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

A. 510(k) Number:

k180577

B. Purpose for Submission:

Modification of traceability for standardization of a previously cleared device (k141114)

C. Measurand:

Total 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

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
MRG	Class II	862.1825 Vitamin D Test System	Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:

   The BioPlex 2200 25-OH Vitamin D Kit is a multiplex 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 as an aid in the assessment of vitamin D sufficiency.

   The BioPlex 2200 25-OH Vitamin D kit is intended for use with the Bio-Rad BioPlex 2200 System.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   For use with the Bio-Rad BioPlex 2200 System

I. Device Description:

The BioPlex 2200 25-OH Vitamin D kit consist of the following reagents:

- One 10 mL vial, containing dyed beads coated with anti-25-OH Vitamin D (sheep), an internal standard bead (ISB), a serum verification bead (SVB) in buffer with protein stabilizers (bovine). ProClin 950 (< 1.0%) and sodium azide (< 0.1%) as preservatives.
- One 10 mL 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.
- One 5 mL vial of conjugate 1, containing biotinylated 25-OH Vitamin D conjugate and biotinylated anti-human FXIII antibody conjugate (murine) in buffer with protein stabilizers (bovine). ProClin 950 (< 1.0%) and 5-bromo-5-nitro-1,3-dioxane (< 0.1%) as preservatives and chemical blockers.
- One 5 mL vial conjugate 2 containing phycoerythrin conjugated streptavidin (SA-PE) in a buffer comprising protein stabilizer (bovine). ProClin 950 (< 1.0%) and sodium azide (< 0.1%) as preservatives, chemical blockers and detergent (Tween20).

J. Substantial Equivalence Information:

1. Predicate device name(s):

   BioPlex 2200 25-OH Vitamin D Kit

2. Predicate 510(k) number(s):

   k141114

3. Comparison with predicate:

Similarities		
Item	Candidate Device BioPlex 2200 25-OH Vitamin D Kit k180577	Predicate device BioPlex 2200 25-OH Vitamin D Kit k141114
Intended Use	For the quantitative determination of 25-hydroxyvitamin D	Same
Sample Type	Serum	Same
Antibody	Sheep antibody against 25-OH Vitamin D	Same
Solid Phase	Antibody-coated paramagnetic microbeads	Same
Conjugate	Biotinylated 25-hydroxyvitamin D and phycoerythrin conjugated streptavidin	Same
Reagent Storage	On-board or in refrigerator at 2-8ºC	Same
Instrumentation	Bio-Rad BioPlex 2200 System	Same
Open pack stability	60 days	Same

Differences		
Item	Candidate Device BioPlex 2200 25-OH Vitamin D Kit k180577	Predicate device BioPlex 2200 25-OH Vitamin D Kit k141114
Measuring range	7.0 – 160.0 ng/mL	6.5 – 125.0 ng/mL
Traceability/ Standardization	Internal standards which are traceable to CDC and Ghent University's ID-LC-MS/MS 25 OH vitamin D Reference Method Procedure (RMP) that is traceable to the National Institute of Standards and Technology NIST SRM 2972	Internal standard (stock) using UV absorbance spectrometry

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

CLSI EP05-A3: Evaluation of Precision Performance of Quantitative Measurement Procedures

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

CLSI EP07-A2: Interference Testing in Clinical Chemistry

CLSI EP15-A3: User Verification of Performance for Precision and Estimation of Bias

CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurements Procedure

CLSI EP28-A3c: Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition

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.

A population of dyed paramagnetic beads is coated with anti-25-OH Vitamin D antibody. The BioPlex 2200 System combines an aliquot of patient sample with the Vitamin D release buffer to dissociate the 25-OH Vitamin 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 25-OH Vitamin D-Biotin conjugate 1. The excess conjugate 1 is removed during a wash cycle and the streptavidin-phycoerythrin (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 detected fluorescence of the SA-PE in relative fluorescence intensity (RFI) is inversely proportional to the concentration of 25-OH Vitamin D in the sample. Two additional dyed beads, an internal standard bead (ISB) and a serum verification bead (SVB), are present in each reaction mixture to verify detector response and the addition of serum to the reaction vessel.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

   The precision of the BioPlex 2200 25-OH Vitamin D assay was evaluated according to CLSI EP05-A3 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 2 runs per day for 20 days (N=80) on one reagent lot.

The results are summarized below.

Sample	n	Mean (ng/mL)	Within-Run		Between Run		Between Day		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	80	16.4	1.02	6.2	1.19	7.2	0.45	2.7	1.62	9.9
2	80	18.9	1.36	7.2	0.76	4.0	0.73	3.9	1.72	9.1
3	80	37.2	1.66	4.4	1.61	4.3	0.00	0.0	2.31	6.2
4	80	46.5	3.66	7.9	3.07	6.6	1.35	2.9	4.96	10.7
5	80	81.4	2.79	3.4	3.42	4.2	0.00	0.0	4.41	5.4
6	80	100.4	3.89	3.9	5.34	5.3	0.00	0.0	6.60	6.6
Control 1	80	21.5	0.88	4.1	1.75	8.1	0.00	0.0	1.96	9.1
Control 2	80	54.1	1.62	3.0	2.45	4.5	0.00	0.0	2.94	5.4

A lot-to-lot precision study was conducted by testing 6 serum samples using 2 different reagent sets. Samples were assayed in replicates of 3 per run with 2 runs per day for 5 days on three instruments with two reagent sets. The results are shown below:

Mean (ng/mL)	Within run		Between run		Between day		Between lot		Total	
	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
18.7	1.195	6.4	0.199	1.1	0.753	4.0	1.146	6.1	2.133	11.4
21.2	1.097	5.2	0.517	2.4	0.513	2.4	1.198	5.6	2.453	11.5
44.6	1.846	4.1	0.773	1.7	1.547	3.5	1.600	3.6	3.812	8.5
57.2	3.390	5.9	2.199	3.8	0.00	0.0	1.541	2.7	5.097	8.9
95.2	3.1478	3.3	1.077	1.1	2.011	2.1	10.586	11.1	11.277	11.8
125.9	3.880	3.1	1.405	1.1	2.904	2.3	3.759	3.0	7.907	6.3

An additional precision study was conducted by testing 8 serum samples and 2 levels of control in duplicate on 2 runs per day for 5 days using 1 lot of 25-OH Vitamin D Reagent Pack at 1 testing facility (for a total of 20 replicates per sample). The results are summarized below:

Sample	Mean (ng/mL)	Within-Run		Between-Run		Between-Day		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 1	15.9	0.96	6.0	0.72	4.5	0.00	0.0	1.20	7.6
Sample 2	17.3	1.22	7.0	0.90	5.2	0.00	0.0	1.52	8.8
Sample 3	23.7	2.22	9.4	0.00	0.0	0.84	3.6	2.37	10.0
Sample 4	25.5	1.49	5.8	1.29	5.1	0.00	0.0	1.97	7.7
Sample 5	52.4	1.86	3.5	1.82	3.5	1.74	3.3	3.13	6.0
Sample 6	56.5	3.58	3.7	0.00	0.0	1.88	3.3	4.04	7.1
Sample 7	88.9	3.66	4.1	0.00	0.0	1.64	1.8	4.01	4.5
Sample 8	94.9	4.27	4.5	0.00	0.0	0.57	0.6	4.31	4.5
QC L1	23.4	1.28	5.5	1.06	4.5	0.00	0.0	1.67	7.1
QC L2	67.9	2.04	3.0	0.00	0.0	2.46	3.6	3.19	4.7

b. *Linearity/assay reportable range:*

A linearity study was conducted according to CLSI EP06-A guideline. A high 25-OH Vitamin D level sample was diluted with a low level sample of 25-OH Vitamin D to create 11 level samples with approximate concentrations of 5, 15, 32, 50, 70, 92, 108, 127, 142, 158, 175 ng/mL. Samples were evaluated in replicates of four on a single analyzer using BioPlex 2200 25-OH Vitamin D kit. The mean value of the observed results were plotted against the expected values. The results of the regression analysis are shown below:

Slope	Intercept	r^2	Sample range tested
1.0002	-0.0143	0.9956	5.4-166.5 ng/mL

The results of the linearity study support the sponsor claimed that the candidate assay is linear from 7 to 160 ng/mL.

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

Traceability:

The BioPlex 2200 25-OH Vitamin D Assay was originally cleared under 510(k) k141114. Bio-Rad has modified the assay by standardizing the cleared vitamin D assay in accordance with the Vitamin D Standardization Program (VDSP). The VDSP is an international collaborative effort to standardize the laboratory measurement of serum 25-OH vitamin D. Please refer to http://ods.od.nih.gov/Research/VitaminD.aspx for more information on the VDSP program.

The BioPlex 2200 25-OH Vitamin D assay is standardized (calibrated) using internal standards which are traceable to CDC and Ghent University's ID-LC/ MS/MS 25(OH) vitamin D Reference Method Procedure (RMP) that is traceable to the National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 2972.

d. *Detection limit:*

The limit of blank (LoB), limit of detection (LoD), and the limit of quantitation (LoQ) were determined following CLSI EP17-A2 guideline.

LoB: LoB was performed by testing 5 blank samples using 2 BioPlex 25-OH Vitamin reagent lots on 1 instrument in 4 replicates per day for 5 days yielding 100 data points per reagent lot. LoB was calculated using a non-parametric statistical analysis at 95th percentile.

LoD: Six low level natural human serum samples were tested with two BioPlex 25-OH Vitamin D reagent lots on one instrument in 10 replicates per day for five days yielding 50 data points per sample per reagent lot. The LoD was determined to be using the following equation: $LoD=LoB + C_p \text{ X } SD_{LoD}$.

LoQ: The LoQ was determined using 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 the lowest value with a precision ≤ 20% CV.

The results of the LoB, LoD, and LoQ are summarized below:

LoB ng/mL	LoD ng/mL	LoQ ng/mL
5.4	7.0	7.0

The sponsor claimed that the candidate assay has a measuring range from 7.0 to 160 ng/mL.

e. Analytical specificity:

Interference studies were conducted to measure the effects of endogenous serum components and exogenous molecules on the 25-OH Vitamin D assay, following CLSI EP07-A2 guideline.

Two sample pools were prepared to achieve a low (10-20 ng/mL) and medium (30-50 ng/mL) concentration of 25-OH Vitamin D. The samples were spiked with the interfering substances.

Biotin interference was tested in 3 samples with low (20-25 ng/mL), medium (50-60 ng/mL) and high (110-130 ng/mL) Vitamin D concentrations. Samples were spiked with 3 biotin concentrations of 1200, 2000, 3600 ng/mL. No significant interference was observed up to 3600 ng/mL biotin level.

Substances are considered interfering if their presence in a sample results in more than ± 10% deviation in quantitation relative to the value determined in the absence of the substance. The substances and the highest 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)	30 mg/dL
Bilirubin (unconjugated)	20 mg/dL
Triglycerides	350 mg/dL
Total protein	12 g/dL
Cholesterol	450 mg/dL
Uric acid	20 mg/dL

Substances	Highest concentration of substance tested which demonstrated no significant interference
HAMA	100 ng/mL
Rheumatoid factor	350 IU/mL
Ascorbic acid	3 mg/dL
Biotin	3600 ng/mL
Lithium heparin	8000 units/dL
Sodium heparin	8000 units/dL
EDTA	800 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 31.5 ng/mL and 43.2 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 using 2 reagent sets to calculate the cross reactivity using the following formula:

% 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

The results of the cross-reactivity study are shown below:

Cross Reactant	Spiked Concentration (ng/mL)	% Cross Reactivity
25-hydroxyvitamin D2	30	93.2 %
25-hydroxyvitamin D3	30	90.8 %
Vitamin D2	1000	0.3 %
Vitamin D3	1000	0.3 %
1,25-dihydroxyvitamin D2	30	92.2 %
1,25-dihydroxyvitamin D3	30	>100 %
3-epi 25-hydroxyvitamin D3	30	70.8 %
24,25-dihydroxyvitamin D3	20	15.5 %
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. Patients on Zemplar should be tested with another methodology"

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was performed to compare the candidate device BioPlex 2200 25-OH Vitamin D to the predicate device. A total of 184 human serum samples with 25-OH Vitamin D values ranging from 9.1 ng/mL to 122.8 ng/mL were analyzed in singlicate using one reagent lot of the candidate and predicate device. Less of 10% of the samples were contrived. Deming regression was used for the regression analysis and the results are summarized below:

N	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient (r)	Sample Range Tested (ng/mL)
184	1.048 (1.009 - 1.088)	-0.885 (-2.211 - 0.441)	0.987	9.1 - 122.8

Method comparison to reference method:

An additional method comparison study was conducted to evaluate the accuracy between the candidate device and the Reference Method Procedure (RMP), University of Ghent's ID-LC/MS/MS method.

A total of 119 native single donor patient serum samples (reference sample set provided by the Vitamin D Standardization and Certification Program with assigned values by the RMP at CDC, independent from the samples used for standardization) were tested with one replicate on the BioPlex 2200 25-OH Vitamin D kit. Results of the Deming regression analysis are summarized in the table below:

N	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient (r)	Sample Range Tested (ng/mL)
119	0.993 (0.913 - 1.073)	-0.775 (-2.705 - 1.155)	0.949	7.4 - 153.2

The method comparison against the RMP supports accuracy at the upper end of the claimed measuring range of the device (up to 160 ng/mL).

b. Matrix comparison:

Not applicable, only serum samples are recommended for use with this assay.

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 conducted following CLSI EP28-A3c guideline. Two hundred and eighty-eight (288) samples from apparently healthy donors including 161 males ranging in age from 21 to 79 and 127 females ranging in age from 21 to 66 were collected from three regions (Northern, Central, and Southern) in the US . The 288 samples from apparently healthy donors met the following inclusion/exclusion criteria as follows:

   - Age from 21 to 90
   - Roughly 50% female and 50% male
   - 20% from Northern, 20% from Central and 60% from Southern region
   - 40% collected in spring, 30% in summer and 30% in winter
   - Study included at least 30% African Americans and 30% Caucasians
   - 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, liver disease, and no bariatric surgery.

   Samples were tested with the BioPlex 25-OH Vitamin D kit in singlicate. The observed median, mean, and range between 2.5th to 97.5th percentile are summarized below:

N	Mean	Median	2.5th – 97.5th percentile
288	31.9 ng/mL	29.2 ng/mL	14.0 – 76.3 ng/mL

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable.

O. Conclusion:

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