Vitamin D-CHECK-1

Quantitative determination of Vitamin D in whole blood, plasma or serum samples
-FOR EASY READER+® USE ONLY –
Ref. 13091/GB_RUO (20 tests)
FOR RESEARCH USE ONLY

I. PRINCIPLE

Vitamin D is a lipid-soluble steroid hormone that exists in two primary forms: vitamin D_2 (Ergocalciferol) and vitamin D_3 (cholecalciferol). The Vitamin D_3 is naturally produced in the skin through the exposure to ultraviolet light or found in certain food sources such as oily fish and variably fortified foods (milk, juices, soy, etc…) while Vitamin D_2 is found in certain plants and mushrooms. The Vitamin D_3 and D_2 have a relatively high affinity for a α2-globulin which functions as a transport protein (Vitamin D binding protein) present in the blood. They must undergo a series of metabolic transformations in the liver and then in the kidney to its biologically active form 1-25-dihydroxyVitamin D (1, 2). The major circulating form of vitamin D is 25-OH-Vitamin D. For this reason, the bloodstream concentration of 25-OH-Vitamin D is considered as the best for Vitamin D status assessment and very well reflects Vitamin D_3 and D_2 inputs (3).

The well-known action of this hormone is the regulation of calcium and phosphors homeostasis by enhancing intestinal absorption of calcium. This system plays an important role in bone metabolism. Abnormalities of this system can lead to bone deformation (commonly known as rickets) in infants and children while in adults, proximal muscle weakness, bone pain and increasing risk of osteoporosis may develop (4, 5). On the other hand, vitamin D also has non-phosphocalcic actions via an autocrine action that affects numerous physiological processes other than bone and mineral metabolism. Thus, vitamin D deficiency is also associated to non-musculoskeletal risks including increased risk of various chronic diseases, auto-immune diseases or cardiovascular risks etc… (6, 7). Deficiency in Vitamin D is therefore linked to various diseases and its prevalence is high in numerous countries, making it a worldwide problem (8, 9).

Vitamin D-CHECK-1 test is a quantitative manual assay for the detection of 25-OH-Vitamin D in whole blood, plasma or serum samples. The method is based on a unique combination of monoclonal dye conjugate and solid phase antibodies to selectively detect 25-OH-Vitamin D with a high degree of sensitivity and specificity.

Depending on the 25-OH-Vitamin D concentration in the sample, different lines intensities will appear on the test allowing its quantitative measurement when used in combination with VEDALAB's EASYREADER+® instrument.

II. VITAMIN D-CHECK-1 KIT COMPONENTS

Each kit contains everything needed to perform 20 tests.

- Aluminium pouches containing:	20
1 Vitamin D-CHECK-1 test device	
1 desiccant bag	
- Disposable plastic pipettes in a protective plastic bag	20
- Diluent in a dropper bottle	5 mL
- Leaflet	1

Material required but not provided:
- Timer

III. STORAGE AND STABILITY

1- All Vitamin D-CHECK-1 kit components should be stored at room temperature (+4°C to +30°C) in the sealed pouch.
2- ***Do not freeze the test kit.***
3- The Vitamin D-CHECK-1 kit is stable until the expiry date stated on the package label.

IV. PRECAUTIONS

1- This test is designed for ***research use only*** and not for diagnostic use**,** nor for self-testing nor for near patient use.
2- Read the instructions for use carefully before proceeding the test.
3- Handle all specimens as if containing infectious agents. When the assay procedure is completed, dispose of specimens carefully after autoclaving them for at least one hour. Alternatively, they can be treated with 0.5% to 1% solution of sodium hypochlorite for one hour before disposal.
4- Wear protective clothing such as laboratory coats and disposable gloves while assaying samples.
5- Do not eat, drink nor smoke in the area where specimens and kit reagents are handled.
6- Avoid any contact between hands and eyes or nose during specimen collection and testing.
7- Do not use beyond the expiration date which appears on the package label.
8- Do not use a test from a damaged protective wrapper or if one of the components is damaged.
7- After opening:
- the reaction device should be used immediately,
- the diluent in the dropper bottle could be used until expiry date.

9- If anticoagulants are needed, only EDTA or heparin should be used. Do not use citrated samples.

V. SPECIMEN COLLECTION AND PREPARATION

1- Vitamin D-CHECK-1 test is to be performed using human whole blood, plasma or serum samples. **Do not use haemolyzed samples.**
2- The specimen should be collected under the standard laboratory conditions (aseptically in such a way as to avoid haemolysis).
3- Patients serum or plasma samples are best performed if tested immediately.
4- Whole blood samples should be tested immediately (<4 hours). Fingerprick samples should be assayed just after collection.
5- If the test is to be run within 48 hours after collection the specimen (serum or plasma) should be stored in the refrigerator (+2°C to +8°C). If testing is delayed more than 48 hours, the specimen should be frozen. The frozen specimen must be completely thawed, thoroughly mixed and brought to room temperature prior to testing. Avoid repeated freezing and thawing.
6- Samples showing cloudiness, high viscosity or particulate matter, should not be tested.

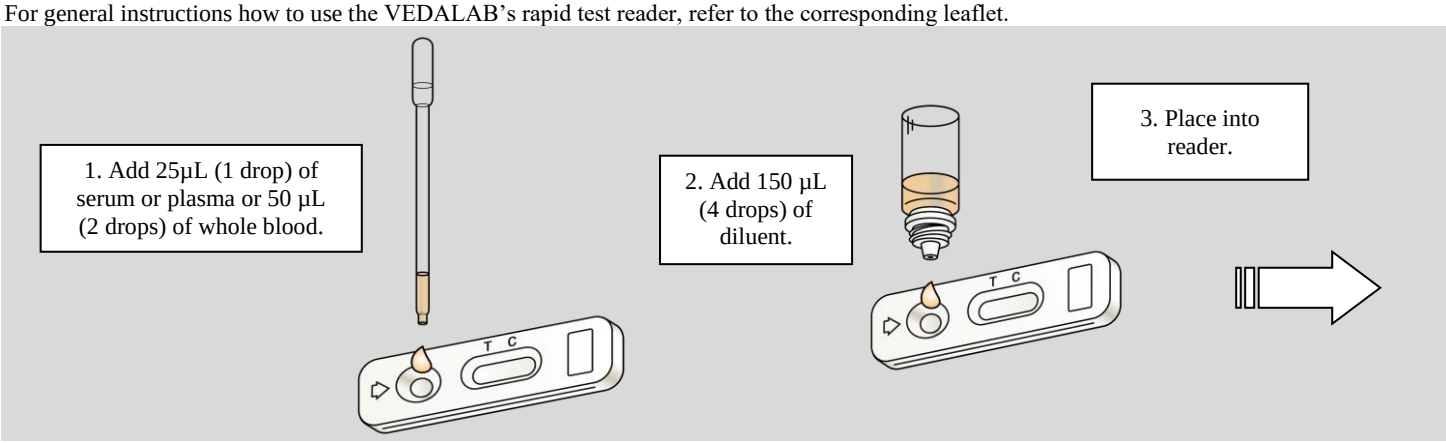
VI. ASSAY PROCEDURE

IMPORTANT: Switch the reader on and allow it to warm up for at least 30 minutes before performing any measurements.

Follow the below instructions or refer to the picture n°1.

1- Allow samples and Vitamin D-CHECK-1 test devices to come to room temperature prior to testing.
2- Remove the reaction device from its protective wrapper by tearing along the split.
3- Label device with the patient's name.

4- Fill the plastic pipette with specimen (serum or plasma) and by holding it vertically, dispense 1 drop (25 µL) into sample well (▷). If whole blood sample is assayed, dispense 2 drops (50 µL) into the sample well (▷) and wait for the whole blood sample to be completely absorbed before adding diluent.
5- Hold the diluent bottle vertically and add exactly 4 drops (150 µL) of diluent into the sample well **with an interval of 2-3 seconds between each drop.**
6- Read the result **(in ng/mL**) after 15 minutes either using the immediate or countdown reading mode.
For general instructions how to use the VEDALAB's rapid test reader, refer to the corresponding leaflet.



Picture n° 1

VII. PERFORMANCES CHARACTERISTICS

a) Measuring range

The measuring range is 8-60 ng/mL.
For the Vitamin D concentration lower than 8 ng/mL, the result will be shown as "<8 ng/mL".
For the Vitamin D concentration higher than 60 ng/mL, the result will be shown as ">60 ng/mL".

b) Linearity

A study has been performed using serum samples have been spiked with Vitamin D reference material NIST (972a standard) and other vitamin D standards (metrologically related to the NIST standard). Theses samples have been assayed using a reference method (Vidas Biomerieux) Analyser). The results show that the values obtained using the Vitamin D-CHECK-1 test and VEDALAB's EASYREADER+® instrument correlate well with the reference method. The dose-response curve fits a linear regression in the range of 8 to 60 ng/mL as follows:

$$y = 1.0163x - 0.1686$$

Linear regression coefficient (R^2= 0.9945)

c) Accuracy

Serial dilution of Vitamin D reference material NIST (972a standard) and other vitamin D standards (metrologically related to the NIST standard) in Vitamin D negative serum have been tested using Vitamin D-CHECK-1 quantitative test. The obtained results are summarized in Table 1.

	Vitamin D concentration (ng/mL)					
Total 25-OH-vitamin D theorical concentration of vitamin D standards	0	9.8	14.7	22.0	33.0	50.0
Vitamin D concentration measured using VIDAS analyser	<8.1	9.2	17.7	23.6	30.0	50.9
Mean value obtained using Vitamin D-CHECK-1 device	<8	10.0	16.0	23.8	31.6	51.3
CV* (%)	NA**	13.9	14.9	11.8	14.9	5.0
Bias (%)	NA	+8.7	-9.6	+0.8	+5.3	+0.8

*CV: Coefficient of variation
**NA: Not applicable

Table 1: Accuracy

The bias between nominal and measured values is statistically (95% t-test) non-significant and Vitamin D concentrations determined using Vitamin D-CHECK-1 test are accurately measured when compared to NIST 972a reference material.

d) Analytical sensitivity

The analytical sensitivity of Vitamin D-CHECK-1 is 8 ng/mL when using the NIST 972a Standard.

e) Analytical specificity (cross reactivity)
The following substances have been tested for cross reaction at 1,000 ng/mL (table 2). The data have been communicated by the supplier of the anti-Vitamin D antibodies.

Substances	Reactivity (%)
Vitamin D_2 (Ergocalciferol)	None
Vitamin D_3 (cholecalciferol)	None

Table 2: Analytical specificity

f) Expected values
There is currently debate over the optimal values of 25 (OH) Vitamin D in serum. In 2011, the Clinical Guidelines Subcommittee of the Endocrine Society Task Force established the guidelines below for recommended serum 25 (OH) Vitamin D levels [3]. Other clinical reference citations may show different values.

Vitamin D Status	25 (OH) Vitamin D Concentration Range (ng/mL)	25 (OH) Vitamin D Concentration Range (nmol/L)
Deficient	<20	<50
Insufficient	20 to <30	50 to <75
Sufficient	30 - 100	75 – 250

Table 3: Expected values

g) Diagnostic sensitivity and specificity and overall correlation
A panel of 148 human pre-assayed serum samples (Biomerieux VIDAS analyser) is assayed using the Vitamin D-CHECK-1 quantitative test and VEDALAB's EASYREADER+®.
A summary of obtained results (using VEDALAB reader) is reported in the tables 4 and 5 (negative samples for vitamin D deficiency correspond to samples for which the vitamin D concentration is over 20 ng/mL and positive samples correspond to samples for which the vitamin D concentration is below 20 ng/mL).

		VIDAS		
		Positive	Negative	**Total**
Vitamin D-CHECK-1	Positive	49	3	**52**
	Negative	2	94	**96**
	Total	**51**	**97**	**148**

Table 4: Summary of results

	Vitamin D-CHECK-1
Diagnostic sensitivity	(49/(49+2)) x 100 = 96.08% (CI 95%*** [85.86 – 100.00])
Diagnostic specificity	(94/(94+3)) x 100 = 96.91% (CI 95% [90.79 – 99.60])
Global correlation	((49+94)/148) x 100 = 96.62% (CI 95% [91.97 – 98.98])
Positive predictive value	(49/(49+3)) x 100 = 94.23% (CI 95% [83.26 – 99.26])
Negative predictive value	(94/(94+2)) x 100 = 97.92% (CI 95% [92.28 – 100.00])
Positive likelihood ratio (LR+)	(49/(49+2))/(3/(94+3)) = 31.07
Negative likelihood ratio (LR-)	(2/49+2))/(94/(94+3)) = 0.04

Table 5: Results
****CI 95%: 95% Confidence Interval*

The positive and negative likelihood ratios of 31.07 and 0.04 respectively show that the Vitamin D-CHECK-1 quantitative test greatly helps in the identification of vitamin D deficiency.

h) Hook effect
High positive serum samples having Vitamin D concentration up to 1,000 ng/mL constantly showed positive results (">60 ng/mL") and did not show any hook effect.

i) Matrix effect
31 whole blood, plasma and serum samples have been assayed using the Vitamin D-CHECK-1 quantitative test. There was no matrix effect observed between whole blood, plasma or serum samples.

j) Intra-lot repeatability
Within run precision was evaluated by performing 25 replicates of three serum samples with different concentrations (13, 32.7 and 47.7 ng/mL) using Vitamin D-CHECK-1 quantitative test. Coefficients of variations obtained are respectively 19.7%, 14.4% and 9.7%.

k) Reproducibility
Reproducibility performance was determined by assaying three serum samples with different concentration (15, 30 and 45 ng/mL), using three different lots of Vitamin D-CHECK-1 quantitative test, three different lots of diluent, on consecutive 3 days or with 3 different operators. Results obtained with Vitamin D-CHECK-1 quantitative test show an excellent reproducibility whatever the test or diluent lot, the performing day or the operator.

l) Interferences

1- Rheumatoid factor (RF)
Three different Vitamin D negative serum samples having a RF concentration of 931 IU/mL, 950 IU/mL and 1,360 IU/mL have been tested with the Vitamin D-CHECK-1 test. The obtained results were constantly "<8 ng/mL" therefore showing no interferences with RF up to 1,360 IU/mL.

2- HAMA
Negative Vitamin D serum samples spiked with type 1 or type 2 HAMA (Human anti-mouse antibodies) were tested with the Vitamin D-CHECK-1 test. The obtained results were constantly "<8 ng/mL" therefore showing no interferences with type 1 or type 2 HAMA.

3-Anticoagulants
Three serum samples, spiked with either EDTA dipotassium (final concentration: 1.8 mg/mL), citrate trisodic (final concentration: 32 mg/mL) or heparin lithium (final concentration: 17 U/mL) have been tested with Vitamin D-CHECK-1. The EDTA dipotassium and heparin lithium are not showing any effect on Vitamin D-CHECK-1 quantitative test results (negative or positive). However, the Citrate trisodic are interfering with the sample, and should not be use.

4-Hemoglobin, bilirubin and triglycerides
Three serum samples containing, either hemoglobin (final concentration: 3 g/L), bilirubin (final concentration: 30 mg/L) or triglycerides (final concentration: 15 g/L) did not show any effect on Vitamin D-CHECK-1 quantitative test results (negative or positive).

VIII. LIMITATIONS
1- As for any ~~diagnostic~~ procedure, the physician should evaluate the data obtained using this kit in the light of the other clinical information available.
2- This format of test is to be only used with VEDALAB EASY READER+® instrument.
3- If the reading time (15 minutes) is not strictly respected, wrong results will be obtained.
4- This format of test should not be used for visual reading.
5- As for any ~~diagnostic~~ method or for any measurements through analysers, there is a variability of the obtained result. Therefore, a confidence range of +/- 25% should be considered for the final value and for the clinical significance of the result.

6- Do not use the reader for measurements before at least 30 minutes warm-up after having switched on.
7- Some serum specimens with high rheumatoid factor concentration (RF) may yield non-specific positive results during testing. Such cases should be discriminated before testing.
8- The test is designed to eliminate the potential interference of human anti-mouse antibodies (HAMA). However high level of HAMA could show falsely positive results.
9- Use only fresh whole blood samples (<4 hours) when the test is to be performed using whole blood samples.
10- If anticoagulants are needed, only EDTA or heparin should be used. Do not assay citrated samples.

IX. BIBILIOGRAPHY

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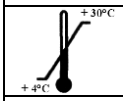
CHANGES DESCRIPTION

Changes type:

- N/A	Not Applicable (creation)
- Technical change	Addition, revision and/or removal of information related to the product.
- Administrative	Implementation of non-technical changes noticeable to the end-user.

Changes type	Change description
Technical	Addition of 4) in Chap. V. Addition of 10- in Chap. VIII. Modification of Chap. I, IV, V, VI, VII, VIII. Addition of µL with number of drops in Picture n°1

Note: Minor typographical, grammar, spelling and formatting changes are not reported in the change details.

	Read the instructions before use		Do not reuse
	Temperature limitations		Manufacturer
	Device not for near-patient-use		Device not for self-testing use

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