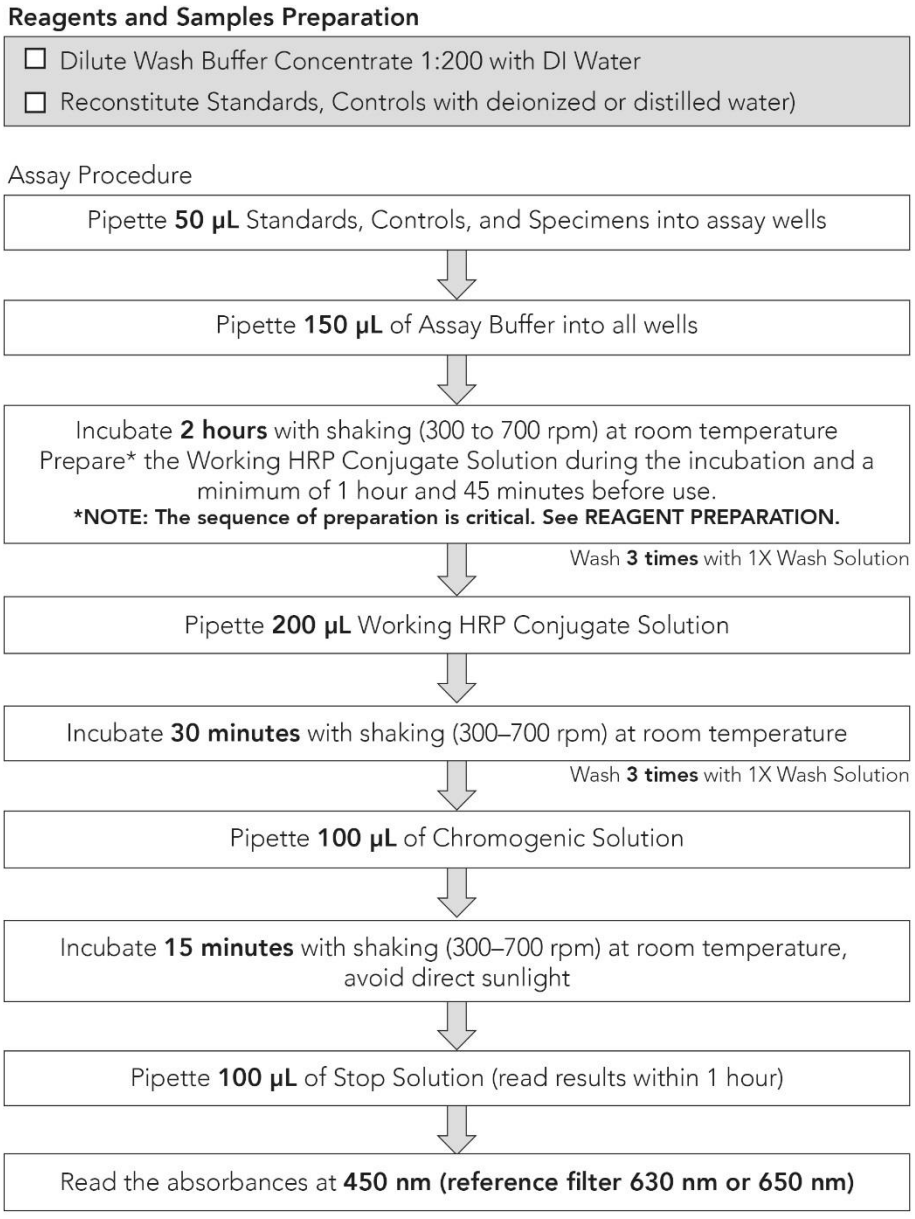
QUIDEL

MicroVue™ Bone 25-OH Vitamin D EIA

Immunoenzymetric assay for the *in vitro* quantitative measurement of 25-hydroxyvitamin D_2 and D_3 (25OH-D_2 and 25OH-D_3) in serum.

SUMMARY



INTENDED USE

The MicroVue 25-OH Vitamin D EIA Test is intended for the quantitative determination of 25-hydroxyvitamin D_2 and D_3 (25OH D_2 and 25OH D_3) in human serum. The results are to be used in conjunction with other clinical and laboratory findings to assess the Vitamin D status of a patient.

SUMMARY AND EXPLANATION

Vitamin D is the generic term used to designate Vitamin D_2 or ergocalciferol and Vitamin D_3 or cholecalciferol. Humans naturally produce Vitamin D_3 when the skin is exposed to ultraviolet sun rays. In the liver mainly, Vitamin D_3 is metabolised into 25-Hydroxyvitamin D_3 (25OH D_3) which is the main form of Vitamin D circulating in the body. 25OH D_3 is a precursor for other Vitamin D metabolites and has also a limited activity by itself. The most active derivative is 1, 25-hydroxyvitamin D_3, produced in the kidney (or placenta) by 1-hydroxylation of 25OH D_3. 25OH Vitamin D stimulates the intestinal absorption of both calcium and phosphorus and also bone resorption and mineralisation. 25OH Vitamin D might also be active in other tissues responsible for calcium transport (placenta, kidney, mammary gland …) and endocrine gland (parathyroid glands, beta cells …).

Vitamin D_3 and Vitamin D_2 are also available by ingestion through food or dietary supplementation. As Vitamin D_2 is metabolised in a similar way to Vitamin D_3, both contribute to the overall Vitamin D status of an individual. It is the reason why it is very important to measure both forms of 25OH Vitamin D equally for a correct diagnosis of Vitamin D deficiency, insufficiency or intoxication.

Vitamin D deficiency is an important risk factor for rickets, osteomalacia, senile osteoporisis, cancer and pregnancy outcomes. The measurement of both 25OH Vitamin D forms is also required to determine the cause of abnormal serum calcium concentrations in patients. Vitamin D intoxication has been shown to cause kidney and tissue damages.

PRINCIPLE OF THE PROCEDURE

The MicroVue 25-OH Vitamin D EIA is a solid phase Enzyme Linked Immunosorbent Assay performed on Microtiter plates. During a first 2-hour incubation step, at room temperature, total 25OH Vitamin D (D_2 and D_3) present in standards, controls and samples is dissociated from binding serum proteins to fix on binding sites of a specific monoclonal antibody. After 1 washing step, a fixed amount of 25OH Vitamin D-labelled with biotin in presence of horseradish peroxidase (HRP) compete with unlabelled 25OH Vitamin D_2 and 25OH Vitamin D_3 present on the binding sites of the specific monoclonal antibody. After a 30-minute incubation at room temperature, the Microtiterplate is washed to stop the competition reaction. The Chromogenic Solution (TMB) is added and incubated for 15 minutes. The reaction is stopped with the addition of Stop Solution and the Microtiter plate is then read at the appropriate wavelength. The amount of substrate turnover is determined colourimetrically by measuring the absorbance, which is inversely proportional to the total 25OH Vitamin D (D_2 and D_3) concentration.

A calibration curve is plotted and the total 25OH Vitamin D (D_2 and D_3) concentrations of the samples are determined by dose interpolation from the calibration curve.

REAGENTS AND MATERIALS PROVIDED

MicroVue 25-OH Vitamin D EIA contains the following:

A	**25-OH Vitamin D Standard** (Calibrator 0) Lyophilized. Zero standard is biological matrix (human plasma) with gentamycin and ProClin®. Reconstitute with 2 mL DI water.	**Part A**	**1 ea x 2 mL (Std A)**
B-F	**25-OH Vitamin D Standards B-F** (Calibrators 1-5) Lyophilized. Horse serum with gentamycin and Proclin®. Reconstitute each vial with 1 mL DI water.	**Part B-F**	**1 ea x 1 mL (Std B-F)**
L	**25-OH Vitamin D Control** (Control 1) Lyophilized. Human serum with ProClin®. Reconstitute with 1 mL DI water.	**Part 4219716**	**1 ea x 1 mL**
H	**25-OH Vitamin D Control** (Control 2) Lyophilized. Human serum with ProClin®. Reconstitute with 1 mL DI water.	**Part 4219717**	**1 ea x 1 mL**
❶	**Microassay Plate** (Microtiterplate) Microtiter plate with 96 Mab anti-25OH Vitamin D_2 and D_3 coated wells.	**Part 4219708**	**12 x 8 wells**
❷	**Stop Solution** Contains 1M Hydrochloric Acid (HCl).	**Part SS04**	**12 mL**
❸	**200X Wash Buffer Concentrate** (Wash Solution) Contains TRIS-HCl. Dilute with DI water.	**Part 4219711**	**10 mL**
❹	**TMB Substrate** (Chromogenic Solution TMB) Ready to use. Contains 3,3',5,5'-tetramethylbenzidene (TMB).	**Part SB04**	**12 mL**
❺	**Biotinylated 25-OH Vitamin D** (Concentrated Conjugate) 25OH Concentrated Conjugate. Dilute with Reconstitution Solution.	**Part of 4119703**	**0.4 mL**
❻	**Concentrated HRP** Contains Concentrated HRP.	**Part 4119713**	**0.2 mL**
❼	**Reconstitution Solution** (Conjugate Buffer) Ready to use. Conjugate Buffer with casein and ProClin®.	**Part 4119705**	**30 mL**
❽	**Assay Buffer** (Incubation Buffer) Ready to use. Incubation Buffer with casein and ProClin®.	**Part 4219713**	**20 mL**

ProClin® is a registered trademark of Rohm and Haas Company.

Note: Use 25-OH Vitamin D Standard A (Calibrator 0) for dilution of samples with values above the highest standard.
No international reference material is available

MATERIALS REQUIRED BUT NOT PROVIDED

The following material is required but not provided in the kit:

- Deionized or distilled water
- Pipettes for delivery of: 50 µL, 150 µL, 200 µL and 1 mL (the use of accurate pipettes with disposable plastic tips is recommended)
- Vortex mixer
- Magnetic stirrer
- Plate shaker (300 to 700 rpm)

- Washer for Microassay Plates
- Microassay Plate reader capable of reading at 450 nm and 650 nm or 630 nm (bichromatic reading)

WARNINGS AND PRECAUTIONS

Safety

- For *in vitro* diagnostic use only.
- The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HBsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures.
- All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.
- Avoid any skin contact with all reagents. Stop Solution contains HCl. In case of contact, wash thoroughly with water.
- Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

STORAGE

- Before opening or reconstitution, all kits components are stable until the expiry date, indicated on the label, if kept at 2°C to 8°C.
- After reconstitution, standards and controls are stable for eight weeks at 2°C to 8°C. For longer storage periods, aliquots should be made and kept at –20°C for maximum 4 months. Avoid subsequent freeze-thaw cycles.
- Freshly prepared Working Wash Solution should be used on the same day.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

REAGENT PREPARATION

Wash Buffer

Prepare an adequate volume of Working Wash solution by adding 199 volumes of DI water to 1 volume of Wash Solution (200x). Use a magnetic stirrer to homogenize. Discard unused Working Wash Solution at the end of the day.

Standard A

Reconstitute the Standard A with 2 mL distilled water.

Standards B-F

Reconstitute the Standards B-F with 1 mL distilled water.

Controls

Reconstitute the Controls with 1 mL distilled water.

Working HRP Conjugate Solution

The Working HRP Conjugate Solution is to be prepared during the 2-hour incubation and at a minimum of 1 hour and 45 minutes before use.

Prepare an adequate volume of Working HRP Conjugate Solution by mixing the three (3) reagents in the following sequence:

1. Reconstitution solution (Conjugate buffer)
2. Biotinylated 25-OH Vitamin D (Concentrated conjugate)
3. Vortex
4. Concentrated HRP
5. Vortex

The order of addition of the three (3) reagents is critical and should be rigorously respected to obtain reproducible Optical Densities.

Prepare an adequate volume of Working HRP Conjugate Solution according to the number of used strips, as indicated below:

- For example, for 6 strips (48 wells): 100 µL of Concentrated Conjugate and 50 µL of Concentrated HRP to 10 mL of Conjugate Buffer.
- Use a vortex to homogenize.
- Until its use keep the working HRP conjugate at room temperature and avoid direct sunlight or use a brown glass vial for its preparation.
- The preparation of working HRP conjugate is not stable and must be discarded if not used.

Number of Strips	Volume of Reconstitution Solution (mL)	Volume of Biotinylated 25OH Vitamin D (µL)	Volume of Concentrated HRP (µL)
1	3	30	15
2	5	50	25
3	6	60	30
4	8	80	40
5	9	90	45
6	10	100	50
7	12	120	60
8	14	140	70
9	16	160	80
10	18	180	90
11	20	200	100
12	22	220	110

SPECIMEN COLLECTION AND STORAGE

This kit is suitable for serum samples.
Serum samples must be kept at 2°C to 8°C.
If the test is not run within 24 hrs, sampling and storage at –20°C is recommended.
Avoid subsequent freeze-thaw cycles.

ASSAY PROCEDURE

Handling notes

- Do not use the kit or components beyond expiry date.
- Do not mix materials from different kit lots.
- Bring all the reagents to room temperature prior to use.
- Thoroughly mix all reagents and samples by gentle agitation or swirling.
- Perform standards, controls and samples in duplicate. Vertical alignment is recommended.
- Use a clean plastic container to prepare the Wash Solution.
- In order to avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample.
- For the dispensing of the TMB Substrate and the Stop Solution, avoid pipettes with metal parts.
- High precision pipettes or automated pipetting equipment will improve the precision.
- Respect the incubation times.
  - **To avoid drift, the time between pipetting of the first standard and the last sample must be limited to the time mentioned in section *SPECIFICITY* (Time delay).**
- Prepare a standard curve for each run; do not use data from previous runs.
- Dispense the TMB Substrate within 15 minutes following the washing of the Microassay Plate.
- During incubation with TMB Substrate, avoid direct sunlight on the Microassay Plate.

Procedure

1. Select the required number of Microassay Plate strips for the run. The unused Microassay Plate strips should be resealed in the bag with a desiccant and stored at 2°C to 8°C.
2. Secure the strips into the holding frame.
3. Pipette 50 µL of each Standard, Control and Sample into the appropriate wells.
4. Pipette 150 µL of Assay Buffer into all the wells.
5. Incubate for 2 hours at room temperature, on a plate shaker (300 to 700 rpm)
6. Prepare the Working HRP Conjugate Solution once the incubation is started (within 15 minutes).
7. Aspirate the liquid from each well.
8. Wash the plate 3 times by:
   - Dispensing 0.4 mL of Wash Solution into each well
   - Aspirating the content of each well
9. Pipette 200 µL of the Working HRP Conjugate Solution into each well. Incubate the Microassay Plate for 30 minutes at room temperature, on a plate shaker (300 to 700 rpm).
10. Aspirate the liquid from each well.
11. Wash the plate 3 times by:
    - Dispensing 0.4 mL of Wash Solution into each well
    - Aspirating the content of each well
12. Pipette 100 µL of the TMB Substrate into each well within 15 minutes following the washing step.
13. Incubate the Microassay Plate for 15 minutes at room temperature, on a plate shaker (300 to 700 rpm), avoid direct sunlight.
14. Pipette 100 µL of Stop Solution into each well.
15. Read the absorbances at 450 nm (reference filter 630 nm or 650 nm) within 1 hour and calculate the results as described in section Interpretation of Results.

INTERNAL QUALITY CONTROL

- If the results obtained for Control L and/or Control H are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.

- If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots. Controls which contain azide will interfere with the enzymatic reaction and cannot be used.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises
- It is recommended that Controls be routinely assayed as unknown samples to measure assay variability. The performance of the assay should be monitored with quality control charts of the controls.
- It is good practise to check visually the curve fit selected by the computer.

INTERPRETATION OF RESULTS

Calculation of Results

1. Read the plate at 450 nm against a reference filter set at 650 nm (or 630 nm).
2. Calculate the mean of duplicate determinations.
3. Calculate for each standard, control and sample:

$$B/B0(\%) = \frac{\text{OD (Standard B-F, Control or Sample)}}{\text{OD (Standard A (zero calibrator))}} \text{ X } 100$$

4. Using either linear-linear of semi-logarithmic graph paper, plot the (B/B0(%)) values for each standard point as a function of the 25OH Vitamin D concentration of each standard point. Reject obvious outliers.
5. Computer assisted methods can also be used to construct the calibration curve. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.
6. By interpolation of the sample (B/B0 (%)) values, determine the 25OH Vitamin D concentrations of the samples from the calibration curve.

TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

Standard	Absorbance (OD)	Result (ng/mL)
A	2.66	0
B	2.39	5.3
C	1.83	15
D	1.46	25.7
E	0.81	54.3
F	0.21	133

EXPECTED VALUES

Dietary intake, race, season and age are known to affect the normal levels of 25OH Vitamin D_3. Each laboratory should establish its own range based on their local population. Review of the current literature has suggested the following ranges for the classification of 25OH Vitamin D status:

Level	ng/mL
Deficient	<10
Insufficient	10-29
Sufficient	30-100
Potential Toxicity	>100

REFERENCE RANGE

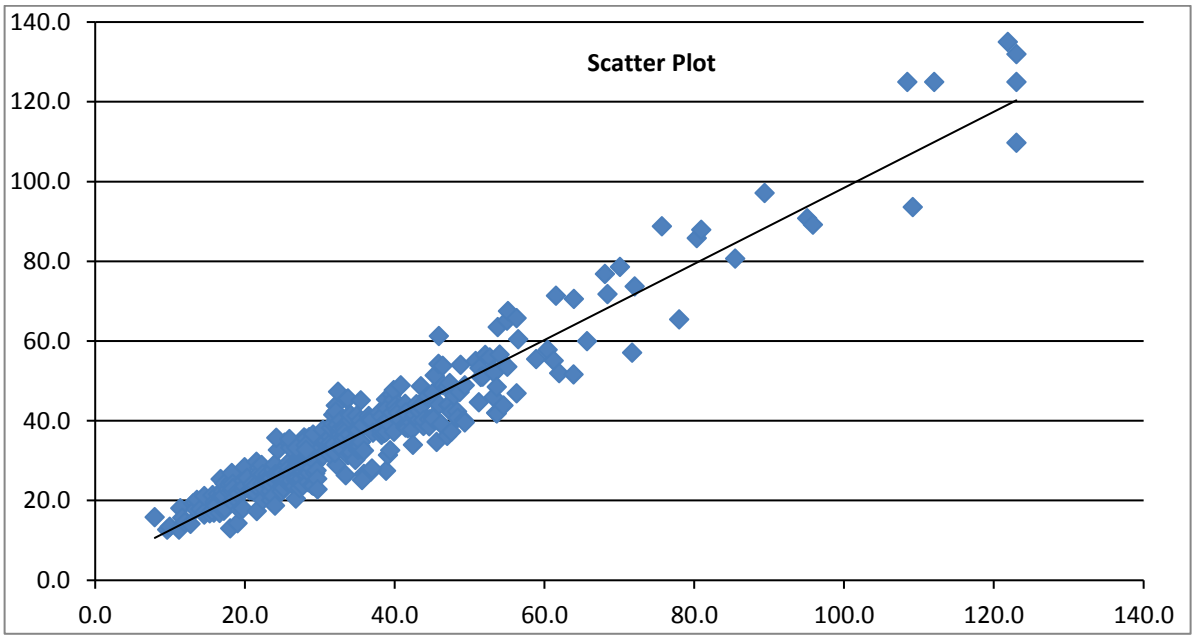
Reference ranges have been established based on 150 apparently healthy individuals. The individual patient serum samples used were obtained from a certified commercial source and were collected from an FDA Licensed Donor Center with informed consent. 50 samples were from Northern U.S. (Pennsylvania), 50 samples were from Central U.S. (Tennessee), and 50 samples were from Southern U.S. (Florida). Samples collected in the winter months (January-March), were between the ages of 21-92 years old and included both light skin and dark skin populations. The samples collected were not taking vitamin D supplements, had no family history of parathyroid, or calcium regulatory disease, had no history or Kidney, Liver, Parathyroid, Calcium related disease or bariatric surgery, and were not taking any medications known to affect absorption or catabolism of Vitamin D. The following table is the summary or results:

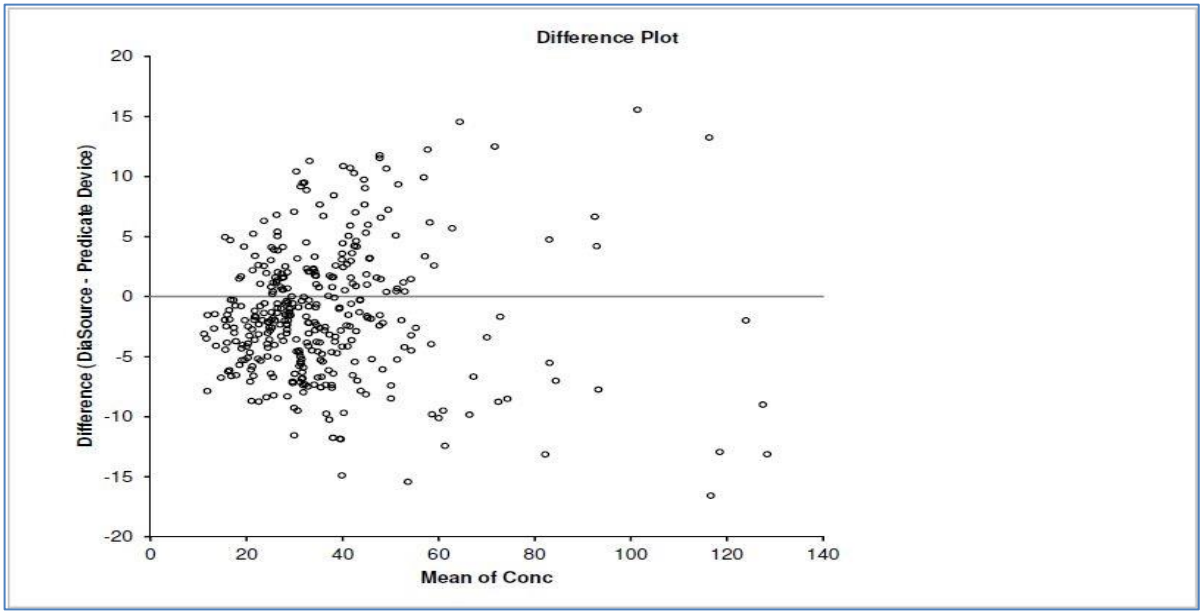
Concentration	**Florida**	**Tennessee**	**Pennsylvania**	**Overall**
Highest Conc. (ng/mL)	88.6	71.7	54.6	88.6
Lowest Conc. (ng/mL)	6.1	4.9	5.9	4.9
Median Conc. (ng/mL)	20.8	17.2	14.3	17.3

Only Central 95% (2.5% - 97.5%) of the results observed were used.

METHOD COMPARISON

The performance of the MicroVue 25-OH Vitamin D EIA Test was determined by conducting a correlation study tested at three different sites using a total of 356 samples. The samples were tested on both the MicroVue 25-OH Vitamin D EIA Test and a commercially available 25OH Vitamin D EIA test. The results ranged from 8.0ng/mL to 123.0ng/mL, the correlation coefficient between the two methods was 0.917, with the 95% confidence interval of 87.6% to 93.6%, a slope of 0.954 and the y-intercept of 3.05. The following graphs summarize the results:





PERFORMANCE OF THE TEST

Limitation of the Test

1. The test is an aid in the diagnosis and is to be used in conjunction with clinical findings.
2. The performance of this assay has not been established in a pediatric population.
3. Samples suspected of containing concentrations above the highest calibrator should be assayed in dilution.
4. Hemolysed samples should not be used.

Limits of Detection

The Limit of Blank (LOB), Limit of Detection (LOD), and the Limit of Quantitation (LOQ), were determined in accordance with the CLSI guideline EP17-A.

- The LOB was calculated by measuring the blank several times and calculating the 95th percentile of the distribution of the test values. The LOB was calculated to be 1.69 ng/mL.
- The LOD was calculated as described in the guideline. The LOD was calculated to be 2.81 ng/mL.
- The LOQ was calculated by testing 5 samples of low value 14 times in different test.
- The LOQ was calculated to be 4.39 ng/mL with CV of 20%.

SPECIFICITY

Cross Reactivity

Cross reactivity of the MicroVue 25-OH Vitamin D EIA was determined by testing sera with spiked and unspiked cross reactants. The results are summarized in the following table:

Compound and Concentration	% Cross Reaction
25 OH-Vitamin D_3 at 10 ng/mL	100
25 OH-Vitamin D_2 at 10 ng/mL	86
1, 25$(OH)_2$-Vitamin D_3 at 200 ng/mL	20
1, 25$(OH)_2$-Vitamin D_2 at 690 ng/mL	1.9
Vitamin D_3 at 200 ng/mL	2.9
Vitamin D_2 at 200 ng/mL	1.3

Compound and Concentration	% Cross Reaction
24,25$(OH)_2$-Vitamin D_3 at 20 ng/mL	>100
25,26$(OH)_2$-Vitamin D_3 at 4 ng/mL	>100
3-epi-25OH-Vitamin D_3 at 20 µg/mL	0.1

Interfering Substances

The effect of potential interfering substances on samples using the MicroVue 25-OH Vitamin D EIA Test was evaluated. Different levels of Hemoglobin, Bilirubin, Triglyceride, Vitamin C, Bilirubin Conjugate and Unconjugated and Zemplar in serum samples were tested on samples with different 25OH Vitamin D concentrations. Our acceptance criteria was to have interference of less than 10%. The tested substances did not affect the performance of the MicroVue 25-OH Vitamin D EIA Test.

Substance	25OH Vitamin D (ng/mL)	Concentration of Interferent (mg/dL)	Mean % Variation
Hemoglobin	7.6	250	–0.6%
		500	
	29.3	250	
		500	
	42.5	250	
		500	
Bilirubin Conjugated	6.0	50	–3.4%
		100	
	21.5	50	
		100	
	38.6	50	
		100	
Bilirubin Unconjugated	7.6	50	2.5%
		100	
	29.3	50	
		100	
	42.5	50	
		100	
Triglyceride	7.6	7.5	–4.3%
		125	
		250	
		500	
	29.3	7.5	
		125	
		250	
		500	
	42.5	7.5	
		125	
		250	
		500	
Vitamin C	6.0	1	2.5%
		10	
		100	

Substance	25OH Vitamin D (ng/mL)	Concentration of Interferent (mg/dL)	Mean % Variation
	21.5	1	
		10	
		100	
	38.6	1	
		10	
		100	
Biotin	8.7	0.2	4.7%
		2	
		4	
	19.8	0.2	
		2	
		4	
	36.1	0.2	
		2	
		4	
Zemplar	17.6	0.0013	–4.4%
		0.0025	
		0.0050	
	33.5	0.0013	
		0.0025	
		0.0050	

Precision

The assay precision was calculated by running samples for a span of at least 20 days on three different lots. The results are summarized in the table below:

Intra-assay				Inter-assay			
Sample	**N**	**<X> ± SD (ng/mL)**	**C.V. (%)**	**Sample**	**N**	**<X> ± SD (ng/mL)**	**C.V. (%)**
A	24	5.5 ± 0.4	7.8	A	39	17.7 ± 1.3	7.4
B	35	27.4 ± 1.5	5.7	B	10	26.3 ± 1.2	4.7
C	35	43.0 ± 1.2	2.7	C	10	42.1 ± 1.8	4.3
D	24	81.2 ± 2.0	2.5	D	21	85.4 ± 7.8	9.2

SD: Standard Deviation, CV: Coefficient of variation

Reproducibility

The reproducibility of the assay was done by testing three samples in duplicate for five days, twice a day, at three sites with two technicians per site. The mean results are summarized in the table below:

Sample	n	ng/mL		Within-Run	Between-Run	Between-Day	Between-Tech	Between-Site	Total
1	57	25.5	SD	0.22	0.61	0.98	1.54	2.21	2.59
			CV	0.3%	0.9%	3.8%	6.0%	8.7%	10.2%

Sample	n	ng/mL		Within-Run	Between-Run	Between-Day	Between-Tech	Between-Site	Total
2	57	52.9	SD	0.64	1.57	1.11	2.28	4.29	5.19
			CV	0.9%	2.3%	2.1%	4.3%	8.1%	9.8%
3	57	124.9	SD	1.00	1.74	1.84	3.39	4.98	6.25
			CV	1.4%	2.5%	1.5%	2.7%	4.0%	5.0%

Recovery

Recovery was assessed by adding different levels of 25OH Vitamin D to samples. The results are summarized in the table below:

Recovery Test	
Added 25OH-Vit D_3 (ng/mL)	**Recovery (%)**
0	100
25	96
50	92
Added 25OH-Vit D_2 (ng/mL)	**Recovery (%)**
0	100
25	105
50	95

Linearity

Two samples with concentrations known to be distributed throughout the measurable range were tested at equidistant dilutions to determine the linear range of the assay. A linear regression analysis was performed. The results are summarized in the following table:

Sample 1

Sample Dilution	Theoretical Concentration (ng/mL)	Measured Concentration (ng/mL)	Slope	Y-Intercept	R^2	Recovery (%)
1/1	96.7	96.7	1.00	–0.30	0.99	100
1/2	48.4	47.6				99
1/4	24.2	24.5				101
1/8	12.1	11.1				92
1/16	6.0	6.2				102

Sample 2

Sample Dilution	Theoretical Concentration (ng/mL)	Measured Concentration (ng/mL)	Slope	Y-Intercept	R^2	Recovery (%)
1/1	122.9	122.9	1.01	0.44	0.99	100
1/2	61.5	64.5				105
1/4	30.7	31.5				103
1/8	15.4	15.0				98
1/16	7.7	7.6				99

The linear range of the assay was found to be 7.7 ng/mL to 122.9 ng/mL.

Time Delay

Time delay test between the last standard and sample dispensing results is shown in the following table.

Time Delay			
	0 min (ng/mL)	10 min (ng/mL)	20 min (ng/mL)
Sample 1	27.9	30.5	30.2
Sample 2	49.5	47.5	49.0

Assay results remain accurate even when Assay Buffer is dispensed 10 and 20 minutes after the standard has been added in the coated wells.

ASSISTANCE

To place an order or for technical support, please contact a Quidel Representative at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern Time. Orders may also be placed by fax at 740.592.9820. For e-mail support, contact customerservice@quidel.com or technicalsupport@quidel.com.

For services outside the U.S., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website quidel.com.

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REF 8046 – MicroVue 25-OH Vitamin D EIA Kit

IVD

CE

EC REP

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PI8046000EN00 (05/17)

Glossary

REF Catalogue number	CE CE mark of conformity
EC REP Authorized Representative in the European Community	LOT Batch code
 Use by	 Manufacturer
 Temperature limitation	 Intended use
 Consult e-labeling instructions for use	IVD For *In Vitro* diagnostic use
 Contains sufficient for 96 determinations	CONT Contents/Contains
CONTROL Control	