

**EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
Vitamin D 200M Assay**

DECISION SUMMARY

A. DEN Number:

DEN170019

B. Purpose for Submission:

De Novo request for evaluation of automatic class III designation for the Vitamin D 200M Assay for the Topaz System

C. Measurand:

Total 25-hydroxyvitamin D

D. Type of Test:

Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

E. Applicant:

AB SCIEX

F. Proprietary and Established Names:

Vitamin D 200M Assay

G. Regulatory Information:

1. Regulation:

21 CFR 862.1840

2. Classification:

Class II (special controls)

3. Product code(s):

PSL

4. Panel:

Clinical Chemistry (75)

H. Indications For Use:

1. Indications for Use:

The Vitamin D 200M Assay for the Topaz System is intended for in vitro diagnostic use in the quantitative determination of total 25-hydroxyvitamin D (25-OH-D) through the measurement of 25-hydroxyvitamin D3 (25-OH-D3) and 25-hydroxyvitamin D2 (25-OH-D2) in human serum using LC-MS/MS technology by a trained laboratory professional in a clinical laboratory. The Assay is intended for use with the Topaz System. The Vitamin D 200M Assay for the Topaz System is intended to be used in conjunction with other clinical or laboratory data to assist the clinician in making individual patient management decisions in an adult population in the assessment of vitamin D sufficiency.

2. Special conditions for use statement(s):

For *in vitro* diagnostic use only.
For prescription use only.

3. Special instrument requirements:

Topaz System

I. Device Description:

The Vitamin D 200M Assay for the Topaz System employs LC-MS/MS technology (Topaz System) in conjunction with reagents and sample preparation components to extract, separate by chromatography, detect, and quantify total Vitamin in human serum.

The Topaz System is a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system intended to for use in a clinical laboratory environment to identify inorganic or organic compounds in human specimens by ionizing the compound under investigation and separating the resulting ions by means of an electrical and magnetic field according to their mass.

Each assay kit contains enough material for 1000 tests, and includes reagents, sample extraction and purification components, calibrators, controls and the Vitamin D 200M Assay specific file which contains all necessary parameters to process the assay.

The assay contains calibrators to calibrate the Vitamin D 200M Assay for the Topaz System. The matrix base of the calibrators is human serum. Human source material is tested for the presence of antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) and Type 2 (HIV-2), Hepatitis B Surface Antigen and antibodies to Hepatitis C Virus (HCV) and found to be negative/non-reactive.

J. Standard/Guidance Documents Referenced:

CLSI EP25-A Evaluation of Stability of In Vitro Diagnostic Reagents

CLSI EP28-A3c Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory

CLSI EP17-A2 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures

CLSI EP9-A3 Measurement Procedure Comparison and Bias Estimation Using Patient Samples

CLSI EP07-A2 Interference Testing in Clinical Chemistry

CLSI EP06-A Evaluation of the Linearity of the Quantitative Measurement Procedures: A Statistical Approach

CLSI EP05-A3 Evaluation of Precision Performance of Quantitative Measurement Methods

CLSI C62-A Liquid Chromatography-Mass Spectrometry Methods

IEC 61010-1:2001 IEC Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use - Part 1: General Requirements

IEC 61010-2-101:2002 IEC Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use - Part 2-101: Particular Requirements for In Vitro Diagnostic (IVD) Medical Equipment

IEC 61010-2-061: 2005 IEC Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use - Part 2-061: Particular Requirements for Laboratory Atomic Spectrometers with Thermal Atomization and Ionization 2005 May 2005 8-28

K. Test Principle:

The Vitamin D 200M Assay for the Topaz System employs LC-MS/MS technology in conjunction with reagents and sample preparation components to extract, separate by chromatography, detect and quantify total Vitamin D in human serum.

During the sample preparation stage, an isotopic 25-hydroxyvitamin D3 analog is used as an internal standard and is added to the sample. The analytes of interest are extracted from the sample matrix by protein precipitation and centrifugation.

Analyte separation, ionization, detection, and quantitation and reporting are achieved through LC-MS/MS technology on the Topaz System. The liquid chromatography (LC) component of the Topaz System separates chemicals by conventional chromatography on a column. The analyte separation method is reverse phase chromatography, where the analytes bind to the chromatography column by hydrophobic interactions in the presence of a hydrophilic solvent (for instance water) and are eluted off by a more hydrophobic solvent (methanol or acetonitrile). As the analytes elute from the end of the column they enter the mass spectrometer, where the solvent is removed and the analytes are ionized. The analytes must be ionized as the mass spectrometer can only detect ions, not neutral molecules. The mass spectrometer then scans the analytes of interest by mass and produces a full high-resolution spectrum, separating all ions that have different masses. The ions can be further fragmented to produce daughter ions (MS/MS). Monitoring the transitions of the original (parent) and daughter ions provides a highly specific identification of the desired analytes.

The parent ions of 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 and major fragment ions are monitored and detected in the mass spectrometer. The concentration of 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 is interpolated from a calibration curve established with calibrators of known standard concentrations. Total Vitamin D concentrations is calculated as a total of the concentrations of 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2.

L. Performance Characteristics:

1. Analytical performance:

a. *Reproducibility/Precision:*

Data was analyzed according to the multi-site evaluation study outlined in CLSI EP05-A3 guideline. The study was performed at three sites. Five serum samples were assayed on five calendar days, with one run per day and five replicates per sample. The analyte concentrations of the samples approximated both low (b) (4) and high (b) (4) levels, as well as medical decision points. The samples were either diluted with serum that did not contain vitamin D or spiked with 25-OH-vitamin D standards to achieve the low and high concentrations. The sixth sample was a native patient sample (unmodified).

Results obtained are summarized below:

Sample	Mean (ng/mL)	Repeatability		Within-Laboratory		Reproducibility	
		SD	%CV	SD	%CV	SD	%CV
1	14.9	0.57	3.8%	1.05	7.0%	1.10	7.3%
2	13.7	0.65	4.7%	0.80	5.8%	0.80	5.8%

Sample	Mean (ng/mL)	Repeatability		Within-Laboratory		Reproducibility	
		SD	%CV	SD	%CV	SD	%CV
3	31.0	1.28	4.1%	2.03	6.5%	2.03	6.5%
4	67.5	3.58	5.3%	3.99	5.9%	5.85	8.7%
5	100	6.37	6.3%	6.46	6.4%	10.4	10.4%
Native Patient Sample	28.4	1.44	5.1%	2.04	7.2%	2.10	7.4%

b. Linearity/assay reportable range:

A linearity study was performed according to CLSI EP06-A to assess the linearity of the Vitamin D 200M Assay across the assay measuring range (4 to 140 ng/mL). A serum sample with a high concentration of vitamin D was serially diluted with a low concentration serum sample to generate nine samples with vitamin D concentration values of 3.4, 47.7, 91.9, 136, 180, 225, 269, 313, 357 ng/mL, respectively.

The results of the linear regression analyses are summarized below:

$$y = 0.9974x + 1.1737 \quad R^2 = 0.9984$$

The linearity study data support the claimed measuring range of 4.0 to 140 ng/mL.

c. Traceability, Stability, Expected values:

Traceability:

The assigned 25-hydroxyvitamin D of the Vitamin D 200M Assay for the Topaz System is certified with the CDC Vitamin D Standardization-Certification Program (VDSCP). See VDSCP website for current certification at https://www.cdc.gov/labstandards/hs_standardization.html (last accessed on May 18, 2017; Note this website is not controlled by FDA).

Stability:

Unopened reagents are stable until the expiry date stipulated on the label, provided that the storage conditions indicated on the label are complied with.

d. *Detection limit:*

Low level determinations were made for each of six samples that were prepared using two kits from different lots. The lower limit of the measuring interval (LLMI) for each lot was determined to be the lowest concentration of analyte that achieved both the bias and precision goals (<20% bias and <20% CV). The maximum LLMI values from the two lots were set as the values for the method. The LLMI for this assay was determined to be 2.9 ng/mL for total 25-OH-Vitamin D.

f. *Analytical specificity:*

The design of the analytical specificity study was based on CLSI EP07-A2 guideline. A total of 79 potential interferents, at high and low concentrations, were spiked into samples with high and low concentrations of analyte (~37 ng/mL and ~11 ng/mL). Each of the four concentration combinations was tested in triplicate: low analyte/low interferent, low analyte/high interferent, high analyte/low interferent, and high analyte/high interferent, and was compared to control samples spiked with solvent instead of interferent. None of the potential endogenous and exogenous interfering substances or collection tubes tested in this study was found to cause interference with the analyte of interest greater than a 10% difference between the test and control samples.

The table below lists all substances tested at concentrations with non-significant (<10%) interference as defined by the sponsor, when compared to the control sample.

Substances	Highest Concentration tested that did not demonstrate significant interference
Vitamin-D2	750 ng/mL
Vitamin-D3	750 ng/mL
1-Hydroxyvitamin-D2	750 ng/mL
1-Hydroxyvitamin-D3	750 ng/mL
3-Epi-25-hydroxyvitamin-D2	100 ng/mL
3-Epi-25-hydroxyvitamin-D3	100 ng/mL
1, 24 (R)-dihydroxyvitamin-D3	750 ng/mL
1, 25-Dihydroxyvitamin-D2	10000 pg/mL
1, 25-Dihydroxyvitamin-D3	10000 pg/mL
3-Epi-1, 25-dihydroxyvitamin-D2	10 ng/mL
3-Epi-1, 25-dihydroxyvitamin-D3	10 ng/mL
23 (R), 25-dihydroxyvitamin-D3	10 ng/mL
24 (R), 25-dihydroxyvitamin-D3	150 ng/mL
25, 26-Dihydroxyvitamin-D3	10 ng/mL
Cholesterol	500 mg/dL
Cholesterol, 7-oxo-	68.1 ng/mL
7-Dehydrocholesterol	250 ng/mL

Substances	Highest Concentration tested that did not demonstrate significant interference
25-Hydroxycholesterol (25-OHC)	100 ng/mL
7 α -Hydroxy-4-cholesten-3-one (7 α C4)	200 ng/mL
7 α ,27-Dihydroxycholesterol	1470 pg/mL
Dehydrocholic acid	147 μ g/mL
Calcipotriene (Dovenex)	750 ng/mL
Dihydrotachysterol (drug)	750 ng/mL
Maxacalcitol	750 ng/mL
Paracalcitol (Zemplar)	750 ng/mL
Taurocholic acid	9.28 ng/mL
Biocytin	3.73 ng/mL
Coenzyme A	38.4 mg/L
β -D-Glucose	990 mg/dL
Ascorbic acid	5 mg/dL
Bilirubin (unconjugated)	20 mg/dL
Biotin	1000 ng/mL
Hemoglobin	500 mg/dL
Human Serum Albumin	6 g/dL
IgG	6000 mg/dL
Triglyceride standards	600 mg/dL
Uric acid	20 mg/dL
2'-Deoxyadenosine-5'-triphosphate	982 ng/mL
2'-Deoxycytidine 5'-triphosphate	934 ng/mL
2'-Deoxyguanosine 5'-triphosphate	1010 ng/mL
2'-Deoxyuridine 5'-triphosphate	936 ng/mL
Adenosine 5'-triphosphate	1010 ng/mL
Cytidine 5'-triphosphate	966 ng/mL
Guanosine 5'-triphosphate	1050 ng/mL
Uridine 5'-triphosphate	968 ng/mL
β -Nicotinamide adenine dinucleotide phosphate (NADP)	743 ng/mL
Aminopterin	749 ng/mL
Benazepril	20.4 μ g/mL
Bimatoprost isopropyl ester	732 ng/mL
Bradykinin	1800 ng/mL
Bromocriptine	1110 ng/mL
Budesonide	732 ng/mL
Calcipotriol	701 ng/mL
Cefotaxime	307 μ g/mL
Cephalothin	301 μ g/mL
Cetirizine	2990 ng/mL

Substances	Highest Concentration tested that did not demonstrate significant interference
Clindamycin	723 ng/mL
Dexamethasone phosphate	709 ng/mL
Etoposide	1000 ng/mL
Flurazepam	659 ng/mL
Fluticasone propionate	851 ng/mL
Fusidic acid	878 ng/mL
Isradipine	631 ng/mL
Latanoprost	735 ng/mL
Loratadine	299 ng/mL
Mifepristone	730 ng/mL
Minocycline	778 ng/mL
Nimodipine	711 ng/mL
Nisoldipine	660 ng/mL
10(S),17(S)-DiHDoHE (Protectin DX)	3610 ng/mL
Raltitrexed	779 ng/mL
Rescinnamine	1080 ng/mL
Reserpine	1030 ng/mL
Tadalafil	662 ng/mL
Tafluprost	769 ng/mL
Trandolapril	732 ng/mL
Treprostinil	664 ng/mL
Triamcinolone	670 ng/mL
Zopiclone	661 ng/mL

2. Comparison studies:

a. *Method comparison with reference method:*

The sponsor performed an accuracy study to the CDC Vitamin D Standardization-Certification Program (VDSCP). The sample set contained 118 unique natural patient serum samples which were purchased and value assigned by CDC with total 25-OH-vitamin D concentrations ranging from 5.6 ng/mL to 133 ng/mL. Twelve specimens were contrived (10.2%).

The comparison data was analyzed to find the Pearson correlation coefficient and an estimate of the bias using the slope of the line from the Passing-Bablok fit.

	Passing-Bablok regression results
n	118
Slope	1.008
Intercept	-0.3949
Correlation Coefficient	0.991
Range (ng/mL)	5.6 – 133 ng/mL

b. Matrix comparison:

Not applicable.

3. Clinical studies:

Not Applicable.

4. Expected Values

A reference range study was conducted with reference to the CLSI EP28-A3 guideline. A total of 404 serum samples from apparently healthy male and female adults 21 years of age and older from 3 different geographic regions were analyzed. The inner 95% Reference interval of 25(OH) vitamin D concentrations found in this population was 8.6 to 49 ng/mL.

M. Instrument Name:

Topaz System

N. System Description:

1. Modes of Operation:

Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device? Yes X or No _____.

Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission: Yes ___ or No X ___.

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes X or No _____

3. Specimen Identification:

Samples are given a unique identifier.

4. Specimen Sampling and Handling:

Human serum, in liquid form, is the only type of sample that should be used in this assay. Collect samples in clot-activating collection tubes with red tops (or closures).

5. Calibration:

The 4 calibrators are run with each cartridge. The device software calculates a slope, intercept and R^2 by linear regression for every run. If the calibration for a run is flagged as “failed” no activity values are reported for the entire run and the entire run must be repeated.

6. Quality Control:

It is expected that a clinical laboratory using the Topaz System adheres to procedures that cover issues such as, but not limited to, operator training, assay development and validation, and external audits of laboratory assay performance.

O. Other Supportive Instrument Performance Characteristics Data Not Covered In the “Performance Characteristics” Section above:

Carryover:

Studies were performed to determine if the level of carryover for the assay is acceptable. Samples with high and low concentrations of analyte were injected in a predetermined order to determine if the carryover was acceptable. Studies were performed at a single internal site using 3 instruments and 3 kit lots over a period of 1 day. There was no carry-over demonstrated in the study. This study was sufficient to demonstrate that there was no significant carry-over.

Electrical Safety and Electromagnetic Compatibility (EMC):

Electrical safety and EMC testing were conducted on the Topaz System. The system complies with IEC 61010-1 and IEC 61010-2-040 standards for safety and IEC 61326 for EMC.

P. Proposed Labeling:

The labeling is sufficient and satisfies the requirements of 21 CFR Parts 801 and 809, as applicable, and the special controls for this type of device.

Q. Patient Perspectives:

This submission did not include specific information on patient perspectives for this device.

R. Identified Risks to Health and Identified Mitigations:

Identified Risks to Health	Identified Mitigations
Clinical action based on falsely elevated inaccurate Vitamin D results may lead to unnecessary supplementation of Vitamin D	General controls and special controls (1) and (2)
Clinical action based on falsely low inaccurate Vitamin D results may lead to a delay in supplementation of Vitamin D	General controls and special controls (1) and (2)
Clinical action based on uninterpretable results due to lack of established device specific reference range values for the representative population	General controls and special controls (3)
Clinical action based on the misinterpretation of Vitamin D2 or Vitamin D3 results as total Vitamin D results	General controls and special controls (4)

S. Benefit/Risk Analysis

Summary	
Summary of the Benefit(s)	<p>Adults may potentially benefit from the use of the device for assessment of vitamin D sufficiency. The results of the device, when used in conjunction with other clinical and laboratory data, may assist healthcare providers in making individual management decisions related to vitamin D sufficiency. Treatment for vitamin D deficiency may improve clinical complications related to bone fracture risk and osteomalacia. Intervention for potential vitamin D toxicity may be helpful in avoiding complications associated with vitamin D toxicity.</p> <p>The technological characteristics of this device may reduce concerns specific to immunoassays, including interference and assay drift. The lack of antibody in this device combined with the two-step separation of 25-hydroxy vitamin D2 and 25-hydroxy vitamin D3 by chromatography followed by selection by mass increases the specificity of the assay thereby mitigating concerns from structurally similar compounds known to interfere with immunoassay-based vitamin D assays. In addition, the lack of reliance on antibody production and maintenance reduces concerns of assay drift over time.</p>

<p style="text-align: center;">Summary of the Risk(s)</p>	<p>A patient with a falsely elevated result which crosses a medical decision making threshold may result in a missed opportunity to benefit from vitamin D treatment, and potentially be at risk for complications associated with vitamin D deficiency. The healthcare provider ordering the test may be misled into believing that vitamin D treatment is not necessary based on a vitamin D result that is reported in the “sufficient” or “toxic” range. This scenario is of particular concern in patients who are particularly likely to benefit from treatment with vitamin D, including those patients with osteoporosis, patients at high risk for bone fractures, or patients suffering from osteomalacia. This scenario is also of concern in patients who have an increased risk of vitamin D deficiency including obesity, certain medications that enhance the catabolism of 25(OH)D and 1,25(OH)2D, as well as conditions that increase metabolism of 25(OH)D to 1,25(OH)2D, including chronic granuloma-forming disorders, some lymphomas, and primary hyperparathyroidism.</p> <p>A patient with a falsely low result which crosses a medical decision making threshold may result in a delay or failure for clinical intervention for potential vitamin D toxicity. The healthcare provider ordering the test may be misled into believing that vitamin D treatment is necessary based on a vitamin D result that is reported in the “deficient” or “sufficient” range. The potential clinical consequences of long-term untreated vitamin D toxicity may be nephrocalcinosis, bone demineralization, and bone pain. This scenario is of particular concern in patients with chronic granuloma-forming disorders (i.e., sarcoidosis or tuberculosis, lymphoma, chronic fungal infections), who may be more sensitive to vitamin D treatment.</p> <p>There is a risk relating to unknown performance of this device outside of the intended use population. Risks relating to utilization outside of the intended use population (i.e., pediatric population) are mitigated by appropriate labeling and requirement for prescription use. Similarly, over-reliance on device results should be mitigated by appropriate labeling and requirement for prescription use.</p>
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Summary of Other Factors	<p>As specified by labelling, a total 25-hydroxy vitamin D level may be used in conjunction with other clinical or laboratory data to assist healthcare providers in making individual management decisions related to vitamin D sufficiency.</p> <p>Further analytical risks are mitigated by labelling and the requirement for professional use.</p> <p>Analytical performance studies were considered as part of the device review.</p>
Conclusions	
Do the probable benefits outweigh the probable risks?	
Yes. Given the device's indications for use, required general controls and special controls established for this device (e.g., labeling and clinical studies performed), the probable benefits outweigh the probable risks.	

T. Conclusion:

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 862.1840. FDA believes that special controls, along with the applicable general controls, provide reasonable assurance of the safety and effectiveness of the device type. This device is classified under the following:

Product Code: PSL
Device Type: Total 25-hydroxyvitamin D Mass Spectrometry Test System
Class: II (special controls)
Regulation: 21 CFR 862.1840

(a) Identification. A total 25-hydroxyvitamin D mass spectrometry test system is a device intended for use in clinical laboratories for the quantitative determination of total 25-hydroxyvitamin D (25-OH-D) in serum or plasma to be used in the assessment of vitamin D sufficiency.

(b) Classification. Class II (special controls). Vitamin D test systems must comply with the following special controls:

(1) The device must have initial and annual standardization verification by a certifying vitamin D standardization organization deemed acceptable by FDA.

(2) The 21 CFR 809.10(b) compliant labeling must include detailed descriptions of performance testing conducted to evaluate precision, accuracy, linearity, interference, including the following:

- (i) Performance testing of device precision must, at a minimum, use intended sample type with Vitamin D concentrations at medically relevant decision points. At least one sample in the precision studies must be an unmodified patient sample. This testing must evaluate repeatability and reproducibility using a protocol from an FDA-recognized standard.
- (ii) Performance testing of device accuracy must include a minimum of 115 serum or plasma samples that span the measuring interval of the device and compare results of the new device to results of a reference method or a legally marketed standardized mass spectrometry based vitamin D assay. The results must be described in the 21 CFR 809.10(b)(12) compliant labeling of the device.
- (iii) Interference from vitamin D analogs and metabolites including vitamin D₂, vitamin D₃, 1-hydroxyvitamin D₂, 1-hydroxyvitamin D₃, 3-Epi-25-Hydroxyvitamin D₂, 3-Epi-25-Hydroxyvitamin D₃, 1,25-Dihydroxyvitamin D₂, 1,25-Dihydroxyvitamin D₃, 3-Epi-1,25-Dihydroxyvitamin D₂, and 3-Epi-1,25-Dihydroxyvitamin D₃, 25, 26-Dihydroxyvitamin-D₃, 24 (R), 25-dihydroxyvitamin-D₃, 23 (R), 25-dihydroxyvitamin-D₃ must be described in the 21 CFR 809.10(b)(7) compliant labeling of the device.
- (3) The 21 CFR 809.10(b) compliant labeling must be supported by a reference range study representative of the performance of the device. The study must be conducted using samples collected from apparently healthy male and female adults at least 21 years of age and older from at least 3 distinct climatic regions within the United States of America in different weather seasons. The ethnic, racial, and gender background of this study population must be representative of the US population demographics.
- (4) The results of the device as provided in the 21 CFR 809.10(b) compliant labeling and any test report generated must be reported as only total 25-hydroxyvitamin D.