25-OH Vitamin D ELISA
Test instruction

For In Vitro Diagnostic Use CLIA Complexity: High

ORDER NO.	ANTIGEN	SUBSTRATE	FORMAT
EQ 6411-9601	25-OH vitamin D	Ab-coated microplate wells	96 x 01 (96)

Intended use: The EUROIMMUN 25-OH Vitamin D ELISA is intended for the quantitative determination of 25-OH Vitamin D and other hydroxylated vitamin D metabolites in human serum and plasma (EDTA, Li-heparin). Results are to be used in conjunction with other clinical and laboratory data to assist the clinician in the assessment of vitamin D sufficiency in adult populations.

Summary and explanation

Clinical significance: The 25-OH Vitamin D ELISA is designed for the serological determination of the vitamin D concentration in the human organism. Types of vitamin D that are differentiated are vitamin D2 (ergocalciferol) that is contained in plant food (mushrooms, avocado) and vitamin D3 (cholecalciferol) that is produced from 7-dehydrocholesterol in the skin under ultra-violet irradiation or found in animal food or products (sea fish, egg yolk, butter). These two forms of vitamin D, which are not yet biologically active, are bound by a protein called VDBP (vitamin D binding protein) in the bloodstream, then metabolised in the liver and converted into 25-OH vitamin D2 (calcidiol) and 25-OH vitamin D3 (calcitriol), respectively, which are storage forms of the vitamin with little activity. In contrast to other commercially available tests, the ELISA uses a newly designed monoclonal antibody which is equally specific for both forms of the vitamin. This is necessary because sometimes vitamin D2 instead of D3 is used in therapy.
It is only with another conversion step in the kidneys that the vitamin becomes the biologically active metabolite 1.25-dihydroxy vitamin D, which functions as a hormone (D-hormone). D-hormone regulates uptake of calcium from the intestinal tract, mineralisation of the bones, differentiation of osteoblasts and bone matrix synthesis. D hormone also influences neuromuscular function, among other things.
The serum level of 25-OH vitamin D, which of all vitamin D metabolites in the blood stores most vitamin D, is the best indicator of the vitamin D supply in the human body.
Vitamin D deficiency is a worldwide problem with serious health effects. Slight vitamin D deficiency, defined by a 25-OH vitamin D level of 12-30 ng/ml (30-75 nmol/l) in the blood, is enough to cause a secondary increase in parathormone and a higher osteolysis rate. Vitamin D deficiency is one of the most important risk factors for osteoporosis. Early detection of vitamin D deficiency allows effective prevention of bone fractures (particularly femoral neck and radius) by vitamin D supplementation. Severe vitamin D deficiency at 25-OH vitamin D levels of <12 ng/ml (<30 nmol/l) leads, for example, to rickets in children and osteomalacia in adults, which are both characterised by defective bone growth and matrix mineralisation.
Abnormal vitamin D serum levels can be a single or accidental causative factor in many diseases.

Antibodies: The reagent wells are coated with monoclonal antibodies which identify specifically 25-OH vitamin D_3 and 25-OH vitamin D_2.

Principles of the test: This ELISA test kit is designed for the in vitro determination of 25-OH vitamin D in human serum or plasma samples. In the first analysis step, the calibrators and patient samples are diluted with biotin-labelled 25-OH vitamin D and added to microplate wells coated with monoclonal anti-25-OH vitamin D antibodies. During the incubation an unknown amount of 25-OH vitamin D in the patient sample and a known amount of biotin-labelled 25-OH vitamin D compete for the antibody binding sites in the microplate wells plate. Unbound 25-OH vitamin D is removed by washing. For the detection of bound biotin-labelled 25-OH vitamin D, a second incubation is performed using peroxidase-labelled streptavidin. In a third incubation using the peroxidase substrate tetramethylbenzidine (TMB) the bound peroxidase promotes a colour reaction. The colour intensity is inversely proportional to the 25-OH vitamin D concentration in the sample. Results for the samples can be calculated directly using a standard curve.

Materials supplied in kit

Contents of the test kit:

Component	Color	Format	Symbol
1. Antibody-coated (Sheep Monoclonal) **microplate wells** 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	STRIPS
2. Calibrator 1 (0 ng/mL 25-OH vitamin D) liquid in horse serum with preservatives	red-brown	1 x 1.0 ml	CAL 1
3. Calibrator 2 (4 ng/mL 25-OH vitamin D) liquid in horse serum with preservatives	red-brown	1 x 1.0 ml	CAL 2
4. Calibrator 3 (10 ng/mL 25-OH vitamin D) liquid in horse serum with preservatives	red-brown	1 x 1.0 ml	CAL 3
5. Calibrator 4 (25 ng/mL 25-OH vitamin D) liquid in horse serum with preservatives	red-brown	1 x 1.0 ml	CAL 4
6. Calibrator 5 (60 ng/mL 25-OH vitamin D) liquid in horse serum with preservatives	red-brown	1 x 1.0 ml	CAL 5
7. Calibrator 6 (120 ng/mL 25-OH vitamin D) liquid in horse serum with preservatives	red-brown	1 x 1.0 ml	CAL 6
8. Control 1 (25-OH vitamin D) liquid in horse serum with preservatives	red-brown	1 x 1.0 ml	CONTROL 1
9. Control 2 (25-OH vitamin D) liquid in horse serum with preservatives	red-brown	1 x 1.0 ml	CONTROL 2
10. Biotin (100x concentrate) 25-OH vitamin D labelled with biotin in solution	blue	1 x 1.2 ml	BIOTIN 100x
11. Sample buffer MES based liquid buffer pH 5.5	yellow	1 x 100 ml	SAMPLE BUFFER
12. Enzyme conjugate, ready for use, BSA based liquid buffer with Streptavidin-Peroxidase	blue	1 x 12 ml	CONJUGATE
13. Wash buffer (10x concentrate) liquid PBS based buffer with Tween	colorless	1 x 100 ml	WASH BUFFER 10x
14. Chromogen/substrate solution (TMB/H_2O_2), ready for use	colorless	1 x 12 ml	SUBSTRATE
15. Stop solution (0.5 M H_2SO_4), ready for use	colorless	1 x 12 ml	STOP SOLUTION
16. Test instruction	---	1 booklet	
17. Quality control certificate	---	1 protocol	
LOT Lot			Storage temperature
IVD In vitro diagnostic medical device			Unopened usable until

Calibrators and controls are supplied in liquid form and are horse serum based with active ingredients of 0.09% ProClin 950 and 0.09% sodium azide. Calibrators are 25-OH vitamin D3 spiked. Calibrators, controls and all used components should be treated as potentially infectious agents and disposal of in accordance with local and state regulations. No special treatment necessary for the use of the calibrators and controls except reagents must be mixed thoroughly before use either manually or by vortexing.

Additional materials and equipment (not supplied):

- **Automatic Microplate Plate Washer:** This is recommended, however, plate washing can be performed manually.
- **Plate Reader:** Capable of measuring optical densities at 450 nm and a reference wavelength of between 620 nm and 650 nm.
- **Calibrated Micropipettes:** For dispensing 500, 200 and 20 µl.
- **Multichannel pipette:** Recommended for dispensing 100 µl volumes of conjugate, substrate and stop solution.
- **Glass/Plastic tubes:** For sample dilution.
- **Vortex mixer.**

Warnings and precautions

1. **For in vitro diagnostic use. For use by laboratory professionals in a clinical or research laboratory setting.**
2. Before starting the assay, carefully read the instructions. Use only the valid version provided with the kit.
3. Observe prudent laboratory practice and safety guidelines. Avoid eye and skin contact with specimens and reagents. In case of eye or skin contact, wash off thoroughly with plenty of clean running water. Remove and wash contaminated clothing. In case of ingestion, obtain medical attention.
4. The serum contained in the calibrators and controls are of animal origin (horse). Handle kit reagents as if capable of transmitting an infectious agent. Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. Disposal of kit reagents should be in accordance with local regulations.
5. Calibrators and controls are supplied in liquid form and are horse serum based with active ingredients of 0.09% ProClin 950 and 0.09% sodium azide. Avoid skin contact. Calibrators are 25-OH vitamin D3 spiked. No special treatment necessary for the use of the calibrators and controls except reagents must be mixed thoroughly before use either manually or by vortexing.
6. Some of the reagents contain sodium azide at a concentration of ≤ 0.09%. Sodium azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions. Rinse sink thoroughly with water after disposing of solutions containing azide.
7. **Storage and stability:** The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.
8. **Waste disposal:** Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.
9. Material safety data sheets for this product are available upon request.

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months, but not longer than the indicated expiry date.
- **Calibrators and controls:** The reagents must be mixed thoroughly before use.
- **Biotin:** The biotin is a 100x concentrate. Mix thoroughly before diluting. The required volume should be removed with a clean pipette tip and diluted in sample buffer (1 part biotin plus 99 parts sample buffer). Example: 1 ml biotin concentrate plus 99 ml sample buffer.
  The working-strength biotin is stable for 2 weeks when stored at +2 °C to +8 °C. For longer storage freeze at -20 °C.
- **Sample buffer:** It can be used for sample dilution after adding the biotin concentrate.

- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallization occurs in the concentrated buffer, warm it to 37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionized or distilled water (1 part reagent plus 9 parts distilled water).
  The working-strength wash buffer is stable until the expiry date when stored at +2 °C to +8 °C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue colored.

- **Stop solution:** Ready for use.

Preparation and stability of the patient samples

Sample material: Human serum or EDTA or heparin plasma.

Stability: CLSI (formerly NCCLS) Document H18-A2 recommends the following storage conditions for samples: Samples should be stored at room temperature no longer than 8 hours. If the assay will not be completed within 8 hours, the samples should be refrigerated at 2-8°C. If the assay will not be completed within 48 hours, or for shipment of the sample, samples should be frozen at -20°C or lower. Frozen samples must be mixed well after thawing and prior to testing. Diluted samples should be incubated within 8 hours. Do not use bacterially contaminated samples.

Please note: Diluted samples should only be used for one test run and subsequently discarded. Always use fresh samples and calibrator dilutions for every test run!

Performance: The calibrators/controls and patient samples for analysis are diluted 1:26 in working strength biotin.
Always pipette the samples and calibrators into the dilution tubes first (not included in kit; adequate are common commercial available single-use tubes consisting of borosilicateglas, polypropylene or polystyrene). Then add the working strength biotin within 5 minutes and leave to act for 10 minutes at room temperature.

Example: Add 0.5 ml of working strength biotin to 20 µl of sample and mix thoroughly (vortex). Incubate the mixture for at least 10 minutes at room temperature (+18°C to +25°C). The samples can subsequently be pipetted into the reagent wells according to the pipetting scheme.

Procedure

(Partly) manual test performance

Sample incubation: (1st step)
Pipette **200 µl** of sample diluted in biotin/sample buffer into each of the microplate wells. Incubate for **2 hours** at room temperature (+18 °C to +25 °C).

Washing:
Manual: Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Wash reagent wells 3 times with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Modus").
Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Enzyme conjugate incubation: (2nd step)
Pipette **100 µl** of enzyme conjugate (streptavidin-peroxidase) into each of the microplate wells and incubate for **30 minutes** at room temperature (+18 °C to +25 °C).

Washing:
Empty the wells. Wash as described above.

Substrate incubation: (3rd step)
Pipette **100 µl** of chromogen/substrate solution into each of the microplate wells. Incubate for **15 minutes** at room temperature (+18°C to 25°C) (protect from direct sunlight).

Stopping the reaction:
Pipette **100 µl** of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution.** Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Test performance using fully automated analysis devices
Sample dilution and test performance are carried out fully automatically using the analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the EUROIMMUN Analyzer I, Analyzer I-2P or the DSX from Dynex and this EUROIMMUN ELISA. Validation documents are available on inquiry.
Automated test performance using other fully automated, open system analysis devices is possible, however, the combination should be validated by the user.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	**C 1**	**P 1**	**P 9**									
B	**C 2**	**P 2**	**P 10**									
C	**C 3**	**P 3**	**P 11**									
D	**C 4**	**P 4**	**P 12**									
E	**C 5**	**P 5**	**P 13**									
F	**C 6**	**P 6**	**P 14**									
G	**Co 1**	**P 7**	**P 15**									
H	**Co 2**	**P 8**	**P 16**									

The pipetting protocol for microtiter strips 1-6 is an example for the **quantitative analysis** of 16 patient sample (P 1 to P 16).

The calibrators (C 1 to C 6), the control 1 (Co1) and control 2 (Co2), and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.

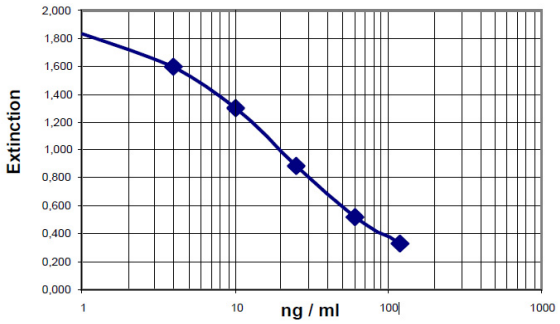
The controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

Calibration: As there is no international standard, the standards and controls are calibrated gravimetrically using UV-Vis (264nm) verified stock standards and compared with NIST standards (National Institute of Standards and Technology, USA), DEQAS (Vitamin D External Quality Assessment Scheme, UK) quality assessment data and in-house quality control sera.

For every group of tests performed, the values of the concentrations must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.

Quantitative analysis: The standard curve from which the 25-OH vitamin D concentrations in the serum samples can be taken is obtained by point-to-point plotting of the OD values measured for the 6 calibration sera against the corresponding units (linear/log). Use “4-PL” or “cubic-spline” plotting for calculation of the standard curve by computer.
For correct logarithmic representation it might be necessary to set the concentration of calibrator 1 from 0 to e.g. 0.1 ng/ml. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of concentrations in patient samples.



If the optical density (OD) of a patient sample lies below the value of calibrator 6 (120 ng/ml), the result should be given as “>120 ng/ml“. It is recommended that the sample be re-tested at an initial dilution of 1:2 with calibrator 1 before following the test instruction. The result in ng/ml read from the calibration curve for this sample must then be multiplied by a factor of 2.
If the optical density (OD) of a patient sample lies above the functional sensitivity (4 ng/ml), the result should be given as “<4 ng/ml“.

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another the sample should be retested.

Therapeutic decisions should not be made on the basis of results from this test, but only under consideration of clinical findings and further diagnostic values.

Calculation:
25-OH vitamin D_3 (ng/mL) x 2.5 = 25-OH vitamin D_3 (nmol/L)

Limitations of the procedure

1. This kit is used as an aid in diagnosis only. A positive result should be interpreted with clinical findings and other serological tests.
2. The results obtained from this assay are not diagnostic proof of the presence or absence of a disease.
3. The activity of the enzyme used is temperature-dependent and the OD values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the OD values. Corresponding variations apply also to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.
4. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
5. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect OD values.

Expected values

Reference range: The levels of 25-OH vitamin D were analyzed in a panel of 206 samples from healthy subjects (70 men and 136 women with an average age of 64 years; age range: 22 – 99 years) from a commercial source from the US. The observed median, minimum and maximum values as well as the 2.5% and 97.5% percentiles were similar to those reported for other devices cleared in the US.

n = 206	25-OH Vitamin-D ELISA	
	ng/ml	nmol/l
Median	19.4	48.4
Range	< 4 to 64.8	< 10 to 162.0
2.5% percentile	5.4	13.6
97.5% percentile	47.0	117.4

This data is provided for guidance only. It is important for each laboratory to establish its own reference ranges, representative of its typical population. Also published studies representing the local population can be taken into consideration.

Interpretation criteria are provided by the US CDC/NCHS National Health and Nutrition Examination Surveys (NHANES) [14]:

Status	Serum 25-OH vitamin-D	
	ng/ml	nmol/l
At risk of vitamin D deficiency	< 12	< 30
At risk of vitamin D inadequacy	12 – 19	30 – 49
Sufficient in vitamin D	20 – 50	50 – 125
Possibly harmful vitamin D	> 50	> 125

The Endocrine Society Clinical Practice Guideline (2011) recently suggested a higher target level of at least 30 ng/ml [15]:

Status	25-OH vitamin-D	
	ng/ml	nmol/l
Deficiency	< 20	< 50
Insufficiency	20 – 29	50 – 75
Sufficiency	30 – 100	75 – 250

Performance characteristics

Limit of blank (LoB), limit of detection (LoD) and functional sensitivity (FS): LoB was determined as the concentration corresponding to the mean OD of the zero calibrator minus 1.645 times the standard deviation using the mean of 60 replicates for calibrator 1 (0 ng/ml) and the mean of 20 replicates for calibrator 2 (4 ng/ml). LoB was found to be 0.4 ng/ml.
LoD was determined as the LoB plus 1.645 times the standard deviation of 200 determinations from 5 samples in the low range of 2 to 10 ng/ml, measured in 5 independent runs with 8 replicates per run. The mean LoD was found to be 1.8 ng/ml.
FS is defined as the lowest concentration at which the potential regression line crosses the 20% CV line and was determined from a plot of the mean concentrations (X-axis) vs. % CVs (Y-axis). FS was found to be 4.0 ng/ml. Results below 4 ng/mL are reported as "< 4 ng/mL".

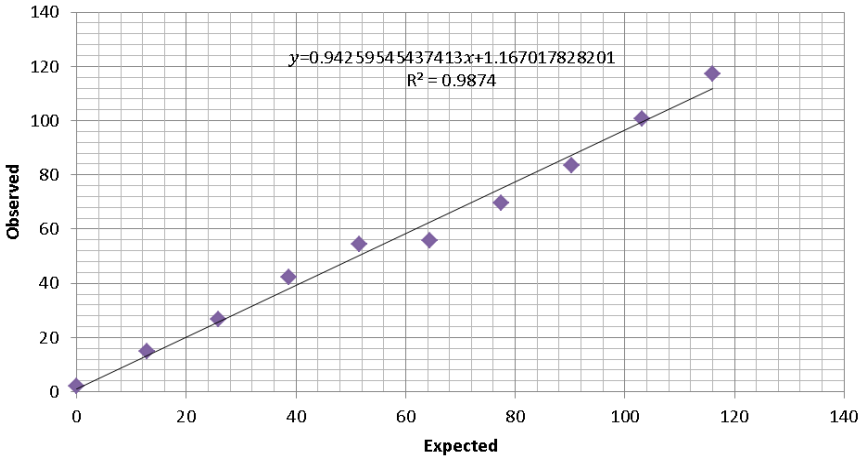
Reproducibility: The reproducibility of the test was investigated by determining the intra-, inter-assay and lot-to-lot coefficients of variation using 8 sera from different areas of the calibration curve. The intra-assay CVs are based on 40 measurements for each serum and the inter-assay CVs on four measurements performed in ten different test runs. The inter-lot CVs are based on 32 determinations performed in 8 different runs on 4 different lots (with 2 runs per lot and 4 replicates per run).

Intra-Assay Variation n = *40*		
Serum	**Mean (ng/ml)**	**CV (%)**
1	4.1	12.4
2	16.8	5.5
3	24.6	6.9
4	28.8	7.4
5	42.9	4.2
6	46.3	6.0
7	68.7	5.1
8	93.3	6.7

Lot-to-Lot Assay Variation n = *32*		
Serum	**Mean (ng/ml)**	**CV (%)**
1	7.3	12.2
2	18.5	10.2
3	24.8	7.3
4	37.4	6.1
5	47.6	8.9
6	58.0	7.0
7	74.3	8.9
8	97.3	9.5

Inter-Assay Variation n = *10 x 4*		
Serum	**Mean (ng/ml)**	**CV (%)**
1	5.8	16.2
2	16.6	7.8
3	22.3	8.1
4	34.8	8.6
5	43.5	7.0
6	55.3	6.7
7	67.8	8.6
8	94.4	8.3

Linearity: Sets of sample preparations were prepared by mixing of a natural negative sample (0 ng/ml) and high positive samples, covering the concentration range of 2 to 129 ng/ml. Each sample preparation was run in double determinations and polynomial regression performed of mean observed results vs expected results. The amount of non-linearity above the functional sensitivity of 4 ng/ml was found acceptable below 15%. The assay is sufficiently linear from 4 to 120 ng/ml.



Cross reactivity: This ELISA detects 25-OH Vitamin D_2 and D_3 specifically. Cross reactions with other metabolites are given in the following table.

Potential Cross-Reacting Substance	**Conc. Spiked** (ng/ml)	**Conc. Observed** (ng/ml)	**Cross Reactivity** (%)
25-OH Vitamin D3	10.0	10.0	100
25-OH Vitamin D2	25.0	24.3	100
24,25-OH Vitamin D3	100	0.3	0.3
Cholecalciferol (Vit. D3)	10,000	3.4	0.03
Ergocalciferol (Vitamin D2)	10,000	5.1	0.05
1,25-OH Vitamin D3	10.0	4.3	45*
1,25-OH Vitamin D2	10.0	19.8	212*
3-epi-25-OH Vitamin D3	10.0	1.7	17

*Expected concentration in natural samples is below 100 ng/ml, so the obtained cross reactivity has no significant influence on results of 25-OH Vitamin D.

Interferences: Samples spiked with potential interfering substances showed no influence on the result up to a concentration of 750 mg/dl for hemoglobin, 2000 mg/dl for triglycerides, 40 mg/dl for bilirubin, 400 mg/dl for cholesterol, 1000 mg/dl for biotin and 10.0 mg/ml for ascorbic acid in this ELISA. Significant interference (above 10 % deviation from unspiked sample) was seen with 1000 mg/dl of hemoglobin.

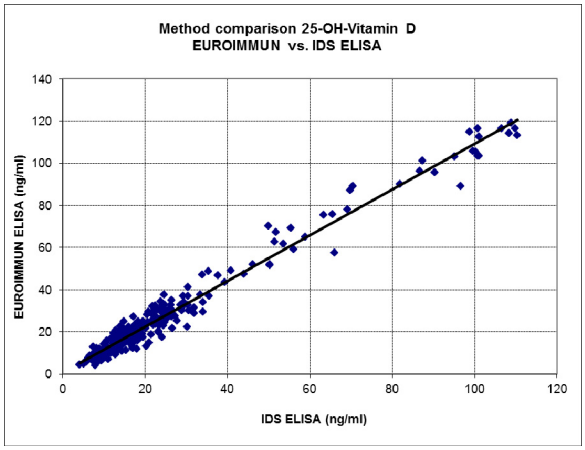
Serum/plasma comparison: The usability of plasma was determined by assaying 38 serum samples (covering the concentration range of 9 – 105 ng/ml) and corresponding EDTA and heparin plasma samples drawn simultaneously. To ensure distribution across the reportable range, 3 sample pairs in the set were spiked with 25-OH Vitamin D. Passing-Bablok regression was calculated for each type of plasma. The results of the Passing-Bablok regression analysis are given below.

	EDTA-Plasma	**Heparin-Plasma**
Regression equation	y = 0.29 + 0.99 x	y = 0.55 + 0.97 x
95% C.I. of intercept	-0.37 to 1.18	-0.65 to 1.33
95% C.I. of slope	0.93 to 1.02	0.93 to 1.04
Coefficient of determination R^2	0.996	0.993
Mean %recovery	100 %	101 %

y = concentration in serum (ng/ml), x = concentration in plasma (ng/ml)

Method comparison: Serum samples were obtained from different sources (141 prospective samples sent in for 25-OH-Vitamin D testing from a clinical laboratory, 28 samples from 25-OH-Vitamin D quality assessment programs, 5 samples from a 25-OH-Vitamin D quality control panel and 30 samples from normal blood donors). To ensure distribution across the reportable range, 36 samples (15 %) in the set were spiked with 25-OH Vitamin D. In total, 240 samples were collected, ranging from 4.1 to 119.1 ng/ml, and tested with the EUROIMMUN 25-OH Vitamin D ELISA and with a commercially available FDA-cleared ELISA assay. Results of linear regression analysis are shown in the table below.

n	240
Concentration range (predicate)	4.1 – 110.4 ng/ml
Concentration range (candidate)	4.1 – 119.1 ng/ml
Regression equation (y = candidate, x = predicate)	y = 0.78 + 1.08 x
95% C.I. of intercept	-0.06 – 1.63
95% C.I. of slope	1.06 – 1.11
Correlation coefficient R	0.9858
95% C.I. of R	0.9817 – 0.9890



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