

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY  
ASSAY ONLY TEMPLATE**

**A. 510(k) Number:**

k141114

**B. Purpose for Submission:**

New Device

**C. Measurand:**

25-hydroxyvitamin D [25(OH) Vitamin D]

**D. Type of Test:**

Quantitative multiplexed flow immunoassay

**E. Applicant:**

Bio-Rad Laboratories

**F. Proprietary and Established Names:**

BioPlex® 2200 25-OH Vitamin D Kit

BioPlex® 2200 25-OH Vitamin D Calibrator Set

BioPlex® 2200 25-OH Vitamin D Control Set

**G. Regulatory Information:**

Product Code	Classification	Regulation Section	Panel
MRG	II	862.1825 Vitamin D Test System	Chemistry (75)
JIT	II	862.1150 Calibrator	Chemistry (75)
JJX	I, Reserved	862.1660 Quality Control Material (Assayed and Unassayed)	Chemistry (75)

## H. Intended Use:

1. Intended use(s):

See indication for use below.

2. Indication(s) for use:

The BioPlex 2200 25-OH Vitamin D kit is a flow competitive immunoassay intended for the quantitative determination of 25-hydroxyvitamin D in human serum. The BioPlex 2200 25-OH vitamin D assay is to be used to aid in the assessment of vitamin D sufficiency. The BioPlex 2200 25-OH Vitamin D kit is intended for use with the BioPlex 2200 System.

The BioPlex 2200 25-OH Vitamin D Calibrator Set is intended for the calibration of the BioPlex 2200 25-OH Vitamin D reagent Pack.

The BioPlex 2200 25-OH Vitamin D Control Set is intended for use as an assayed quality control to monitor the overall performance of the BioPlex 2200 system and corresponding BioPlex® 25-OH Vitamin D reagent pack in the clinical laboratory. The performance of the BioPlex 2200 25-OH Vitamin D Control Set has not been established with any other 25-hydroxyvitamin D assays.

3. Special conditions for use statement(s):

For *in vitro* diagnostics

For prescription use only

4. Special instrument requirements:

BioPlex 2200 system

## I. Device Description:

The BioPlex 2200 25-OH Vitamin D Kit consists of the following:

1. One 10mL vial of Bead Set containing dyed beads coated with anti-25-OH D antibody (sheep), an Internal Standard bead (ISB), and a Serum Verification bead (SVB) in buffer with protein stabilizers (bovine). ProClin 950 (<1.0%) and sodium azide (<0.1%) as preservatives.
2. One 10mL vial of Release Buffer containing 25-OH Vitamin D releasing reagents in citrate and trisodium citrate acid buffer at pH 4.1 and ProClin 950 (<1.0%) as preservative.
3. One 5mL vial of Conjugate 1 containing biotinylated 25-OH Vitamin D conjugate and biotinylated anti-human FXIII antibody conjugate (murine) in buffer with protein stabilizer (bovine). ProClin 950 (<1.0%) and 5-bromo-5-nitro-1, 3-dioxane (<0.1%) as preservatives

and chemical blockers.

4. One 5mL vial of Conjugate 2 containing phycoerythrin conjugated streptavidin (SA-PE) in buffer comprising protein stabilizers (bovine). ProClin 950 (<1.0%) and sodium azide (<0.1%) as preservatives, chemical blockers and detergent (Tween 20).

BioPlex 2200 25-OH Vitamin D Calibrator set (sold separately) contains six 0.5 mL 25-OH Vitamin D vials. Calibrator level 1 contains 25% horse serum without 25-OH Vitamin D. The calibrator levels 2 to 6 are provided in a Vitamin D depleted human serum matrix supplemented with known concentration of 25-hydroxyvitamin D<sub>3</sub>. All calibrators contain ProClin 950 (≤0.3%), sodium benzoate (≤0.1%) and 5-bromo-5-nitro-1, 3-dioxane (≤0.1%) as preservatives.

Calibrator Set	Target (ng/mL)
Level 1	0.0
Level 2	10.0
Level 3	30.0
Level 4	75.0
Level 5	110.0
Level 6	165.0

BioPlex 2200 25-OH control set (sold separately) contains two 1.5 mL of Level 1 and two 1.5 mL of Level 2 control vials. Each vial contains 25-OH Vitamin D in human serum matrix. All controls contain ProClin 950 (≤0.3%), sodium benzoate (≤0.1%) and 5-bromo-5-nitro-1, 3-dioxane (≤0.1%) as preservatives.

Control Set	Target (ng/mL)	Range (ng/mL)
Level 1	19.0	14.5 – 23.5
Level 2	55.0	45.0 – 65.0

Calibrator and Control contain human source material. Each donor unit of serum in the preparation of these materials were tested and found negative for the Human Immunodeficiency Virus Antibody (HIV I/II Ab), Hepatitis B Surface Antigen (HBsAg), and Hepatitis C Virus Antibody (HCV).

#### **J. Substantial Equivalence Information:**

1. Predicate device name(s):  
EUROIMMUN 25-OH Vitamin D ELISA
2. Predicate 510(k) number(s):  
k123660

3. Comparison with predicate:

Assay:

<b>Similarities / Differences</b>		
<b>Item</b>	<b>BioPlex® 2200 25-OH Vitamin D Kit Candidate Device</b>	<b>EUROIMMUN 25-OH Vitamin D ELISA Predicate Device (k123660)</b>
Intended Use	For the quantitative determination of 25-hydroxyvitamin D in human serum.	Same
Assay Type	Quantitative	Same
Test Principle	Competitive immunoassay	Same
Antibody	Monoclonal Sheep antibody against 25 OH Vitamin D	Same
Signal Detection	Fluorescence	Same
Unit of Measure	ng/mL	Same
Assay Technology	Automated multiplex flow competitive immunoassay	Manually competitive immunoassay
Conjugate	Biotinylated 25-hydroxyvitamin D and phycoerythrin streptavidin	Biotinylated 25-hydroxyvitamin D and Peroxidase-labeled streptavidin and substrate TMB
Solid Phase	Antibody-coated paramagnetic microbeads	Antibody coated 96 microwell ELISA plate
Measuring Range	6.5 ng/mL – 125.0 ng/mL	4.0 ng/mL – 120 ng/mL
Sample Matrix	Serum	Serum or EDTA or Lithium heparin plasma
Sample Size	10µL	20µL
Open Pack Stability	60 days	Not Applicable
Reagent Storage	On-board or in refrigerator at 2-8°C	Not Applicable
Sample Handling	Automated	Manually
Instrumentation	Bio-Rad BioPlex® 2200 System	ELISA plate reader
Measuring Wavelength	550-610 nm	450/620 nm

Calibrator:

<b>Similarities / Differences</b>		
<b>Item</b>	<b>BioPlex® 2200 25-OH Vitamin D Calibrator Candidate Device</b>	<b>EUROIMMUN 25-OH Vitamin D ELISA Calibrator Predicate Device</b>
Intended Use	For the calibration of the Vitamin D reagent pack.	Same
Calibrator(s) and Calibration	6 calibrator levels (sold separately); 4-PL (parameter logistic) curve fit algorithm	Same
Calibrator Matrix	25% horse serum and depleted human serum with ProClin 950, sodium benzoate and BND	Liquid in horse serum with preservatives
Calibrator Open Storage at 2-8°C	30 days	3 months
Calibration Frequency	Every 30 days	Every 96 well plate

Controls:

<b>Similarities / Differences</b>		
<b>Item</b>	<b>BioPlex® 2200 25-OH Vitamin D Control Candidate Device</b>	<b>EUROIMMUN 25-OH Vitamin D ELISA Controls Predicate Device</b>
Intended Use	Use as an assayed quality control to monitor the overall performance of the Vitamin D reagent.	Same
Storage	Store at 2-8°C until ready to use	Same
Matrix	Human serum with ProClin 950, Sodium benzoate and BND	Liquid in horse serum with preservatives

**K. Standard/Guidance Document Referenced (if applicable):**

*CLSI EP05-A2*: Evaluation of Precision Performance of Quantitative Measurement Methods

*CLSI EP6-A*: Evaluation of Linearity of Quantitative Measurement Procedures

*CLSI EP07-A2*: Interference Testing in Clinical Chemistry

*CLSI EP09-A2IR*: Method comparison and Bias Estimation

*CLSI EP15-A2*: User Verification of Performance for Precision and Trueness

*CLSI EP17-A2*: Evaluation of Detection Capability for Clinical laboratory measurements

## Procedure

*CLSI EP25-A: Evaluation of Stability of In-vitro diagnostic Reagents*

*CLSI C28-A3c: Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory*

### **L. Test Principle:**

The BioPlex 2200 25-OH Vitamin D assay is a multiplex flow competitive immunoassay for the quantitative determination of 25-hydroxyvitamin D in human serum.

The BioPlex 2200 System combines an aliquot of patient sample with the Vitamin D Release Buffer to dissociate the 25-hydroxyvitamin D from its binding protein. After the first Incubation, the bead reagent is added to the reaction vessel and incubated at 37°C. After the second incubation, the BioPlex® 2200 System adds the vitamin D-biotin conjugate 1. The excess conjugate 1 is removed during a wash cycle and the streptavidinphycoerythrin (SA-PE) conjugate 2 is added. The excess conjugate 2 is removed during a wash cycle and the beads are re-suspended in wash buffer. The bead mixture then passes through the detector. The identity of the dyed beads is determined by the fluorescence of the dyes and the amount of 25-hydroxyvitamin D captured is inversely proportional to the fluorescence of the attached SA-PE. Raw data is calculated in relative fluorescence intensity (RFI). Two additional dyed beads, Internal Standard Bead (ISB) and Serum Verification Bead (SVB) is present in each reaction mixture to verify detector response and the addition of serum to the reaction vessel, respectively.

The BioPlex® 25-OH Vitamin D assay is calibrated using a set of 6 distinct calibrators supplied separately by Bio-Rad Laboratories. Results are calculated from a 4-PL (parameter logistic) calibration curve in which an inverse relationship exists between the amounts of 25-hydroxyvitamin D in the patient sample and the amount of RFI detected by the system. The results are expressed in ng/mL.

### **M. Performance Characteristics (if/when applicable):**

#### 1. Analytical performance:

All performance testing were conducted on the Bio-Rad BioPlex 2200 System

##### *a. Precision/Reproducibility:*

The precision of Bio-Rad BioPlex 2200 25-OH Vitamin D assay was evaluated according to CLSI EP5-A2 guideline. Serum samples with low, medium, and high levels (6 total) of 25-OH Vitamin D and two levels of serum controls were assayed in duplicate per run with two runs per day for twenty days (N=80) on one reagent lot.

Sample	N	Mean (ng/mL)	Within-Run		Between Run		Between Day		Total Precision	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	80	15.0	1.19	7.9	0.69	4.60	0.77	5.2	1.58	10.5
2	80	17.2	1.02	5.9	0.86	5.00	1.19	6.9	1.78	10.4
3	80	36.1	1.61	4.5	1.30	3.60	1.56	4.3	2.59	7.2
4	80	47.2	2.20	4.7	1.24	2.60	1.85	3.9	3.13	6.6
5	80	77.9	1.99	2.6	1.42	1.80	1.98	2.5	3.15	4.0
6	80	110.8	3.60	3.2	3.00	2.7	2.62	2.4	5.37	4.8
Control 1	80	22.1	1.30	5.9	0.87	3.9	1.04	4.7	1.88	8.5
Control 2	80	50.0	2.38	4.8	1.18	2.4	1.52	3.0	3.07	6.1

*b. Linearity/assay reportable range:*

Linearity samples were prepared by diluting natural and spiked highly concentrated patient serum samples with a low-level human serum sample (<6.5 ng/mL). Each sample and dilution was evaluated in replicates of four on a single analyzer using one lot of reagent. Linearity was evaluated by calculating a linear regression comparing observed values versus expected values based on the CLSI EP6-A guideline. The regression parameters (slope, intercept, and  $r^2$ ) of the observed values vs. expected values are shown below:

Slope	Intercept	$r^2$	Sample range tested
1.0001	0.0045	0.9988	5.5-168.9

Based on the results of the linearity study the sponsor claimed that the candidate assay is linear from 6.5 to 125 ng/mL

*c. Traceability, Stability, Expected values (controls, calibrators, or methods):*

Traceability:

The BioPlex 25-OH Vitamin D Calibrators are traceable to internal standards, which are determined by UV spectrophotometric analysis using the extinction coefficient of 18,000 mL/mmol/cm at 264 nm wavelength.

The six levels of Master Calibrators are manufactured volumetrically from the internal standards into the depleted human serum except level 1 (25-OH hydroxyvitamin D free) in 25% horse serum. The Master Calibrators are immediately frozen at -70°C.

Value Assignment:

The BioPlex® 25-OH Vitamin D kit calibrators are manufactured for each lot kit with the

same matrix as the Master Calibrators.

The BioPlex 2200 25-OH Vitamin D calibrator value assignments is established for the BioPlex 2200 25-OH Vitamin D kit using Master Calibrators as reference. For each calibrator level except level 1, three vials are tested in replicates of five on three BioPlex analyzers for a total of 45 points. Target values of the calibrators are listed in the table below.

Calibrator Set	Target value (ng/mL)
Level 1	0.0
Level 2	10.0
Level 3	30.0
Level 4	75.0
Level 5	110.0
Level 6	165.0

Controls:

The two levels of BioPlex 2200 25-OH Vitamin D control set are prepared from a pool of native serum specimen. For each control level, three vials are tested in replicates of five using each of the kit lots on three BioPlex 2200 analyzers for a total of 45 replicates per reagent lot. Target values and QC ranges of the controls are listed in the table below.

Control Set	Target value (ng/mL)	QC Range (ng/mL)
Level 1	19.0	14.5 – 23.5
Level 2	55.0	45.0 – 65.0

Stability:

Shelf life stability studies: Real-time stability studies were performed for the BioPlex 2200 25-OH Vitamin D kit. The stability study protocol and the acceptance criteria have been reviewed and found acceptable. The real time ongoing kit stability study supports a stability of 9 months or until expiration date when stored unopened on the instrument or at 2-8C.

Shelf-life stability for calibrators and controls: The stability study used elevated storage temperatures to model potential real time stability under normal conditions (2-8°C). Accelerated stability studies protocol and acceptance criteria have been reviewed and found acceptable. The accelerated stability model estimates that the BioPlex® 2200 25-OH Vitamin D Calibrator and controls are stable for more than two years at 2-8°C. Real-time stability study is on-going. Real-time stability studies protocol and acceptance criteria have been reviewed and found acceptable.

Open vial stability: The open vial stability studies were performed to assess the stability

over time with BioPlex® 2200 25-OH calibrator and control materials stored at 2-8°C. The stability study protocol and the acceptance criteria have been reviewed and found acceptable. The study supports an open vial stability of 30 days for the BioPlex 2200 25-OH calibrator and supports an open vial stability of 60 days for BioPlex 2200 25-OH controls.

*d. Detection limit:*

The limit of blank (LoB), limit of detection (LoD), and the limit of quantitation (LoQ) were determined following CLSI Document EP17-A2 guideline. The LoD is defined as the lowest concentration of 25-OH Vitamin D that can be detected with 95% probability. LoB was performed using two BioPlex 25-OH Vitamin reagent lots on one instrument in 4 replicates per day for 5 days yielding 100 data points per reagent lot. LoD was performed with six serum samples with mean measured concentration ranging from 3.89 to 34.29 ng/mL. The samples were tested with two BioPlex 25-OH Vitamin D reagent lots on one instrument in 12 replicates per day for five days yielding 60 data points per sample per reagent lot. LoQ: The precision was calculated with the same six low level samples used for the LoD. The mean, standard deviation, and coefficient of variation for each sample were calculated. LoQ was defined as precision  $\leq$  20% CV.

The LoB, LoD, and LoQ are summarized below:

LoB	LoD	LoQ
0.8 ng/mL	2.5 ng/mL	6.5 ng/mL

The sponsor claimed that the candidate assay has a measuring range from 6.5 to 125 ng/mL.

*e. Analytical specificity:*

Interference study:

To measure the effects of endogenous serum components and exogenous molecules on the 25-OH Vitamin D assay. Three sample pools were prepared to achieve a low (10-20 ng/mL), medium (30-50 ng/mL), and high (70-90 ng/mL). The samples were spiked with the interfering substances. The tests and controls were evaluated for a total of ten replicates per interferent using BioPlex 2200 25-OH Vitamin D reagent. Substances are considered interfering if their presence in a sample results in more than  $\pm$  10% deviation in quantitation relative to the value determined in the absence of the substance. The protocol and calculations were based on CLSI EP7-A2 guideline. The substances and the maximum levels tested are shown in the table below:

Substances	Highest Concentration of substance tested which demonstrated no significant interference.
Hemoglobin	150 mg/dL
Bilirubin (conjugated)	20 mg/dL
Bilirubin (unconjugated)	30 mg/dL
Triglycerides	400 mg/dL
Total Protein	12 g/dL
Cholesterol	500 mg/dL
Uric Acid	20 mg/dL
HAMA	100 ng/mL
Rheumatoid Factor	350 IU/mL
Ascorbic Acid	3 mg/dL

The sponsor has the following limitations in their labeling:

“Hemoglobin > 150 mg/dL may interfere and cause increased Vitamin D results. Do not use visibly hemolyzed samples.”

Cross-Reactivity:

The study was conducted using 2 serum pools at 25-hydroxyvitamin D concentrations of 20 ng/mL and 35 ng/mL. Nine cross reactants at levels listed below were spiked into the serum pools. The spiked and non-spiked samples were then evaluated in replicates of five to calculate the cross reactivity as shown below:

% cross reactivity = [(mean recovery of test samples in ng/mL) – (mean recovery of control sample in ng/mL) / (concentration of cross reactant in ng/mL)] \* 100

Cross Reactant	Spiked Concentration (ng/mL)	% Cross Reactivity
25-hydroxyvitamin D2	30	103%
25-hydroxyvitamin D3	30	97%
Vitamin D2	1000	0.2%
Vitamin D3	1000	0.0%
1,25-dihydroxyvitamin D2	30	>100%
1,25-dihydroxyvitamin D3	30	79%
3-epi 25-hydroxyvitamin D3	30	59%
24,25-dihydroxyvitamin D3	20	9%
Paricalcitol (Zemplar)	24	>100%

The sponsor has the following limitations in their labeling:

“Paricalcitol (Zemplar) has been found to cross-react and interfere with the BioPlex® 2200 25- OH Vitamin D assay.”

f. Assay cut-off:

Not Applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

A method comparison study using 204 human serum samples was performed to compare the candidate device BioPlex 2200 25-OH Vitamin D to the predicate device EUROIMMUN 25-OH Vitamin D ELISA. 185 samples were unaltered and 19 samples were spiked with 25-hydroxyvitamin D<sub>3</sub>. A total of 196 human serum samples with 25-OH Vitamin D values ranging from 6.6 ng/mL to 124.9 ng/mL were analyzed. There were eight samples with values lower or higher than the measuring range of the predicate method that were excluded in the data analysis. The samples were assayed in singlicate using one reagent lot of the candidate and predicate device. Deming regression was used for the regression analysis and the results are summarized below:

N	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient (r) (95% CI)	Sample Range Tested (ng/mL)
196	1.0039 (0.9365 to 1.0712)	-0.2256 (-2.4121 to 1.9608)	0.9553 (0.9412 to 0.9661)	BioPlex: 6.6 – 124.9

b. *Matrix comparison:*

Not Applicable, only serum is recommended

3. Clinical studies:

a. *Clinical Sensitivity:*

Not Applicable

b. *Clinical specificity:*

Not Applicable

c. Other clinical supportive data (when a. and b. are not applicable):

Not Applicable

4. Clinical cut-off:

Not Applicable

5. Expected values/Reference range:

The study was performed in accordance with CLSI C28-A3c guideline. Two hundred and eighty-seven samples from apparently healthy donors including 160 males and 127 female were collected from three regions (North, Central, and South) in the US and in three seasons (spring, summer and winter), including Caucasians and African American subjects. The 287 samples from apparently healthy donors met the following inclusion / exclusion criteria as follows and tested with the BioPlex 25-OH Vitamin D kit in singlicate. \*One sample <6.5 ng/ml was excluded from the data analysis.

- Age from 21 to 90
- 50% female and 50% male
- 20% from Northern, 20% from Central, and 60% from Southern region
- 40% collected in Summer and 60% in Winter
- At least 30% dark and 30% light skin
- 90% not taking Vitamin D supplements and <30% of those taking Vitamin D supplements at or more than 1000 IU, but less than 2000 IU
- Normal TSH, PTH, and Total Calcium
- No family history of parathyroid or calcium regulatory disease. In addition, no personal history of kidney disease, GI disease, and no bariatric surgery

The observed median, mean, and ranges between 2.5th to 97.5th percentile are summarized below:

N	Mean	Median	2.5 <sup>th</sup> to 97.5 <sup>th</sup> Percentile
286	29.7 ng/mL	27.7 ng/mL	12.7 – 65.7 ng/mL

Each laboratory should establish its own reference range pertinent to their specific populations.

**N. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**O. Conclusion:**

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.