.

Results: In samples with high levels of HIV-1 antibodies, HIV-2 antibodies, or HIV-1 antigen, the BioPlex 2200 HIV Ag-Ab assay did not exhibit a high-dose hook effect.

Dual Infection Samples

Fifteen (15) specimens from seven (7) individuals known to be infected with HIV-1 and HIV-2 were obtained from CIRBA, Centre Integre De Recherches Biocliniques D'Abidjan. The samples were determined to be from co-infected individuals based on testing performed by the vendor. Samples tested on the BioPlex 2200 HIV Ag-Ab assay are interpreted as reactive when the index value is greater than or equal to the cut-off (Index value of 1.0) for any one analyte. The differentiation of HIV-1 and HIV-2 reactivity is as follows: if both HIV-1 Ab and HIV-2 Ab index values are ≥1.00 and the Index ratio of HIV 1 Ab and HIV 2 Ab is < 5 fold, the result for HIV 1 and/or HIV-2 Ab is reported as "Reactive, Undifferentiated". If there is ≥ 5-fold difference in the Indices, the higher Ab Index is reported as Reactive and the lower Ab Index is reported as Non-Reactive.

Results: The BioPlex HIV Ag-Ab assay results were in agreement with the FDA approved HIV-1/HIV-2 differentiation test.

- The BioPlex 2200 HIV Ag-Ab assay was reactive in all dual infection samples.
- The assay correctly identified dual infection in 5 out of 7 dually infected samples, and represents 100% agreement with the FDA approved HIV-1/HIV-2 differentiation test.
- All of the samples from one patient were non-reactive to HIV-1 antibody and highly reactive to HIV-2 antibody and were reported as HIV-2 reactive.
- Both samples from one patient were HIV-1 antibody and HIV-2 antibody reactive; however the testing results were not categorized as dual infected samples, since one replicate had a result of undifferentiated (ratio < 5) and the other replicate had a result of HIV-2 antibody reactive (ratio ≥ 5). The BioPlex 2200 HIV Ag-Ab assay demonstrated acceptable performance.

HIV-1 NAT yield samples

A total of 43 HIV-1 NAT yield samples (collected from blood donors in the US by the American Red Cross) were evaluated with the BioPlex 2200 HIV Ag-Ab Assay. The viral load and subtype information for these samples was provided by the American Red Cross. Each sample was tested in duplicate on one lot of the BioPlex 2200 HIV Ag-Ab assay and the FDA approved HIV Ag/Ab assay. If available, historical EIA data were used.

Results: The BioPlex 2200 HIV Ag-Ab assay and the FDA approved HIV Ag/Ab assay detected 15 of 43 yield specimens (34.9%). Two (2) specimens were detected differently by the two assays. Sample 003S 32686 was repeatedly reactive in BioPlex 2200 HIV Ag-Ab and non-reactive in the FDA approved HIV Ag/Ab assay. Sample 026KG86081 was repeatedly reactive in the FDA approved HIV Ag/Ab assay and

non-reactive in BioPlex HIV Ag-Ab. Overall, both BioPlex 2200 HIV Ag-Ab assay and the FDA approved HIV Ag/Ab assay detected the same number of HIV-1 NAT yield specimens. The diagnostic sensitivity of the BioPlex 2200 HIV Ag-Ab assay in the detection of HIV-1 NAT yield samples collected from blood donors in the US is 34.9%. When compared to the FDA approved assay, the BioPlex 2200 HIV Ag-Ab assay detects the same number of HIV-NAT yield samples. A summary of the results is presented in Table 8.

Table 8: BioPlex 2200 HIV Ag-Ab Results with HIV-1 NAT-yield samples

			BioPlex 2200 HIV Ag-Ab Indices											**FDA Approved Ag/Ab Assay**						
			Rep 1					***Rep 2***						***Rep 1***			***Rep 2***			
Specimen ID	**Viral Load**	**HIV Subtype**	**HIV-1Ag**	**HIV-1 M Ab**	**HIV-2 Ab**	**HIV-1 O Ab**	**HIV Ag-Ab**	**HIV-1 Ag**	**HIV-1 M Ab**	**HIV-2 Ab**	**HIV-1 O Ab**	**HIV Ag-Ab**	***Final Interpretation***	***CO***	***OD***	***Ratio***	***CO***	***OD***	***Ratio***	***Final Interpretation***
054KF99223	750,000	B	6 36	0 07	0 26	0 19	6 36	6 51	0 06	0 20	0 18	6 51	Repeatedly Reactive	0 24	1 13	4 69	0 24	1 14	4 73	Repeatedly Reactive
022LQ82592	390	B	0 26	17 15	0 09	0 31	17 15	0 24	18 82	0 14	0 41	18 82	Repeatedly Reactive	0 24	3 50	14 58	0 24	3 50	14 58	Repeatedly Reactive
003K 16030	790	B	0 25	0 07	0 15	0 17	0 25	0 21	0 04	0 15	0 10	0 21	Nonreactive	0 24	0 05	0 21	0 24	0 05	0 21	Nonreactive
054KC25532	81,000	B	0 94	0 58	0 12	0 15	0 94	1 03	0 51	0 12	0 12	1 03	Reactive	0 24	0 78	3 27	0 24	0 78	3 25	Repeatedly Reactive
029KM27572	2,300	B	0 16	0 05	0 11	0 08	0 16	0 24	0 05	0 07	0 08	0 24	Nonreactive	0 24	0 06	0 24	0 24	0 05	0 21	Nonreactive
036KH11546	4,400,000	B	154 47	0 05	0 07	0 08	154 47	156 42	0 02	0 12	0 03	156 42	Repeatedly Reactive	0 24	3 50	14 58	0 24	3 50	14 58	Repeatedly Reactive
013FY89120	910	B	0 11	0 05	0 05	0 06	0 11	0 13	0 06	0 13	0 07	0 13	Nonreactive	0 24	0 06	0 23	0 24	0 04	0 15	Nonreactive
003GP52279	1,500	B	0 17	0 06	0 12	0 10	0 17	0 21	0 06	0 13	0 09	0 21	Nonreactive	0 24	0 04	0 18	0 24	0 05	0 19	Nonreactive
035FH89864	850	B	0 11	0 04	0 05	0 08	0 11	0 02	0 03	0 11	0 07	0 11	Nonreactive	0 24	0 04	0 18	0 24	0 05	0 19	Nonreactive
036FL20959	1,300	B	0 36	0 06	0 15	0 15	0 36	0 35	0 07	0 16	0 13	0 35	Nonreactive	0 24	0 06	0 23	0 24	0 05	0 23	Nonreactive
022GH05568	200	B	0 31	0 06	0 16	0 16	0 31	0 25	0 06	0 14	0 15	0 25	Nonreactive	0 24	0 05	0 21	0 24	0 05	0 20	Nonreactive
003S 32686	410,000	B	1 50	0 11	0 24	0 16	1 50	1 79	0 12	0 24	0 16	1 79	Repeatedly Reactive	0 24	0 23	0 97	0 24	0 23	0 96	Nonreactive
053GM60877	5,800	B	0 23	0 07	0 13	0 10	0 23	0 09	0 04	0 12	0 06	0 12	Nonreactive	0 24	0 07	0 27	0 24	0 06	0 26	Nonreactive
026KG66346	100	B	0 07	0 04	0 09	0 08	0 09	0 20	0 04	0 09	0 10	0 20	Nonreactive	0 24	0 06	0 23	0 24	0 05	0 20	Nonreactive
011GS88902	46,00	B	0 48	0 08	0 05	0 05	0 48	0 50	0 12	0 09	0 12	0 50	Nonreactive	0 24	0 16	0 65	0 24	0 17	0 72	Nonreactive
042G 71168	2,100,000	B	2 20	109 72	0 14	0 07	109 72	2 23	110 40	0 07	0 09	110 40	Repeatedly Reactive	0 24	3 50	14 58	0 24	3 50	14 58	Repeatedly Reactive
053FW65871	17,000	Unk	0 68	0 03	0 06	0 06	0 68	0 60	0 04	0 06	0 04	0 60	Nonreactive	0 24	0 10	0 42	0 24	0 21	0 88	Nonreactive
036GP25256	610,000	Unk	58 03	0 03	0 13	0 12	58 03	61 15	0 03	0 10	0 10	61 15	Repeatedly Reactive	0 24	3 50	14 58	0 24	3 50	14 58	Repeatedly Reactive
022FS86599	13,000	Unk	1 13	0 03	0 11	0 11	1 13	1 18	0 04	0 08	0 11	1 18	Repeatedly Reactive	0 24	0 27	1 13	0 24	0 26	1 06	Repeatedly Reactive
012LS24073	220,000	Unk	2 27	0 02	0 11	0 05	2 27	2 27	0 06	0 16	0 08	2 27	Repeatedly Reactive	0 24	0 71	2 98	0 24	0 75	3 13	Repeatedly Reactive
022FC76383	3,700	Unk	0 45	0 09	0 25	0 22	0 45	0 44	0 12	0 27	0 20	0 44	Nonreactive	0 24	0 08	0 35	0 24	0 09	0 37	Nonreactive
003L 15178	120,000	Unk	1 85	0 41	0 15	0 12	1 85	1 78	0 39	0 13	0 08	1 78	Repeatedly Reactive	0 30	0 74	2 50	0 30	0 83	2 80	Repeatedly Reactive
024GK71945	203,940	Unk	9 67	0 08	0 17	0 18	9 67	9 56	0 07	0 22	0 14	9 56	Repeatedly Reactive	0 30	2 57	8 70	0 30	2 63	8 90	Repeatedly Reactive
026KG86081	95,180	Unk	0 85	0 06	0 17	0 08	0 85	0 78	0 04	0 13	0 11	0 78	Nonreactive	0 30	0 48	1 60	0 30	0 49	1 60	Repeatedly Reactive
013GT54513	18,750	Unk	0 33	0 04	0 08	0 08	0 33	0 45	0 05	0 11	0 13	0 45	Nonreactive	0 30	0 24	0 80	0 30	0 24	0 80	Nonreactive
053FK43019	345,880	Unk	1 75	0 05	0 11	0 06	1 75	1 59	0 04	0 10	0 08	1 59	Repeatedly Reactive	0 30	0 77	2 60	0 30	0 74	2 50	Repeatedly Reactive
012J 20817	176,800	Unk	1 60	0 11	0 34	0 27	1 60	1 18	0 12	0 30	0 26	1 18	Repeatedly Reactive	0 30	0 47	1 60	0 30	0 47	1 60	Repeatedly Reactive
012GM77609	1,650	Unk	0 31	0 07	0 15	0 12	0 31	0 33	0 05	0 18	0 13	0 33	Nonreactive	0 30	0 08	0 30	0 30	0 10	0 30	Nonreactive
003LM54246	1,050	Unk	0 32	0 06	0 12	0 16	0 32	0 33	0 06	0 12	0 10	0 33	Nonreactive	0 30	0 09	0 30	0 30	0 10	0 30	Nonreactive
003LE75595	3,450	Unk	0 28	0 06	0 16	0 15	0 28	0 32	0 09	0 17	0 17	0 32	Nonreactive	0 30	0 08	0 30	0 30	0 08	0 30	Nonreactive
003GS91125	2,850	Unk	0 12	0 03	0 04	0 07	0 12	0 18	0 02	0 08	0 05	0 18	Nonreactive	0 30	0 10	0 30	0 30	0 09	0 30	Nonreactive
054KC40785	< 100	Unk	0 05	0 03	0 08	0 06	0 08	0 05	0 03	0 04	0 02	0 05	Nonreactive	0 30	0 12	0 40	0 30	0 15	0 50	Nonreactive
003FT69201	3740	Unk	0 41	0 09	0 25	0 27	0 41	0 50	0 14	0 26	0 26	0 50	Nonreactive	0 30	0 08	0 30	0 30	0 09	0 30	Nonreactive
017GS94812	130	Unk	0 19	0 05	0 12	0 06	0 19	0 07	0 02	0 11	0 07	0 11	Nonreactive	0 30	0 08	0 30	0 30	0 08	0 30	Nonreactive
049KE25760	20910	Unk	0 37	179 48	0 22	0 44	179 48	0 31	171 39	0 25	0 43	171 39	Repeatedly Reactive	0 30	3 56	12 00	0 30	3 55	12 00	Repeatedly Reactive
001LV16853	26320	Unk	0 22	0 03	0 13	0 08	0 22	0 30	0 06	0 12	0 08	0 30	Nonreactive	0 30	0 21	0 70	0 30	0 22	0 70	Nonreactive

Table 8 summary: The diagnostic sensitivity of the BioPlex 2200 HIV Ag-Ab assay in the detection of HIV-1 NAT yield samples collected from blood donors in the US is 34.9%. When compared to the FDA approved HIV Ag/Ab assay, the BioPlex 2200 HIV Ag-Ab assay detected the same number of HIV-NAT yield samples (15).

Known HIV-1 Subtype Antibody Positive Samples
One hundred fifty-six (156) known HIV-1 antibody positive Group M non-B subtypes and 3 Group O samples were included in the study. Specimens reactive for HIV antibody were identified as HIV-1 or HIV-2, or were reported as undifferentiated. Initially reactive (IR) samples were retested in duplicate. The samples were also tested with an FDA approved 4th Generation HIV Ag/Ab Assay.

Table 9: HIV-1 Subtype Antibody Testing (N=156)

Known HIV-1 Antibody Subtype	N	BioPlex 2200 HIV Ag-Ab Overall HIV Ag-Ab Test Repeatedly Reactive	FDA Approved 4th Generation HIV Ag/Ab Assay Reactive
A	23	23	23
A1	1	1	1
AE	1	1	1
AG	4	4	4
B	1	1	1
C	5	5	5
CRF01_A/E	3	3	3
CRF02/CRF11_A/G	2	2	2
CRF02_A	63	63	63
CRF02_A/F	1	1	1
CRF02_A/G	2	2	2
CRF06	1	1	1
CRF07	1	1	1
CRF09	1	1	1
CRF09_CPX/A	1	1	1
CRF11_A	3	3	3
CRF11_A/G	3	3	3
CRF11_G	1	1	1
CRF13	1	1	1
CRF13_A/G	1	1	1
D	3	3	3
F	8	8	8
G	11	11	11
H	7	7	7
J	1	1	1
K	3	3	3
U	1	1	1
GROUP O	3	3	3
Total	**156**	**156**	**156**

Table 9 summary: All samples tested were reactive with both the FDA approved 4th generation HIV Ag/Ab assay and the BioPlex 2200 Ag-Ab assay.

Results: The BioPlex 2200 HIV Ag-Ab assay detected 100% (156/156) of the known HIV-1 subtype samples demonstrating acceptable performance in detecting antibodies to multiple HIV-1 subtypes. The reactivity rate for the initial testing of the samples was the

same as that for the repeat testing. All 156 samples were non-reactive for the BioPlex 2200 HIV-2 Ab and HIV-1 p24 Ag.

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 4th generation HIV Ag/Ab assay. The BioPlex 2200 HIV Ag-Ab assay detected 100% of the AIDS with CDC classification samples.

Table 10: AIDS with CDC Stage Classification (N=100)

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

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

Table 10 summary: All samples tested were reactive with both the FDA approved 4th generation HIV Ag/Ab assay and the BioPlex 2200 Ag-Ab assay.

Results: The BioPlex 2200 HIV Ag-Ab assay demonstrated acceptable performance (100/100 reactive) when evaluated using AIDS stage classification samples.

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.

Results: All 63/63 samples were reactive for HIV-1 Antibody with the BioPlex 2200 HIV Ag-Ab assay.

Reactivity in Known HIV-2 Antibody Positive Samples

Two hundred (200) known HIV-2 antibody positive samples obtained from individuals from different geographic locations were tested with the BioPlex 2200 HIV Ag-Ab assay. The samples included 148 plasma and 52 sera.

Table 11: Reactivity in Known HIV-2 Antibody Positive Samples Summary (N = 200)

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

[a] 4 samples were not reportable for HIV-1 p24 Ag due to high Ab level

Table 11 summary: All 200 samples were reactive for HIV on the BioPlex 2200 Ag-Ab assay and the FDA approved 4th generation HIV Ag/Ab assay. The BioPlex 2200 Ag-Ab assay identified 188/200 samples as reactive for HIV-2 Ab and 12/200 as antibody undifferentiated.

Sensitivity in Known HIV-2 Antibody Positive Samples Summary

- The BioPlex 2200 HIV Ag-Ab assay was repeatedly reactive in 200/200 of the known HIV-2 antibody positive samples. One hundred and eighty eight (188) of the known HIV-2 antibody positive samples were reactive for HIV-2 Ab and twelve (12) samples were HIV Ab reactive, undifferentiated.

- Of the 12 BioPlex 2200 HIV Ag-Ab reactive, undifferentiated samples, two (2) were undifferentiated, and the remaining 10 were HIV-2 reactive on a FDA approved HIV-1/HIV-2 differentiation assay.

- Four (4) samples were not reportable for HIV-1 p24 Ag due to high Ab level.

- All 200 samples were reactive for HIV on the 4th generation HIV Ag/Ab assay

Reactivity in Known HIV-1 Antigen positive samples

Four (4) HIV-1 antigen positive samples were tested with the BioPlex 2200 HIV Ag-Ab assay. Two of the samples were serum and two were plasma. Initially reactive samples on the BioPlex 2200 HIV Ag-Ab assay were retested in duplicate and tested by the HIV-1 RNA assay. The samples were also tested with a FDA approved HIV Ag/Ab assay.

Table 12: Reactivity in Known HIV-1 Ag Positive Samples (N = 4)

Population	N	BioPlex 2200 HIV Ag-Ab Reactive			
		Overall HIV Ag-Ab	HIV-1 Ab	HIV-2 Ab	HIV-1 p24 Ag
Known HIV-1 Ag Positive	4	4	1[a]	0	4[a]

[a] One sample was reactive for both HIV-1 p24 Ag and HIV-1 Ab

Table 13: Reactivity in Known HIV-1 Ag Positive Samples versus FDA Approved 4th Generation HIV Ag/Ab Assay and Supplemental Testing

Population	N	BioPlex 2200 HIV Ag-Ab Overall HIV Ag-Ab Test		FDA Approved 4th Generation HIV Ag/Ab Assay Reactive	Reactive Specimens
		Non-Reactive	Repeatedly Reactive HIV Ag-Ab		HIV-1 RNA Assay Pos
Known HIV-1 Ag Positive	4	0	4	4	4

Table 12 and 13 summary: All 4 samples were reactive with the BioPlex 2200 HIV Ag-Ab assay and the FDA approved 4th generation HIV Ag/Ab assay

Reactivity in Known HIV-1 Ag Positive Samples Summary

- The BioPlex 2200 HIV Ag-Ab assay detected 4/4 HIV-1 p24 Ag positive samples.

- One (1) of 4 samples was also reactive for HIV-1 Ab. The remaining Ag positive samples were non-reactive for both HIV-1 Ab and HIV-2 Ab.

Reproducibility

The reproducibility of the BioPlex 2200 Ag-Ab assay was evaluated at three independent clinical sites. Each of the panel members and positive and negative controls were tested in replicates of three (3) on one (1) run per day for five (5) days on three (3) lots of the BioPlex 2200 HIV Ag-Ab assay (45 replicates per member per site x 3 sites =135 samples for each panel member). The data were analyzed for intra-assay and inter-assay reproducibility. The mean Index value, standard deviation (SD) and coefficient of variation (CV) were calculated.

Results: The total CV for the panel members ranged from 4.5 to 11.7% for the BioPlex 2200 Ag-Ab assay. The test results indicate acceptable performance of reproducibility.

Table 14: Reproducibility Testing Panel

#	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

Table 15: Reproducibility Results – BioPlex 2200 HIV Ag-Ab Assay

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 15 summary: The total CV for the panel members ranged from 4.5 to 11.7% for the BioPlex 2200 Ag-Ab assay indicating acceptable reproducibility in the end-user environment for all variables evaluated. The reproducibility of the individual analytes was also analyzed and demonstrated acceptable performance in the end-user environment. The mean index values for HIV-1 Group M Ab low positive, HIV-1 Group O Ab low positive, HIV-2 Ab low positive and HIV-1 p24 low positive panel members ranged between 1.95 and 2.19; and the total CV ranged between 4.5 and 9.2%. The mean index value of the HIV-1 Group M Ab high negative was 0.593 and the total CV was 6.6%. These results demonstrate acceptable performance.

Precision

An in-house precision study was performed at the manufacturing facility 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. The standard deviation (SD) and coefficient of variation (CV) were analyzed for each panel member.

Results: All assays exhibited precision well within the 10% CV limit. The results are summarized in Table16. The test results indicate acceptable performance of precision.

Table 16: Precision Results - BioPlex 2200 HIV Ag-Ab Combination Assay

Panel Member	Mean	Within Run		Between Run		Between Day		Total[a]	
		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[b]	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[b]	0.0	0.052	2.4	0.092	4.3
7	4.77	0.133	2.8	0.000[b]	0.0	0.080	1.7	0.155	3.3
8	1.92	0.081	4.2	0.000[b]	0.0	0.047	2.4	0.094	4.9
9	1.93	0.067	3.5	0.059	3.0	0.033	1.7	0.095	4.9
10	0.60	0.030	5.0	0.005	0.9	0.021	3.5	0.037	6.2
11	2.25	0.070	3.1	0.034	1.5	0.056	2.5	0.096	4.3
12	2.38	0.087	3.7	0.000	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	0.05	0.013	NA	0.007	NA	0.000[b]	NA	0.015	NA
15	0.07	0.017	NA	0.000[b]	NA	0.005	NA	0.017	NA
16	3.39	0.107	3.2	0.000[b]	0.0	0.085	2.5	0.137	4.0
17	4.15	0.186	4.5	0.000[b]	0.0	0.000[b]	0.0	0.186	4.5
18	0.02	0.014	NA	0.000[b]	NA	0.004	NA	0.014	NA

[a] Total variability of the assay performance includes within run, between run and between days.
[b] Negative variances were rounded to zero, per statistical convention.

Table 16 summary: The assay exhibited acceptable precision well within the 10% CV limit for all panel members analyzed.

Stability

Real-time stability data indicated that the test performed according to the specifications when stored for [redacted] months at 2-8°C. Based on this study, expiration dating for the BioPlex 2200 HIV Ag-Ab was established and approved at 9 months when stored at 2-8^{0}C. After initial use, the Reagent Pack is stable for 60 days when stored on-board the instrument [redacted] Calibrators can be used on the BioPlex 2200 for 5 calibration events over [redacted] 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 [redacted] days, with an on-board (open vial) time of 3 hours at room temperature. The BioPlex 2200 HIV Ag-Ab Controls and Calibrators are not lot specific, and can be used with any lot number of the Reagent Pack.

XI. Summary of Clinical Studies:

Specificity

Samples from Low Risk Individuals

Six thousand three hundred ninety-five (6395) samples from populations at low risk for HIV infection (unknown HIV status) were tested with the BioPlex 2200 HIV Ag-Ab assay. The samples included 4358 serum and 2037 plasma samples. Results for the overall HIV Ag-Ab test, as well as individual tests for HIV-1 antibodies, HIV-2 antibodies, and HIV-1 p24 antigen were reported. Specimens reactive for HIV antibody were identified as HIV-1 or HIV-2, or were reported as undifferentiated. Initially reactive samples on the BioPlex 2200 HIV Ag-Ab were retested in duplicate and tested by a FDA licensed HIV-1 RNA assay and a FDA approved HIV-1/HIV-2 differentiation test. The 6395 samples that were tested from low risk individuals included the following populations:

- First Time Blood Donors - One thousand nine hundred ninety-nine (1999) samples from first time blood donors were tested. The median subject age was 18 years with a range of 15-86 years. This population was 41.3% (826/1999) male and 58.7% (1173/1999) female. Six hundred fifty three (653) samples were plasma and 1346 were serum.

- Individuals at Low Risk for HIV Infection - Two thousand two hundred ninety-seven (2297) low risk samples were tested. The median subject age was 49 years with a range of 11-102 years. This population was 58.0% (1331/2297) female and 42.0% (966/2297) male. One thousand three hundred six (1306) samples were plasma and 991were serum.

- Military Recruits - Nine hundred ninety-nine (999) low risk samples from military recruits were collected and tested. Five hundred (500) of these samples were fresh when tested and 499 samples had been previously frozen before testing. Age and gender were not provided with the samples. All samples were serum.

- Low Risk Pregnant Women - One thousand (1000) samples from low risk pregnant women were tested. Seventy-eight (78) samples were plasma and 922 were serum. The median subject age was 30 years with a range of 13 - 52 years.

- Low Risk Pediatrics - One hundred (100) samples from healthy pediatric subjects were tested. The median age was 11 years with an age range of 2 - 21 years. This population was 46.0% (46/100) female and 54.0% (54/100) male.

- Six thousand three hundred ninety-five (6395) samples from populations at low risk for HIV infection (unknown HIV status) were also tested with an FDA approved HIV Ag/Ab assay. The results for these samples with the BioPlex 2200 HIV Ag-Ab assay were compared to results of testing the same samples with the FDA approved HIV Ag/Ab assay.

Table 17: BioPlex 2200 HIV Ag-Ab Assay Reactivity in Low Risk Populations (N = 6395 samples)

Low Risk Population	N	BioPlex 2200 HIV Ag-Ab Assay											Supplemental Results for HIV Reactive Specimens			
		NR	HIV Ag-Ab		HIV-1 Ab		HIV-2 Ab		HIV Ab Un-differentiated		HIV-1 p24[a]		HIV-1 RNA Reactive	HIV-1/HIV-2 Ab Differentiation Assay		
			IR	RR	IR	RR	IR	RR	IR	RR	IR	RR		HIV-1 Pos	HIV-2 Pos	HIV Ab Undifferentiated
First Time Blood Donors - Serum	1346	1344	2	2	1	0	0	0	0	0	2	2	NT	0	0	0
First Time Blood Donors - Plasma	653	653	0	0	0	0	0	0	0	0	0	0	NT	0	0	0
Normal Healthy Individuals - Serum	991	988	3	3	3	3	0	0	0	0	0	0	2	2	0	0
Normal Healthy Individuals - Plasma	1306	1289	17	17	15	15	0	0	1	1	2	2	14	14	0	0
Military Recruits - Fresh Serum	500	499	1	1	1	1	0	0	0	0	0	0	NT	1	0	0
Military Recruits - Frozen Serum	499	498	1	1	1	1	0	0	0	0	0	0	0	0	0	0
Pregnant Women - Serum	922	918	4	4	2	2	0	0	1	1	2	2	NT	1	0	0
Pregnant Women - Plasma	78	78	0	0	0	0	0	0	0	0	0	0	NA	0	0	0
Healthy Pediatric Subjects - Serum	100	100	0	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA
Total	6395	6367	28	28	23	22	0	0	2	2	6	6	16	18	0	0

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

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

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

[a] One of the plasma samples from the normal healthy individuals population was not reportable for HIV p24 Ag due to high Ab level

Table 17 summary: 99.56% (6367/6395) of the samples from the low risk populations were non-reactive and 0.44% (28/6395) initially and repeatedly reactive for the overall HIV Ag-Ab test.

Table 18: BioPlex 2200 HIV Ag-Ab versus FDA Approved 4th Generation HIV Ag/Ab Assay Low Risk Populations–Assay Comparison Summary

BioPlex 2200 HIV Ag-Ab	FDA Approved 4th Generation HIV Ag/Ab Assay	N	HIV-1 RNA Assay Pos	FDA Approved HIV-1/HIV-2 Differentiation Test		
				HIV-1 Positive	HIV-2 Positive	HIV Positive Undifferentiated
Repeatedly Reactive	**Repeatedly Reactive**	19[a]	15	18	0	0
Repeatedly Reactive	**Non-Reactive**	9[b]	1	0	0	0
Non-Reactive	**Repeatedly Reactive**	3[c]	0	0	0	0
Non-Reactive	**Non-Reactive**	6364	NA[d]	NA[d]	NA[d]	NA[d]
Total		**6395**	**16**	**18**	**0**	**0**

[a] Of these 19 samples, 1 sample was non-reactive for both the HIV-1RNA assay and the FDA approved HIV-1/HIV-2 differentiation test and 3 had insufficient volume for testing on the FDA licensed HIV-1 RNA assay.
[b] Of these 9 samples, 4 had insufficient volume for testing on the FDA licensed HIV-1 RNA assay and were negative with an HIV-1 Ag EIA.
[c] Of these 3 samples, 1 had insufficient volume for testing on the FDA licensed HIV-1 RNA assay and were negative with an HIV-1 Ag EIA.
[d] NA = Not applicable because no results were reactive on the BioPlex 2200 HIV Ag-Ab assay or an FDA approved 4th generation HIV Ag/Ab assay.

Six thousand three hundred ninety-five (6395) samples were tested on both the BioPlex 2200 HIV Ag-Ab assay and an FDA approved 4th generation HIV Ag/Ab assay. Out of these, six thousand three hundred and sixty four (6364) samples were non-reactive on both the assays. Of nineteen (19) samples that were reactive with both the BioPlex assay and the FDA approved HIV Ag/Ab assay, 15 samples were reactive on both the FDA licensed HIV-1 RNA assay and the FDA Approved HIV-1/HIV-2 Differentiation Test. Three (3) were reactive only on the FDA Approved HIV-1/HIV-2 Differentiation Test (1 was nonreactive on the FDA licensed HIV-1 RNA test and 2 were quantity non-sufficient for the HIV-1 RNA test) and one sample was nonreactive on both the HIV-1 Ag EIA test and the FDA approved HIV-1/HIV-2 Differentiation Test (the sample was quantity non-sufficient for the FDA licensed HIV-1 RNA test). One sample was reactive on the BioPlex 2200 HIV Ag-Ab, however was non-reactive on the FDA licensed HIV-1 RNA assay and the FDA approved HIV-1/HIV-2 Differentiation Test.

Summary of BioPlex 2200 HIV Ag-Ab Specificity in Low Risk Populations:

- 99.56% (6367/6395) of the samples from the low risk populations were non-reactive and 0.44% (28/6395) initially and repeatedly reactive for the overall BioPlex HIV Ag-Ab test.

- All of the samples that were repeat reactive on the BioPlex 2200 HIV Ag-Ab assay and/or the 4th generation FDA approved HIV Ag/Ab assay were tested with the FDA approved HIV-1/HIV-2 differentiation assay.
- Of the 28 initially and repeatedly BioPlex 2200 HIV Ag-Ab reactive specimens, 19 were confirmed positive by supplemental testing. Three (3) of these samples were only reactive by the FDA approved HIV-1/HIV-2 differentiation test and one (1) was only positive by the RNA test. Fifteen (15) samples were positive for HIV-1 Ab with the FDA approved HIV-1/HIV-2 differentiation test and with the FDA licensed HIV-1 RNA test.
- Of the 9 samples that were reactive on BioPlex 2200 HIV Ag-Ab and non-reactive on the FDA approved HIV Ag/Ab assay, one (1) sample was reactive on the FDA licensed HIV RNA test only.
- One sample was BioPlex 2200 HIV Ag-Ab reactive and FDA approved HIV Ag/Ab assay reactive, non-reactive by the FDA licensed HIV-1 RNA assay and the FDA approved HIV-1/HIV-2 differentiation test.
- The 19 confirmed positive samples were removed from the specificity calculations.
- In this study, the specificity of the BioPlex 2200 HIV Ag-Ab assay in the low risk populations was 99.86% (6367/6376) with a 95% CI of 99.73% – 99.93%.

Sensitivity

Reactivity in Known HIV-1 Antibody Positive Samples

One thousand three hundred sixty-three (1363) known HIV-1 antibody positive samples were tested with the BioPlex 2200 HIV Ag-Ab assay. These samples included 997 retrospective HIV-1 antibody positives samples, 100 CDC Staged AIDS samples, 50 samples from HIV-1 infected pediatric subjects, 60 samples from HIV-1 positive pregnant women and 156 samples of known HIV-1 antibody subtype. The samples included 348 sera and 1015 plasma.

Specimens reactive for HIV antibody were identified as HIV-1 or HIV-2, or were reported as undifferentiated. Initially reactive samples were retested in duplicate. The samples were also tested with an FDA approved 4th generation HIV Ag/Ab assay.

Table 19 Summary of Reactivity in Known HIV-1 Antibody Positive Samples (N = 1363)

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

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 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 include 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.

Table 19 summary: All samples tested reactive with both the FDA approved 4th generation HIV Ag/Ab assay and the BioPlex 2200 Ag-Ab assay. Two hundred and forty six (246) samples were not reportable for HIV-1 p24 Ag due to high HIV-1 Ab level.

Sensitivity in Known HIV-1 Antibody Positive Samples Summary

- The sensitivity of the BioPlex 2200 HIV Ag-Ab assay in the known HIV-1 antibody positive population was 100% (1363/1363) with a 95% confidence interval of 99.72 – 100%.
- All known HIV-1 antibody positive samples were non-reactive for HIV-2 antibody.
- There were no samples in the known HIV-1 antibody positive population that were identified as reactive for HIV-1 p24 antigen.
- Two hundred and forty six (246) samples were not reportable for HIV-1 p24 Ag due to high Ab level.
- All 1363 samples were reactive for HIV on the 4th generation FDA approved HIV Ag/Ab assay.

Reactivity in Samples from High Risk Individuals

A total of 1866 samples obtained from individuals at high risk for HIV infection were tested with the BioPlex 2200 HIV Ag-Ab assay. The results were reported for an overall HIV Ag-Ab test as well as individual tests for HIV-1 antibodies, HIV-2 antibodies, and HIV-1 p24 antigen. Specimens reactive for HIV antibody were identified as HIV-1 or HIV-2, or were reported as undifferentiated.

Initially reactive samples on the BioPlex 2200 HIV Ag-Ab assay were retested in duplicate and tested with a FDA licensed HIV-1 RNA assay and an FDA approved HIV-1/HIV-2 differentiation test. The samples were also tested with an FDA approved 4th generation HIV Ag/Ab assay.

These 1729 serum samples and 137 plasma samples (n=1866) included 999 samples from individuals at high risk for HIV infection, plus 383 samples from pregnant women at high risk for HIV infection, and 484 samples from pediatric subjects at high risk for HIV infection.

Table 20: Reactivity in High Risk Individuals (N = 1866)

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] 3 additional samples 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 FDA approved HIV-1/HIV-2 differentiation test and the FDA licensed 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, 56 were confirmed positive by the combination of supplemental testing performed, 15 were non-reactive on supplemental testing, 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.

Table 20 summary: 95.71% (1789/1866) of the individuals at high risk of HIV infection were non-reactive and 4.29% (77/1866) were reactive with the BioPlex 2200 HIV Ag-Ab combination assay. Of the individuals at high risk of HIV infection, 4.02% (75/1866) were repeatedly reactive for HIV-1 Ab and 0.05% (1/866) was repeatedly reactive for HIV-2 Ab. 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.

Table 21: BioPlex 2200 HIV Ag-Ab versus FDA approved HIV Ag/Ab Assay - Individuals at High Risk for HIV Infection (N = 1866)

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.

Table 21 summary: 1786 samples were non-reactive with an FDA approved 4th generation HIV Ag/Ab assay.

Reactivity in High Risk Populations (N = 1866) Summary

- 95.87% (1789/1866) of the individuals at high risk of HIV infection were non-reactive and 4.13% (77/1866) were reactive with the BioPlex 2200 HIV Ag-Ab combination assay.
- Out of the 77 BioPlex 2200 HIV Ag-Ab repeatedly reactive samples, 75 samples were reactive for HIV-1 Ab, one (1) sample was reactive for HIV-1 p24 antigen and one (1) sample was reactive for HIV-2 Ab on the BioPlex 2200 HIV Ag-Ab assay.
- Out of the 77 BioPlex 2200 HIV Ag-Ab reactive samples, 57 samples were confirmed reactive by the combination of supplemental tests. Fifty five (55) samples were positive for HIV-1 antibody, one (1) sample was HIV-1 Ab indeterminate, and one (1) sample was positive for HIV-2 antibody on the FDA approved HIV-1/HIV-2 differentiation test. Fifteen (15) samples 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.
- 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% (1/866) of the samples from the high risk populations. The repeatedly reactive sample was also reactive for HIV-2 on the FDA approved 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 3/1866 (0.16%) 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 an assay for HIV-1 p24 antigen.

- When the same 1866 samples from high risk populations that were tested on the BioPlex 2200 HIV Ag-Ab assay were tested with an FDA approved 4th generation HIV Ag/Ab assay, results indicate that 1786 samples were non-reactive with an FDA approved 4th generation HIV Ag/Ab assay.

Reactivity in Individuals from an HIV-2 Endemic Region

A total of 490 samples from an HIV-2 endemic region were tested with the BioPlex 2200 HIV Ag-Ab assay. Specimens reactive for HIV antibody were identified as HIV-1 or HIV-2, or were reported as undifferentiated. Initially reactive samples on the BioPlex 2200 HIV Ag-Ab assay were retested in duplicate and tested with the HIV-1 RNA assay and the FDA approved HIV-1/HIV-2 differentiation test. The samples were also tested with the FDA approved HIV Ag-Ab assay.

Table 22: HIV-2 Endemic Region: BioPlex 2200 HIV Ag-Ab results and HIV Ag-Ab supplemental testing results summary (N = 490)

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	HIV-1 p24 Ag Not Reportable Due to High Ab Level		HIV-1 Pos	HIV-2 Pos	HIV Undifferentiated
HIV-2 Endemic Region	490	182	308	236	47	25	0	55	129	224	43	24

Table 22 summary: A total of 62.86% (308/490) of the samples from an HIV-2 endemic region were repeatedly reactive and 37.14% (182/490) were nonreactive on the BioPlex 2200 Ag-Ab assay.

Table 23: BioPlex 2200 HIV Ag-Ab versus FDA approved 4th generation HIV Ag/Ab assay Repeat Testing

HIV-2 Endemic Region		FDA approved 4th generation HIV Ag/Ab assay		
		Reactive	**Non-Reactive**	**Total**
BioPlex 2200 HIV Ag-Ab	**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 assay. One (1) of the 6 samples was non-reactive and 5 were not tested on the FDA licensed HIV-1 RNA assay.
[b]All 7 samples were negative for HIV antibody with the FDA approved HIV-1/HIV-2 differentiation assay. Three (3) of the 7 samples were non-reactive and 4 were not tested on the FDA licensed HIV-1 RNA assay.

Table 23 summary: A total of 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.

HIV-2 Endemic Region samples summary

- A total of 62.86% (308/490) of the samples from an HIV-2 endemic region were repeatedly reactive and 37.14% (182/490) were nonreactive when the overall HIV Ag-Ab test was ordered.
- Of the 308 repeatedly reactive specimens, 129 specimens out of the 190 specimens 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.
- A total of 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.
- Out of the 25 samples that were repeatedly undifferentiated with the BioPlex 2200 HIV Ag-Ab assay, 21 were undifferentiated by supplemental testing with the FDA approved HIV-1/HIV-2 differentiation test, 2 were HIV-1 Ab reactive, 1 was HIV-2 Ab reactive, and 1 was nonreactive.
- Out of the 224 samples identified as HIV-1 Ab positive by the supplemental FDA approved HIV-1/HIV-2 differentiation test, the BioPlex 2200 HIV Ag-Ab assay was repeatedly reactive for HIV-1 Ab in 99.11% (222/224) of the samples and reactive undifferentiated in the remaining 0.89% (2/224) samples.
- A total of 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.
- Out of the 47 samples repeatedly reactive for HIV-2 Ab with the BioPlex 2200 HIV Ag-Ab assay 43 samples were identified as HIV-2 Ab positive by the supplemental

FDA approved HIV-1/HIV-2 differentiation test Out of these 43 HIV-2 Ab reactive samples 97.67% (42/43) of the samples were identified by the BioPlex 2200 HIV Ag-Ab assay as HIV-2 Ab positive and reactive undifferentiated in the remaining 2.33% (1/43) of the samples.

- 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.

Pediatric Populations (2-21 years)

1. Low Risk Pediatric Populations

The reactivity rate of the BioPlex 2200 HIV Ag-Ab assay was determined using 100 samples from healthy pediatric subjects. Specimens reactive for HIV antibody were identified as HIV-1 or HIV-2, or were reported as undifferentiated. Initially reactive samples on the BioPlex 2200 HIV Ag-Ab assay were retested in duplicate and tested by the FDA licensed HIV-1 RNA assay and the FDA approved HIV-1 and HIV-2 differentiation test. The samples were also tested with the FDA approved HIV Ag/Ab assay.

Table 24: Healthy Pediatric Population overall HIV Ag-Ab test Summary (N = 100)

Age Range in Years	Gender	N	Non-Reactive	BioPlex 2200 HIV Reactive				FDA Approved HIV Ag/Ab Assay	
				HIV Ag-Ab Assay	HIV-1 Ab Assay	HIV-2 Ab Assay	HIV-1 p24 Ag Assay	Non-Reactive	Reactive
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**

Table 24 summary: Specificity of the BioPlex 2200 HIV Ag-Ab assay in the healthy pediatric population was 100% (100/100).

Healthy Pediatric Population Summary All results were nonreactive and the specificity of the BioPlex 2200 HIV Ag-Ab assay in the healthy pediatric population was 100% (100/100) with a 95% confidence interval of 96.30 - 100%.

2. High Risk Pediatric Population

A total of 573 samples from high risk pediatric subjects were evaluated with the BioPlex 2200 HIV Ag-Ab assay. The 573 samples included 89 samples from the general high risk population that were identified in the age range of 2-21 years. Specimens reactive for

HIV antibody were identified as HIV-1 or HIV-2, or were reported as undifferentiated. Initially reactive samples on the BioPlex 2200 HIV Ag-Ab assay were retested in duplicate and tested with the FDA licensed HIV-1 RNA assay and the FDA approved HIV-1/HIV-2 differentiation test. The samples were also tested with the FDA approved HIV Ag/Ab assay.

Table 25: High Risk Pediatric Population HIV Ag-Ab Test Summary (N = 573)

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.

Table 25 summary: In the high risk pediatric population 0.87% (5/573) samples were repeatedly reactive with the BioPlex 2200 HIV Ag-Ab assay and 99.13% (568/573) were non-reactive. Four (4) samples were repeatedly reactive with the FDA approved HIV Ag/Ab assay.

High Risk Pediatric Population Summary

- In the high risk pediatric population 0.87% (5/573) samples were repeatedly reactive with the BioPlex 2200 HIV Ag-Ab assay and 99.13% (568/573) were non-reactive.
- Four (4) of the 5 BioPlex 2200 HIV Ag-Ab repeatedly reactive samples 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 assay. One (1) sample was reactive on the licensed HIV-1 RNA assay.

3. Known HIV-1 Antibody Positive Samples from Pediatric Subjects

The reactivity rate of the BioPlex 2200 HIV Ag-Ab assay was determined for known HIV-1 antibody positive specimens from pediatric subjects (ranging in age from 2-21 years). A total of 50 HIV-1 antibody positive pediatric samples were tested with the

BioPlex 2200 HIV Ag-Ab assay. Specimens repeatedly reactive for HIV antibody were identified as HIV-1 or HIV-2, or were reported as undifferentiated. The samples were also tested with the FDA approved HIV Ag/Ab assay.

Table 26: Known HIV-1 Positive Pediatric Population Summary (N = 50)

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**

Table 26 summary: All 50 samples were repeatedly reactive with the BioPlex 2200 HIV Ag-Ab assay and with the FDA approved HIV Ag/Ab assay.

Known HIV-1 Positive Pediatric Population Summary

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

Pregnant Women Populations

Low Risk Healthy Pregnant Populations

The reactivity rate of the BioPlex 2200 HIV Ag-Ab assay was determined using 1000 samples from healthy pregnant subjects. Specimens reactive for HIV antibody were identified as HIV-1 or HIV-2, or were reported as undifferentiated.
Initially reactive samples were retested in duplicate, and additionally were tested by the HIV-1 RNA assay and the FDA approved HIV-1/HIV-2 differentiation test. The samples were also tested with the FDA approved HIV Ag/Ab assay.

Table 27: Healthy Pregnant Population Overall HIV Ag-Ab Test Summary (N = 1000)

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 confirmed 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.

Table 27 summary: Four (4) samples were initially and repeatedly reactive with the BioPlex 2200 HIV Ag-Ab assay and two (2) of these samples were repeatedly reactive with the FDA approved HIV Ag/Ab assay.

Healthy Pregnant Population Summary

- Four (4) samples were initially and repeatedly reactive with the BioPlex 2200 HIV Ag-Ab assay.
- Two (2) of these samples were repeatedly reactive with the FDA approved HIV Ag/Ab assay.
- One (1) sample, of the 2 samples that were repeatedly reactive with the BioPlex 2200 HIV Ag-Ab assay and the FDA approved HIV Ag/Ab assay, was confirmed positive for HIV-1 antibody by the FDA approved HIV-1/HIV-2 differentiation test (there was insufficient volume for testing these samples with the FDA licensed HIV-1 RNA assay). The one confirmed positive sample was removed from the specificity calculations. Therefore, the specificity of the BioPlex 2200 HIV Ag-Ab assay in the healthy pregnant population was 99.70% (996/999) with a 95% confidence interval of 99.12% - 99.90%.

High Risk Pregnant Women Populations

The reactivity of the BioPlex 2200 HIV Ag-Ab assay was evaluated using 383 samples from high risk pregnant subjects. Specimens reactive for HIV antibody were identified as HIV-1 or HIV-2, or were reported as undifferentiated.

Samples that were initially reactive on the BioPlex 2200 HIV Ag-Ab assay were retested in duplicate and tested with the FDA licensed HIV-1 RNA test and the FDA approved HIV-1/HIV-2 differentiation test. The samples were also tested with a FDA approved 4th generation HIV Ag/Ab assay.

Table 28: High Risk Pregnant Women Population HIV Ag-Ab Combo Test Summary (N = 383)

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.

Table 28 summary: In the high risk pregnant population 96.9% (371/383) of the samples were non-reactive with the BioPlex HIV Ag-Ab assay and 12 samples were reactive. Thirteen (13) samples were repeatedly reactive on an FDA approved 4th generation HIV Ag/Ab test.

High Risk Pregnant Women Population Summary

- In the high risk pregnant population 96.9% (371/383) of the samples were non-reactive with the BioPlex 2200 HIV Ag-Ab assay.
- Twelve (12) samples were initially and repeatedly reactive on the BioPlex 2200 HIV Ag-Ab assay and 13 samples were repeatedly reactive on an FDA approved 4th generation HIV Ag/Ab test.
- Of the 13 samples, 10 were positive for HIV-1 Ab and one (1) was positive for HIV-2 Ab on the FDA approved HIV-1/HIV-2 differentiation test.
- Ten (10) of the 13 samples were reactive on the FDA licensed HIV-1 RNA assay.

Known HIV-1 Antibody Positive Samples from Pregnant Women

A total of 60 known HIV-1 antibody positive samples from pregnant women were tested to determine the reactivity rate of the BioPlex 2200 HIV Ag-Ab assay. Specimens reactive for HIV antibody were identified as HIV-1 or HIV-2, or were reported as undifferentiated. Samples with initially reactive results on the BioPlex 2200 HIV Ag-Ab assay were retested in duplicate. The samples were also tested with the FDA approved HIV Ag/Ab assay.

Table 29: Known HIV-1 Positive Pregnant Women Population Test Summary (N = 60)

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

Table 29 summary: In pregnant women known to be positive for HIV-1 antibodies, 60/60 of the samples were repeatedly reactive with the BioPlex 2200 HIV Ag-Ab assay and the FDA approved 4th generation HIV Ag/Ab assay.

HIV-1 Positive Pregnant Women Population Summary
In pregnant women known to be positive for HIV-1 antibodies, 60/60 of the samples were repeatedly reactive with the BioPlex 2200 HIV Ag-Ab assay.

Repeat Blood Donors
In addition to the first time blood donors being tested in clinical studies of the BioPlex 2200 HIV Ag-Ab assay to determine specificity of the assay, 1340 repeat blood donors were also tested in support of use of the assay in urgent situations where traditional licensed blood donor screening tests are unavailable. The median subject age was 39 years with a range of 16-84 years. This population was 48.7% male (652/1340) and 51.3% (688/1340) female. Six hundred sixty-five (665) of the samples were plasma and 675 were serum.

Table 30: Reactivity in Repeat Blood Donors Summary (N = 1340)

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 Reactive	HIV-1/HIV-2 Differentiation Assay	
			IR	RR	IR	RR	IR	RR	IR	RR	IR	RR		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

Table 30 summary: Specificity in this population was 99.93% (1339/1340).

Reactivity in Repeat Blood Donors Summary

- A total of 99.93% (1339/1340) of the repeat blood donors were Bioplex 2200 HIV Ag-Ab non-reactive and 1/1340 (0.07%) was repeatedly reactive.

- The one repeatedly reactive specimen did not confirm positive on the FDA licensed HIV-1 RNA assay or reactive on the FDA approved HIV-1/HIV-2 differentiation test. All 1340 samples tested non-reactive with the FDA approved 4th generation HIV Ag/Ab assay.
- The final specificity of the BioPlex 2200 HIV Ag-Ab assay in the repeat blood donors was 99.93% (1339/1340) with a 95% CI of 99.58 – 99.99%.

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).

Table 31 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 FDA approved HIV-1/HIV-2 differentiation assay. Two (2) were undifferentiated, and the remaining 10 were identified as HIV-2 positive.

Table 31 summary: The BioPlex 2200 HIV-1 Ab assay identified 1363/1363 (100%) as HIV-1 Reactive and 188/200 (94 %) as HIV-2 Reactive

HIV-1 and HIV-2 Differentiation Summary

- **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: 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 %) as HIV-2 Reactive (95% CI: 89.80 - 96.54%). The remaining HIV-2 samples were identified as reactive, undifferentiated.

Studies in support of the Organ Donor Screening Claim

Studies were performed using blood specimens collected from normal living donors or from donors who were declared brain dead but whose heart was still beating. Specimens were not hemolyzed, lipemic, icteric, frozen after processing, or considered plasma diluted in accordance with 21 CFR § 1271.80(d). Prior to use in the studies, all normal living donor specimens were tested and found to be HIV Ab negative and HIV NAT negative. All specimens from brain dead individuals were found to be HIV Ab negative.

Interference: The performance of the BioPlex 2200 HIV Ag-Ab assay was assessed in the presence of high, low, and zero levels of the following potential interfering substances: Heparin, Dopamine, Norepinephrine, Triiodothyronine (T3), L-Thyroxine (T4), and Insulin. Blood specimens collected from normal living donors were spiked at low and medium positive index levels with serum known to be reactive on tests for HIV-1 Ab, HIV-2 Ab, HIV-1 group O Ab, or HIV-1 p24 Ag. Each serum pool (8 spiked serum pools + 1 negative pool) was tested against each interfering substance (6 interfering substances at 3 levels each) in replicates of 5 for a total of 810 data points ((8+1)*6*3*5). If the mean index value of spiked specimens is within 2SD of the mean of the control index, then no bias/interference is noted.

Interference results summary:

- Seven hundred and twenty (720) out of 720 spiked specimens were identified as reactive.
- Ninety (90) out of 90 unspiked specimens were identified as nonreactive.
- The mean index value of all spiked specimens was within 2SD of the mean of the control index (except as noted below), and therefore no interference is noted.
- The 95% CI upper limit of the low positive HIV-1 p24 Ag pool spiked with a high level (3000 U/L) of heparin slightly exceeded the specification. Since the mean index level was within the acceptance criteria, a 95% CI upper limit that is slightly above the specification does not indicate a significant risk of erroneous results due to heparin.

Specificity: A total of 100 specimens (50 normal living individuals and 50 from brain dead individuals) collected as K_2 EDTA plasma were each tested using three kit lots for a total of 300 data points ((50+50)*3=300). Clinical results: All 50 normal living specimens and all 50 brain dead specimens were non-reactive on the BioPlex 2200 HIV Ag-Ab test. The overall specificity for normal and brain dead specimens was 100.00% with a 95% CI of 92.8-100.0%.

Specificity results summary: 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% confidence interval 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: A total of 100 specimens (50 normal living individuals and 50 from brain dead individuals) were collected as K_2 EDTA plasma. For spiking, sera from 5 specimens positive for HIV-1 M Ab, HIV-1 O Ab, HIV-1 p24 Ag, or HIV-2 Ab were collected. Each positive serum pool was used to spike 10 normal living and 10 brain dead specimens at a level approximately 1-3 times the assay cutoff. Each specimen was then tested in single replicate using three kit lots.

Sensitivity results summary:
All 50 normal living specimens and all 50 brain dead specimens spiked with HIV-1 M Ab, HIV-1 p24 Ag, or HIV-2 Ab were reactive on the BioPlex 2200 HIV Ag-Ab test. 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.

Reproducibility: Specimens from 40 normal living donors and 40 brain dead individuals were spiked with either HIV-1 p24 Ag or a pool of HIV-1 Ab, HIV-2 Ab, and HIV-1 group O Ab. Each spiked specimen was tested on 3 kit lots on 6 different days for a total of 1440 data points ((20+20)*2*3*6=1440). All specimens were reactive on the BioPlex 2200 HIV Ag-Ab test across all 6 days and all 3 kit lots. However, variance of Index values for specimens from the two populations was not statistically different (p values were >0.05 for all assays and all lots) except for the following:

- HIV-1 p24 antigen spiked samples tested with Lot 120167: The p-value was 0.040, with the brain dead population having a lower CV of 7.1% vs. 8.4% for the living donors population;
- HIV-2 antibody spiked samples tested with Lot 120828: The p-value was 0.021, with the brain dead population having a slightly higher CV of 9.5% vs. 7.7% for the living donors; and
- HIV-1 group O antibody spiked samples: The p-value was 0.046, with the brain dead population having a lower CV of 12.1% vs. 14.9% for the normal population.

Reproducibility Results summary: Variance of Index values for specimens from the two populations were comparable and did not appear to have a clinically significant impact on the overall reactive status of the specimens analyzed.

XII. Inspections

Manufacturing Facilities Review/Inspection
Bio-Rad Laboratories will manufacture the BioPlex 2200 HIV Ag-Ab assay in the currently licensed Bio-Rad Laboratories facilities at [redacted] and the Bio-Rad Laboratories facility at [redacted]. The review of facility and equipment-related issues conducted by the Division of Manufacturing and Product Quality/Office of Compliance and Biologics Quality did not identify any major concerns. A pre-approval inspection of the Bio-Rad manufacturing facilities was not required because of the following:

- The final IVD device manufacturing facilities at [redacted] were inspected by the Biologics Core Team in [redacted]; the inspection was classified as Voluntary Action Indicated (VAI).

- An inspection of the Bio-Rad Laboratories facility at [redacted] responsible for the design controls of the subject IVD assay was conducted by SAN-DO in (b) (4) and was classified as Voluntary Action Indicated (VAI).

Bioresearch Monitoring (BIMO) Inspections
CBER Bioresearch Monitoring (BIMO) issued high-priority inspection assignments at two of four testing sites in the United States. These inspections did not reveal significant problems that impact the data submitted in this PMA. The inspections were classified as No Action Indicated (NAI).

XIII. Conclusions Drawn from the Studies

Risk/Benefit Analysis
As a diagnostic test, the BioPlex 2200 HIV Ag-Ab assay involves removal of blood from an individual for testing purposes. This blood draw represents no greater safety hazard than a blood draw for any other routine in vitro diagnostic evaluation.

The potential risks encountered with in vitro diagnostic tests are not unusual in the clinical laboratory setting. Risks to patients typically relate to false positive and false negative results, which can lead to inappropriate treatment and patient anxiety, or a delay in diagnosis and therapy, respectively. Appropriate warnings for these risks are contained in the labeling and package insert for the BioPlex 2200 HIV Ag-Ab assay.

Standard good laboratory practices are considered sufficient to mitigate the risks to the end user.

Safety and Effectiveness
- The performance of the BioPlex 2200 HIV Ag-Ab was evaluated in a multi-centered clinical study conducted in the U.S., and results were compared to HIV antibody and antigen assays that are currently FDA licensed or approved.
- The effectiveness of the BioPlex 2200 HIV Ag-Ab has been shown in clinical and pre-clinical studies performed with serum and plasma samples.
- Based on these results, the assay has been shown to be effective in identifying individuals with HIV-1 and HIV-2 infection, including pediatric subjects as young as 2 years of age, and those individuals with signs or symptoms of AIDS. The assay has also been shown effective for screening organ donors when specimens are obtained while the donor's heart is still beating,
- The BioPlex 2200 HIV Ag-Ab assay has demonstrated acceptable performance for all specimen types and populations evaluated to establish the safety and effectiveness of the device. The benefit to individuals tested by this assay outweighs potential adverse events or risks to the patient or user due to assay malfunction or operator error.