

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

A. 510(k) Number:

k121608

B. Purpose for Submission:

New Device

C. Measurand:

25-hydroxyvitamin D (25-(OH)-D₂/D₃) and other hydroxylated Vitamin D metabolites

D. Type of Test:

Quantitative chemiluminescent immunoassay

E. Applicant:

Ortho-Clinical Diagnostics, Inc

F. Proprietary and Established Names:

VITROS[®] Immunodiagnostic Products 25-OH Vitamin D Total Reagent Pack
VITROS[®] Immunodiagnostic Products 25-OH Vitamin D Total Calibrators

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
MRG	Class II	21 CFR 862.1825 Vitamin D Test System	Chemistry 75
JIT	Class II	21 CFR 862.1150 Calibrators	Chemistry 75

H. Intended Use:

1. Intended use(s):

See Indications for Use below

2. Indication(s) for use:

For *in vitro* diagnostic use only

For the quantitative measurement of total 25-OH vitamin D in human serum using the VITROS ECI/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated System.

The results of the VITROS 25-OH Vitamin D Total assay are used in the assessment of Vitamin D sufficiency. Assay results may be used in conjunction with other clinical or laboratory data to assist the clinician in patient management.

VITROS Immunodiagnostic Products 25-OH Vitamin D Total Calibrators:

For *in vitro* diagnostic use only.

For use in the calibration of the VITROS ECI/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic Systems and the VITROS 5600 Integrated System for the quantitative measurement of total 25-OH vitamin D in human serum.

3. Special conditions for use statement(s):

For prescription use only.

Assay results should be used in conjunction with other clinical or laboratory data to assist the clinician in making individual patient management decisions in an adult population.

The performance characteristics of this assay have not been established in a pediatric population

4. Special instrument requirements:

To be used with VITROS ECi/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated System only.

I. Device Description:

The 25-OH Vitamin D reagent pack consists of:

- wells coated with antibody against 25-OH Vitamin D
- conjugate reagent consisting of HRP-25-OH VitD, buffer and 0.5% Proclin 950
- dissociation reagent consisting of EDTA buffer, surfactant and 0.5% Proclin 950

The Calibrators consist of:

1 set of VITROS 25-OH Vitamin D Total Calibrators 1 and 2 (liquid, 25-OH Vitamin D in human serum with antimicrobial agent, 2.0 mL); nominal values 28 and 120 ng/mL (70 and 300 nmol/L)

The label includes the following warning:

Human blood products provided as components of the VITROS 25-OH Vitamin D Total Calibrators have been obtained from donors who were tested individually and who were found to be negative for hepatitis B surface antigen, and for antibodies to human immunodeficiency virus (HIV 1+2) and hepatitis C virus (HCV), using FDA approved methods (enzyme immunoassays). Treat as if capable of transmitting infection.

J. Substantial Equivalence Information:

a. Predicate Device name(s)

IDS-iSYS® 25-Hydroxy Vitamin D (25OHD) and IDS-iSYS® Vitamin D Calibrators

b. Predicate 510(k) number(s)

k091849

c. Comparison with predicate for IDS-iSYS® 25-Hydroxy Vitamin D (25OHD):

Similarities		
Item	Device	Predicate (k091849)
Intended use and indications for use	Same	Quantitative determination of 25-Hydroxyvitamin D to be used in the assessment of Vitamin D sufficiency.
Fundamental Scientific Technology	Same	Immunoassay, solid phase antibody capture

Basic Principle	Same	Direct competitive assay
Detection	Same	Light signal measurement
Instrumentation	Same	Automated instrumentation
Sample Type	Same	Serum
Differences		
Item	Device	Predicate (k091849)
Antibody	Anti-25 OH D Sheep Monoclonal IgG	Anti-25 OH D Sheep Polyclonal IgG
Measuring Range	12.8- 126 ng/mL	6-126 ng/mL

Comparison of the VITROS[®] and IDS-iSYS[®] Vitamin D Calibrators

Similarities		
Item	New Device	Predicate (k091849)
Intended use and indications for use	Same.	For use in the calibration of total 25-OH vitamin D in human serum.
Format	Same	Liquid Ready-to-Use
Calibrator Levels	Same	Two
Storage	Same	Refrigerated

Differences		
Item	New Device	Predicate (k091849)
Calibrator Matrix	Human serum and antimicrobial	Horse Serum in a buffer matrix and sodium azide

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

STANDARDS
• Stability Testing of In Vitro Diagnostic Reagents (13640)
• How to Define and Determine Reference Intervals in the Clinical Laboratory; CLSI Approved Guideline - Second Edition (C28-A2)
• Interference Testing in Clinical Chemistry; CLSI Approved Guideline (EP 7-A)
• Method Comparison and Bias Estimation Using Patient Samples; CLSI Approved Guideline (EP9-A2)
• Preliminary Evaluation of Quantitative Clinical Laboratory Methods; CLSI Approved Guideline (EP10-A2)
• Evaluation of Precision Performance of Quantitative Measurement Methods; CLSI Approved Guideline-Second Edition (EP5-A2)

L. Test Principle:

VITROS 25 OH Vitamin D assay is a direct, competitive chemiluminescent immunoassay for quantitative determination of total 25 OH vitamin D in serum. During the first incubation, 25 OH Vitamin D is dissociated from its binding protein and binds to the specific antibody on the solid phase. After 10 minutes the tracer (vitamin D linked to an isoluminol derivative) is added. After additional 10 minute incubation, the unbound material is removed with a wash cycle. Subsequently, the starter reagents are added to initiate a flash chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLU) and is inversely proportional to the concentration of 25 OH vitamin D present in calibrators, controls, or samples. The light signal is measured by a photomultiplier as relative light units (RLU) and is inversely proportional to the concentration of 25 OH Vitamin D present in calibrators, controls, or samples.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

VITROS ECi/ECiQ Immunodiagnostic System

Precision was evaluated consistent with NCCLS document EP5-A2. Two replicates each of 3 patient serum samples and 1 commercial control sample were tested on 2 separate occasions per day on at least 20 different days. The experiment was performed using 2 reagent lots on 2 different systems. The data presented are a representation of the product performance.

VITROS 3600 Immunodiagnostic System and VITROS 5600 Integrated System

Precision was evaluated consistent with NCCLS document EP5-A2. Two

replicates each of 3 patient serum samples and 1 commercial control sample were tested on 2 separate occasions per day on at least 20 different days. The experiment was performed using 3 reagent lots on 2 different systems. The data presented are a representation of the product performance.

System	Units = [ng/mL]							No. Obser v.	No. Days
	Mean [25-OH Vitamin D Total] Conc.	Within-run		Within-calibration		Within-lab			
		SD	CV (%)	SD	CV (%)	SD	CV (%)		
ECi/ECiQ System 1 Lot 1	22.5	1.66	7.4	3.14	14.0	3.43	15.3	80	20
	31.1	2.25	7.2	3.86	12.4	4.13	13.3	80	20
	70.0 [†]	3.86	5.5	5.86	8.4	6.24	8.9	80	20
	121	4.1	3.4	6.1	5.1	6.7	5.5	80	20
ECi/ECiQ System 2 Lot 2	20.7	2.46	12.0	3.32	16.2	3.43	16.4	80	20
	28.1	3.06	11.0	3.34	12.0	3.43	12.1	80	20
	65.0 [†]	5.20	8.1	5.66	8.8	5.94	9.1	80	20
	108	4.1	3.8	5.5	5.2	5.9	5.4	80	20
3600 System 1 Lot 1	22.9	2.26	10.5	2.90	13.5	4.04	16.5	80	20
	31.6	2.66	8.9	3.36	11.2	4.65	14.0	80	20
	72.2 [†]	4.30	6.1	5.73	8.1	6.80	9.2	80	20
	123	4.8	3.9	6.5	5.3	7.4	6.0	80	20
3600 System 1 Lot 3	21.0	3.22	15.3	3.29	15.6	3.32	15.8	80	20
	29.5	3.35	11.3	3.43	11.6	3.62	12.3	80	20
	71.1 [†]	5.93	8.3	6.07	8.5	5.92	8.4	80	20
	120	5.8	4.7	5.9	4.9	5.8	4.8	80	20
5600 System 1 Lot 2	23.5	2.43	10.1	2.95	12.2	2.93	12.8	80	20
	31.9	2.52	7.7	3.22	9.9	3.13	10.1	80	20
	69.4 [†]	3.75	5.3	4.82	6.8	4.39	6.5	80	20
	117	6.1	5.1	6.5	5.4	6.4	5.6	80	20

[†] This sample is a commercial quality control fluid. The other samples are human serum samples

b. *Linearity/assay reportable range:*

The method was based on CLSI EP6-A (“Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical approach; Approved Guideline”). Two lots of VITROS 25-OH Vitamin D Total Assay (tVitD) were tested using three VITROS Systems (one VITROS ECi/ECiQ Immunodiagnostic System, one VITROS 3600 Immunodiagnostic System, and one VITROS 5600 Integrated System). Two pools of VITROS 25-OH Vitamin D Total Assay samples were prepared with patient samples and were selected near the extremes of the calibration range. The low pool had an estimated concentration of 6.73 ng/mL. The high pool had an estimated concentration of 175 ng/mL. The low and high concentration pools were sequentially mixed to give 7 further pools of intermediate concentrations. All results from both Master Lot 1 and 2 and all three VITROS Systems supported a measuring range of 12.8 to 126 ng/mL.

The regression statistics for linearity are as follows:

System	Equation from the linearity study
VITROS ECi/ECiQ Immunodiagnostic System	$Y = 1.039x + 0.626$ ng/mL $R^2 = 0.9954$
VITROS 3600 Immunodiagnostic System	$Y = 1.035x + 0.790$ ng/mL $R^2 = 0.9956$
VITROS 5600 Integrated System	$Y = 1.017x + 3.54$ ng/mL $R^2 = 0.9948$

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

Calibrator traceability and value assignment

VITROS 25-OH Vitamin D Total calibrators are value assigned using in house reference calibrators. The in house reference calibrators are value assigned using patient samples that are traceable to Liquid Chromatography Mass Spectrometry values.

The value assignment was carried out as part of the Master Curve generation. Twenty four individual reference calibrator curve assays were performed. Each assay contains reference calibrators, in-house controls and the selling calibrators, all in singleton. The test is performed using 4 VITROS Systems, 8 VITROS 25-OH Vitamin D Total Reagent Packs, and 12 sets of VITROS 25-OH Vitamin D Total calibrators.

Each assay is checked for validity against the curve-shape limits and in-house control test limits. At least 20 runs must be used to generate the Master Curve. The Master Curve was checked for validity against the curve shape and in-house control release limits. The mean values for the selling calibrators were checked against their release limits. The acceptance criteria for the selling calibrators are shown below.

	Target value (ng/mL)	Release limits (ng/mL)
Calibrator 1	28	20 – 36
Calibrator 2	120	105 – 135

Stability

The calibrator shelf-life and open-vial stability testing protocols and acceptance criteria were described and found to be adequate. Current shelf life studies support an eight week shelf life at 2-8°C (supported by real time studies), with real-time studies on-going.

Once opened calibrators are stable for up to 4 weeks off board at 2-8°C and at -20 °C and onboard stability of 4 weeks.

d. Detection limit:

The Limit of Blank study was designed to run ten (10) replicates of a negative sample on 2 occasions per day for 5 days, giving 100 determinations in total. The 5 testing days also incorporated three calibration events.

These determinations were run using two VITROS 25-OH Vitamin D Total reagent lots run across a VITROS ECi/ECiQ Immunodiagnostic System, a VITROS 3600 Immunodiagnostic System, and a VITROS 5600 Integrated System.

The Limit of Blank (LoB) is 4.34 ng/mL

The Limit of Detection study was designed to run 10 replicates of each of six LoD pools, on 2 occasions per day for 5 days, giving 100 determinations of each pool in total. The 5 testing days incorporated three calibration events. The six LoD pools were made by selecting samples that give estimated 25-OH Vitamin D concentrations of approximately 8.00 to 30.0 ng/mL.

These determinations were run using two VITROS 25-OH Vitamin D Total reagent lots run across a VITROS ECi/ECiQ Immunodiagnostic System, a VITROS 3600 Immunodiagnostic System, and a VITROS 5600 Integrated System.

The Limit of Detection (LoD) for VITROS 25-OH Vitamin D Total Assay is 8.64 ng/mL determined consistent with CSLI document EP17 and with proportions of false positives (α) less than 5% and false negatives (β) less than 5%; based on 700 determinations, with 1 blank and 6 low-level samples.

The Limit of Quantitation (LoQ) is 12.8 ng/mL as determined by the lowest concentration at which precision design of <20%CV. At 12.8ng/mL, the observed imprecision (%CV) is < 20% across lots and analyzers.

The sponsor's claimed measuring range is 12.8 to 126 ng/mL.

e. Analytical specificity:

Potential interfering and cross-reacting substances for the VITROS 25-OH Vitamin D Total assay were studied using the VITROS 3600 Immunodiagnostic System consistent with CSLI EP7-A2. Point estimates of the effects of test levels of potential cross reactants and interferents have been made with patient samples with values near 30 ng/mL and 80 ng/mL Vitamin D. To calculate the % interference or % cross reactivity, the mean value of a solution of each test substance was compared with that of a corresponding "control" in two VITROS 25-OH Vitamin D Total Assay lots. The sponsor defined non-significant interference as $\leq 10\%$ bias between the tested and the control samples. Paricalcitol (Zemplar) interferes with the VITROS 25-OH Vitamin D Total Assay. Of the other compounds tested for interference, none was found to cause a >10% bias at the test concentrations.

The VITROS 25-OH Vitamin D Total test was evaluated for interference consistent with CLSI document EP7-A2. Of the compounds tested, none was found to cause a bias of >10% with the test at the concentrations indicated at 25-OH Vitamin D concentrations of 30-80 ng/mL (75-200nmol/L).

Compound	Concentration of interference substance tested that did not interfere with the assay	
	Acetaminophen	1324 μ mol/L
Acetylsalicylic Acid	3.62mmol/L	65.16mg/dL
Bilirubin (unconjugated)	513 μ mol/L	30 mg/dL
Bilirubin (conjugated)	356 μ mol/L	30 mg/dL
Biotin	61.35nmol/L	1.5 μ g/dL
Hemoglobin (hemolysate)	0.124mmol/L	200 mg/dL
Ibuprofen	0.576mmol/L	12mg/dL
Triolein	33.0mmol/L	3000 mg/dL
Cholesterol	7.91 mmol/L	306 mg/dL
Total Protein	108g/L	10.8g/dL
Triglycerides	5.69 mmol/L	504 mg/dL

The sponsor has included the following limitations in their labeling:

1. Paricalcitol (Zemplar) interferes with the VITROS 25-OH Vitamin D Total test. Paricalcitol, when tested, caused a positive bias at the concentration indicated.
2. Grossly hemolyzed samples should not be used.

The results of the potentially cross-reacting substances listed in the Table below show the % cross-reactivity in the VITROS 25-OH Vitamin D Total assay

Compound	Concentration	Sample 25-OH Vitamin D Concentration		Mean 25-OH Vitamin D Result of Cross-reactant Pool		% Cross-reactivity
		[ng/mL]	[nmol/L]	[ng/mL]	[nmol/L]	
Vitamin D ₂ (Ergocalciferol)	100ng/mL	8.81	22.0	9.77	24.4	1.0
Vitamin D ₃ (Cholecalciferol)	100ng/mL	8.81	22.0	9.66	24.2	0.9
25-OH Vitamin D ₂	100ng/mL	8.10	20.3	113	283	104.9
25-OH Vitamin D ₃	100ng/mL	8.10	20.3	107	268	98.9
1,25 (OH) ₂ Vitamin D ₂	0.2ng/mL*	8.81	22.0	10.1	25.3	>100
1,25 (OH) ₂ Vitamin D ₂	0.2ng/mL*	26.8	67.0	28.5	71.3	>100
1,25 (OH) ₂ Vitamin D ₃	0.2ng/mL*	8.10	20.3	8.09	20.2	-5.0
24,25 (OH) ₂ Vitamin D ₂	10ng/mL**	26.8	67.0	30.2	75.5	34.3
24,25 (OH) ₂ Vitamin D ₃	10ng/mL**	7.92	19.8	11.4	28.5	34.8
3-epi 25-OH Vitamin D ₃	100ng/mL	7.92	19.8	45.3	113	37.4

*Levels tested were 2x to 4x the typical endogenous levels of analyte. 0.2 ng/mL 1,25 (OH)₂ Vitamin D₂ (4 x the upper limit of the reference interval) produced a bias in the measurement of just 1.7 ng/mL at a baseline 25-OH Vitamin D of 30 ng/mL.

**Levels tested were 2x to 4x the typical endogenous levels of analyte.

f. *Assay cut-off:*

Not Applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

The method was consistent with CSLI document EP9-A2-IR (“Method Comparison and Bias Estimation Using Patient Samples”; Approved Guideline - Second Edition) Interim Review.

A minimum of 117 human serum samples were assayed using the VITROS®

Immunodiagnostic Products 25-OH Vitamin D Total assay on the VITROS Systems and the *IDS-iSYS*® 25-Hydroxy Vitamin D assay on the *IDS-iSYS* System All human serum samples were run in singleton on all systems.

Seven (7) -spiked and one pooled sample were used for this test.

Passing & Bablok Regression was performed for all comparisons. In each case, the VITROS 25-OH Vitamin D Total assay results were plotted as the “y” variable and those from the *IDS-iSYS* assay as the “x” variable. For the quantitative comparison, only those data within the measuring range of the compared *IDS-iSYS* and VITROS assays were analyzed (12.8 - 126 ng/mL). The parameters of the Passing & Bablok Regressions were used to state the equations for the product claim.

The comparisons of VITROS to *IDS-iSYS* are as follows:

1. VITROS 5600 Integrated System 25-OH Vitamin D Total = $0.99 x -5.12$ ng/mL, n = 102, r = 0.92. The 95% CI for the slope is 0.86 to 1.12 and for the Intercept is -10.2 to -0.53.
2. VITROS 3600 Immunodiagnostic System 25-OH Vitamin D Total = $1.08 x -7.87$ ng/mL, n = 103, r = 0.93. The 95%CI for the slope is 0.96 to 1.22 and for the Intercept is -12.4 to -2.95.
3. VITROS ECi/ECiQ Immunodiagnostic System 25-OH Vitamin D Total = $0.96 x -9.07$ ng/mL, n = 102, r = 0.94. The 95%CI for the slope is 0.86 to 1.09 and for the Intercept is -14.2 to -4.69.

b. *Matrix comparison:*

Not applicable

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:
A study was conducted using 399 apparently healthy adults between the ages of 21–79. Samples came from individuals who live in the North, South and Central regions of the United States and were collected in both summer and winter. These samples were tested using the VITROS 25-OH Vitamin D Total assay Serum samples from apparently healthy individuals were collected at three US sites using the following criteria:
- Approximately 50% male and 50% female
 - Include ages 21 to 90 years old
 - Approximately 50% of the study samples were collected in the summer and 50% of the study samples were collected in the winter
 - Individual will live in the north, south and central United States
 - The individuals enrolled in the study must include different skin tones (minimum 30% dark skin and 30% light skin)
 - No history of parathyroid or calcium regulatory disease.
 - No history of kidney disease, gastro-intestinal disease, liver disease or had bariatric surgery
 - Currently not taking any medications including Vitamin D supplements greater than 1000IU per day

The data was analyzed for each system separately and as a mean of the system results. The data was also analyzed for mean, median, minimum and maximum for gender, skin type and geographic location by system and as the mean of the three VITROS systems, The VITROS 3600, VITROS 5600, and VITROS ECi/ECiQ. The reference interval was calculated using the mean value across the VITROS systems for each of the 399 samples as this would result in representative data for the VITROS family of analyzers

The observed values are summarized below:

Observed Values		
Median 25 OH Vitamin D	33.4 (ng/mL)	83.5(nmol/L)
Observed Range 2.5 th to 97.5 th Percentile	14.7 to 68.3 (ng/mL)	36.8 – 171 (nmol/L)

It is recommended in the labeling that each laboratory establishes its own expected values for the population it serves. A review of the most recent literature¹ suggests the recommendation for 25-OH Vitamin D levels are:

Level	Range
Deficient	<20 ng/mL (50 nmol/L)
Insufficient	20–29 ng/mL (50–72.5 nmol/L)
Sufficient	30–100 (75–250 nmol/L)
Potential Toxicity	>100 (250 nmol/L)

1. Holick, MF et al. Evaluation, Treatment, and Prevention of Vitamin D Deficiency: an Endocrine Society Clinical Practice Guideline; J Clin Endocrin Metab. July 2011, 96(7).

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.