






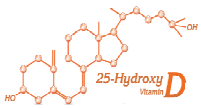








VIDAS® Vitamin D

Reference 30463 - V1 - 2013/09

BIOMÉRIEUX UNIVERSITY

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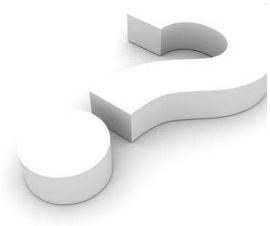
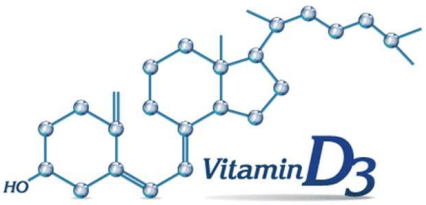


1 General questions

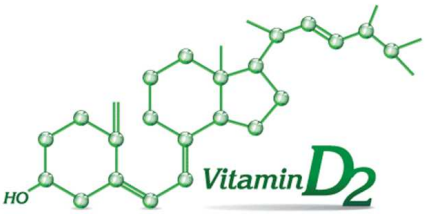
1.1 What is Vitamin D ?

Vitamin D is a fat-soluble **steroid pro-hormone**.

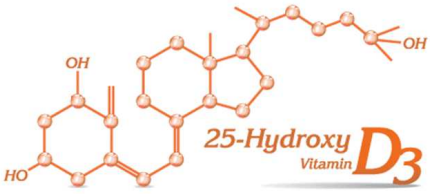
Vitamin D is found mainly in two forms: vitamin **D2** (ergocalciferol) and vitamin **D3** (cholecalciferol).



Vitamin D3 is synthesized from 7-dehydrocholesterol by action of solar ultraviolet radiation on the skin. It is also present in food (mostly in fatty fish).



Vitamin D2 is from exogenous origin only. Small amounts of vitamin D2 are present in food (mushrooms, vegetables).



The **active form** of the molecule is the **1,25-$(OH)_2$vitamin D** (calcitriol) which is obtained from vitamin D through two successive hydroxylation reactions. The first hydroxylation occurs in the liver to yield 25-(OH) vitamin D (calcidiol). The second hydroxylation occurs in the kidneys and other tissues as well to yield biologically active 1,25-$(OH)_2$vitamin D.

Both vitamin D2 and D3 are used for medical supplementation and are identically metabolized by the body.

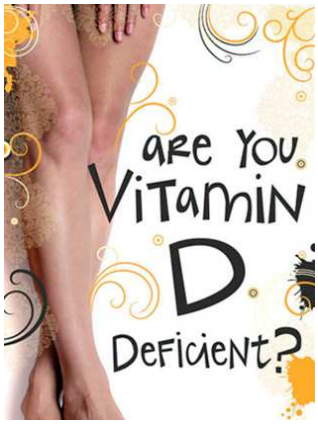
1.2 VIDAS® 25 (OH) Vitamin D TOTAL assay: intended use and kit information

VIDAS® 25 OH Vitamin D Total (VITD) is an automated quantitative test for use on the instruments of the VIDAS® family for the determination of 25-hydroxyvitamin D Total in human serum or plasma using the ELFA technique (Enzyme Linked Fluorescent Assay).

The VIDAS® 25 OH Vitamin D Total assay is to be used as an **aid in the assessment of Vitamin D sufficiency**.

The VIDAS® 25 OH Vitamin D Total assay reference **30463** comes in a **60 tests kit** format under, with a **15 months shelf life**.

1.3 Why should we perform Vitamin D testing ?



Vitamin D deficiency can be associated with rickets in children, osteoporosis and secondary hyper-parathyroidism in adults. Recent studies have established a link between low circulating vitamin D levels and an increasing risk of diabetes, cardiovascular or autoimmune diseases as well as various forms of cancer (1-8).

Vitamin D testing has become an **assay of general health status** (9).

As the active form have a very short half-life and as blood titers are highly related to phosphocalcic metabolism, especially considering the influence of the parathyroid hormone (PTH), this form is not a good marker.

The **25-(OH) vitamin D is the main storage form** of vitamin D in the human body. It is found in high concentrations in serum or plasma, which makes 25-(OH) vitamin D the preferred analyte for the determination of **vitamin D nutritional status** (10).

1.4 What is the difference between deficiency and insufficiency ?

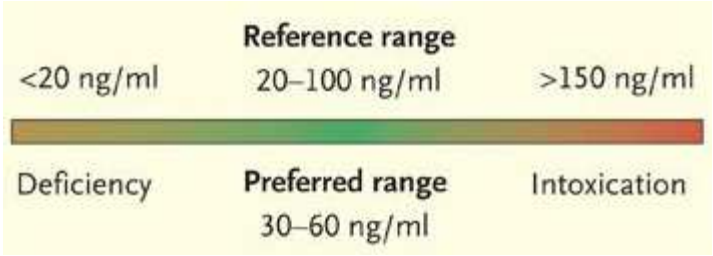
A blood test is required to find out your own vitamin D status. The results will be used to guide you regarding any changes required in diet, sun exposure, or vitamin supplementation you may need.

However, clinicians usually prescribe Vitamin D testing assays to check for **deficiency** .
Depending of their specialty, you may find different consensus in the literature. For example endocrinologists may have a deficiency threshold different from dermatologists.

The difference between insufficiency and deficiency in vitamin D is a matter of degree:

- Vitamin D **insufficiency** is defined as the range were blood level of 25 OH vitamin D are below 20 ng/ml.
- Vitamin D **deficiency** occurs when 25 OH vitamin D levels are between 21 - 29 ng/mL.

25(OH) Vitamin D normal range has been historically defined and recently recommended by the Institute of Medicine (1 & 11) as :



- Vitamin D **deficiency** is **estimated** as a 25(OH)D **below 20 ng/ml** (50 nmol/liter)
- Vitamin D **insufficiency** a is **estimated** s a 25(OH)D of **21–29 ng/ml** (525–725 nmol/liter)
- Vitamin D **intoxication** is extremely rare, done by **over treatment.**

2 Technical points

2.1 What is the test format ?

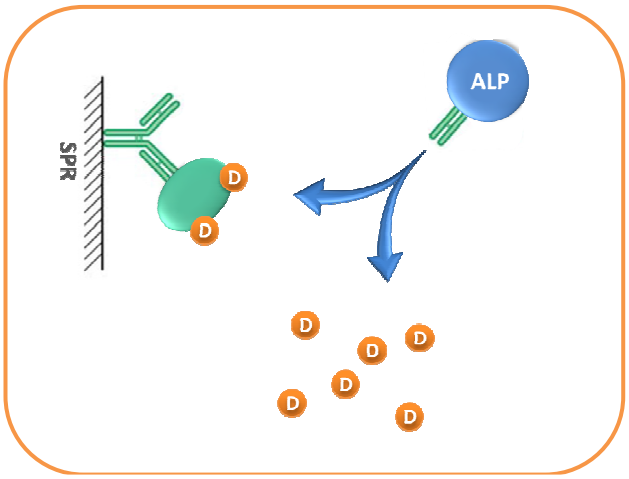
The assay principle combines an enzyme immunoassay **competition method** with a final fluorescent detection (ELFA).

No sample pre-treatment is needed !

Both the dissociation step and the dosage are processed automatically in the VIDAS® 25 OH vitamin D strip. This ensure a reliable and reproducible assay which leads to maximum performances.

Practically, the sample is mixed with pre-treatment reagent present in the strip to **separate vitamin D from its binding protein**.

The pre-treated sample is then collected and transferred into the well that contains an alkaline phosphatase **(ALP) labeled anti-vitamin D antibody (conjugate)**.



VIDAS 25 (OH) Vitamin D Total: a competition format

The vitamin D antigen present in the sample and the vitamin D antigen coating the interior of the SPR® compete for binding sites on the anti-**vitamin D antibody-ALP conjugate**.

The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent residue. The **intensity of the fluorescence is inversely proportional to the concentration of vitamin D** antigen present in the sample.

2.2 Protocol compatibility

The **VIDAS® 25 OH Vitamin D Total (VITD)** assay use a dedicated and **unique protocol** which is not compatible with other assay at the time we write this FAQ sheet.

2.3 What about sample stability ?

In vivo, half-life of vitamin D2 is short while half-life of vitamin D3 is longer.

In vitro, both vitamins D2 and D3 bind to their carrier protein and hence are found to be very **stable**.

However, you should refer to **package insert for specimen stability** key points to know what are the conditions validated for the use of the VIDAS® 25 OH Vitamin D total testing:

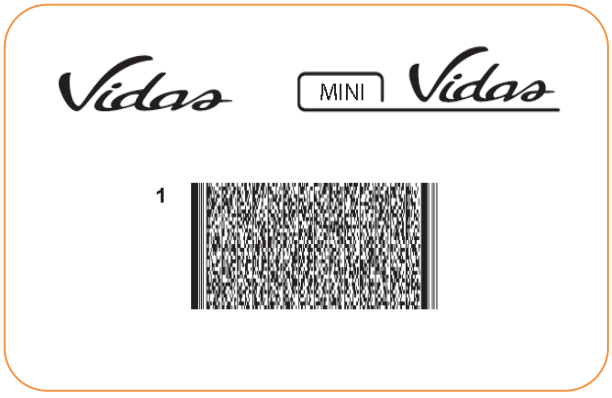
"Serum and plasma samples can be stored in primary tube at 18-25°C for up to 8 hours or aliquoted and stored at 2-8°C up to 5 days; if longer storage is required, freeze the sear or plasma at - 25 ± 6°C.
Serum-type samples can be stored for 3 months at - 25 ± 6°C, with 3 freeze/thaw cycles. Plasma-type samples can be stored for 3 months at - 25 ± 6°C, with 3 freeze/thaw cycles."

2.4 Is the Vitamin D protocol already available for my VIDAS® ?

For **miniVIDAS** and **VIDAS PC**, the Vitamin D protocol can be updated via reading the 2 dedicated PTC available at the end of the package insert.

For **VIDAS 3**, the Vitamin D protocol is embedded in the system knowledge base from **version 1.2**
If you are running an older version of the VIDAS 3 software, you can update the protocol using the dedicated PTC available at the end of the package insert or downloadable from http://www.biomerieux.com/techlib.

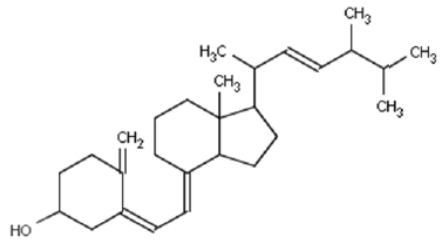
Please note that there is now 2 different PTC barcode at the end of the PI:
- the first PTC is dedicated to **miniVIDAS and VIDAS PC** update and comes in a **single barcode**
- the second PTC is dedicated to **VIDAS 3** update and comes in a **2 parts barcode**



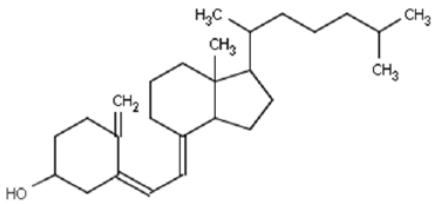
PTC scanning must be performed from the package insert !

2.5 What is detected by the VIDAS® 25 (OH) Vitamin D assay?

Vitamin D2 and vitamin D3 are the 2 main Vitamin D isomers.
They are structurally different in C22-23 and C-24:



Vitamin D2



Vitamin D3

Both vitamin D2 and D3 can be metabolized *in vivo*, hence both isomers can potentially be prescribed by clinicians.

The **VIDAS® 25 OH Vitamin D Total** assay can detect both vitamin D2 and Vitamin D3, hence this test allow **Vitamin D deficiency** status determination **and Vitamin D supplementation follow up**.

2.6 Do we claim equal D2 and D3 detection ?

Clinicians will check patients status about vitamin D and, if there is deficiency of insufficiency, they will determine the appropriate quantity of vitamin D to prescribe.

Afterwards, patients vitamin D status will be followed to check the dosage and eventually adjust it.
Depending of the country, prescriptions can be made of vitamin D2 (used for many years) or vitamin D3 (used more and more widely)

Equimolarity detection is mandatory to provide all clinicians with the most **accurate result**, whatever their prescription is.

We can define **equimolarity** as the ability of a method **to detect both vitamin D2 and vitamin D3 isomers** with **same performances**.

For this assay, equimolarity calculation is done using the following formula:

$$\text{D2 cross reaction (\%)} = \frac{(\text{VIDAS 25(OH)D Total dose}) - (\text{LC-MS/MS 25(OH)D3 dose})}{(\text{LC-MS/MS 25(OH)D2 dose})} \times 100$$

The **VIDAS® 25 OH Vitamin D Total** assay demonstrate performances at the "state of the art" as the mean 25(OH) Vitamin D2 cross reactivity is 91%.

Company / Assay	D2 cross reaction
IDS iSYS [1]	100 %
Diasorin Liaison [1]	100 %
Roche Cobas [1]	92 %
Abott Architect [2]	82 %
Siemens Centaur [1]	106 %
Ortho Vitros [1]	105 %
Tosoh AIA [1]	101 %
bioMerieux VIDAS [2]	91 %

[1] *values from the PI, obtained with spiked D2*
[2] *values obtained with endogenous D2 and calculation formula*

2.7 How do the VIDAS® 25 OH Vitamin D total match with the international standard ?

The National Institute of Standard and Technology (NIST) has developed a **Standard Reference Material** (SRM) for vitamin D metabolites in human serum, applicable to a LC-MS/MS dosage method (12).

The **VIDAS® 25 OH Vitamin D Total** assay is traceable to the LC-MS/MS reference method.

2.8 Can we have cross reactivity ?

The **VIDAS® 25 OH Vitamin D Total** assay is designed to dose the circulating forms of Vitamin D which are 25 (OH) Vitamin D2 and 25 (OH) Vitamin D3.

However, other analogs can be found *in vivo* and may present cross reactivity.
We can quote:

- 24, 25 (OH) Vitamin D
- 1, 25 (OH) Vitamin D

These compounds, like the 24, 25 (OH) Vitamin D, are products from the vitamin D metabolic cycles.

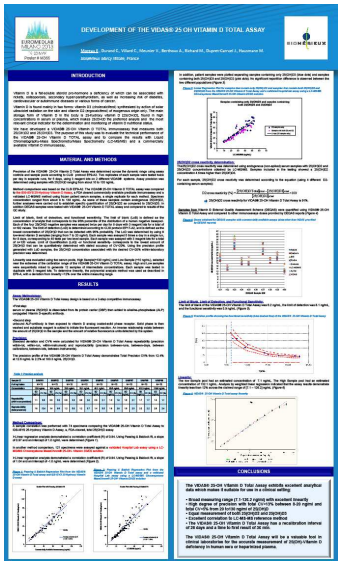
Let's go further with this example: we state that 24, 25 (OH) Vitamin D got a 577 % cross reaction factor which can seems huge. But such compounds are present at pg/mL levels in serum, which is 100 to 1000 fold lower than levels detected for 25 (OH) vitamin D2 and 25 (OH) vitamin D3 in our assay.

As the VIDAS® 25 OH Vitamin D Total assay will give a result in ng/mL, the **impact of cross reactivity is insignificant**.

To go further, please note that our **VIDAS® 25 OH Vitamin D Total assay do not have cross reaction with 3 epi 25 (OH) Vitamin D**.
From a clinical point of view, it is **important not to ensure the 3 epi 25 (OH) Vitamin D is not detected so that the Vitamin D reserve is not overestimated**.

In support of this, current clinical cut-offs for vitamin D insufficiency have been established using immunoassays that are incapable of detecting the C3-epimer. On the same note, it is important to recognize that clinical evidence does not exist at the current time to support the quantitation of 3-epi-25(OH) Vitamin D3, and it can therefore be argued that its quantitation can obscure the clinical picture presented by 25(OH) Vitamin D3 alone (13).

2.9 Performances


DEVELOPMENT OF THE VIDAS® 25 OH VITAMIN D TOTAL ASSAY

INTRODUCTION

MATERIAL AND METHODS

RESULTS

CONCLUSIONS

Development of the VIDAS® 25 OH VITAMIN D TOTAL assay
EUROMEDLAB, Milano, 2013
Poster #M366

The VIDAS® 25-OH Vitamin D Total Assay exhibits excellent analytical data which makes it suitable for use in a clinical setting:

- Broad measuring range (7.1-126.2 ng/ml) with excellent linearity
- High degree of precision with total CV<13% between 8-20 ng/ml and total CV<5% from 20 to130 ng/ml of 25(OH)D
- Equal measurement of both 25(OH)D2 and 25(OH)D3
- Excellent correlation to LC-MS-MS reference method
- The VIDAS® 25-OH Vitamin D Total Assay has a recalibration interval of 28 days and a time to first result of 36 min. The VIDAS® 25-OH Vitamin D Total Assay will be a valuable tool in clinical laboratories for the accurate measurement of 25(OH)-Vitamin D deficiency in human sera or heparinized plasma.

CLICK TO OPEN

3 Results and Interpretations

3.1 Can I have false results ?

High performances of the **VIDAS® 25 OH Vitamin D Total** assay are closely linked to the dissociation step efficiency.

This step can be influenced by the matrix quality. Pregnancy, dialyzed patient, lipemic, hemolyzed or icteric samples, may present under estimated titers.

Please refer to package insert "Limitations of the test" for more information.

3.2 What are the reference values ?

Reference values of a particular analyte are usually related to a specific population (e.g. male / female, child / adult, pregnancy,...(11) but it is not the case with this assay. Why ?

Vitamin D3 production in the skin is related to sun exposure. So this source is highly variable depending of sun exposure (e.g. seasons, fog frequency, geographic area, clothing,...) and skin pigmentation.
So it is just **not possible to define reference values** for each case. Moreover, some factor like obesity can impact the range of expected value of a population type (16)

3.3 What are the expected values ?

For reasons mentioned above, it is recommended that every lab establish its own expected values for the population it serves.

Please refer to package insert for further details.

3.4 If there is a high limit where Vitamin D can become toxic?

Depending on the intensity of UV rays and the minutes of exposure, an equilibrium can develop in the skin, and vitamin D degrades as fast as it is generated. So it is hardly possible to be intoxicated with high levels of Vitamin D.

Despite this equilibrium, the concentration of plasma 25(OH) Vitamin D3, a good biomarker for toxicity, has been studied and the threshold for **toxic symptoms was established at 750 nmol/L** (14).

This threshold value implies that 25(OH) Vitamin D concentrations up to the currently considered upper limit of the normal range, namely 250 nmol/L, are safe and still leave a broad margin for error because values significantly higher than this value have never been associated with toxicity

So, to reach toxic concentrations of 25(OH) Vitamin D a patient has to take 50 000 u/day during several months, whereas the prescription recommendation is 4000 u/day !
Scientific publications reports only 1 to 2 cases per year.

3.5 Measurement range: min, max, dilution

The **VIDAS® 25 OH Vitamin D Total** assay will provide results between **8,1 and 126 ng/mL** where actual ranges explored by clinician are usually 12 - 48 ng/mL [PI]

As 95% of the population will be between 8,8 - 10,8 ng/mL [95% CI] and depending of the method accuracy, it is possible to found people naturally below 8 ng/mL in approximately 1 to 5 % of the population :

	95% Reference limit	90% CI
Lower (ng/ml)	9.3	8.8 to 10.8
Upper (ng/ml)	48.5	41.5 to 55.5

According to publications, we usually retrieve 1 to 2 % of the population below 8 ng/mL.

If the VIDAS® 25 OH Vitamin D Total assay give a result > 126 ng/ml, then you have to dilute the patient sample using a low titer serum.

Please note that as it may contains traces of Vitamin D in its binded form, the use of bioMerieux **Serum Free** (# 66581) as diluent is **not validated** for this assay.

4 References

1. HOLICK, M.F. (2007). Vitamin D deficiency. N Engl J Med,357:266-281.

2. DOBNIG H, et al. Independent association of low serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels with all-cause and cardiovascular mortality. Arch Intern Med. 2008 Jun 23;168(12):1340-9.

3. FREEDMAN BI, et al. Vitamin D, adiposity, and calcified atherosclerotic plaque in african-americans. J Clin Endocrinol Metab. 2010 Mar;95(3):1076-83.

4. REIS JP, VON MÜHLEN D, MILLER ER 3rd, MICHOS ED, APPEL LJ. Vitamin D status and cardiometabolic risk factors in the United States adolescent population. Pediatrics. 2009 Sep;124(3):e371-9.

5. ZIPITIS CS, AKOBENG AK, 5- Vitamin D supplementation in early childhood and risk of type 1 diabetes: a systematic review and meta-analysis. Arch Dis Child. 2008 Jun;93(6):512-7.

6. PITTAS AG, et al; 6-Vitamin D and calcium intake in relation to type 2 diabetes in women., Diabetes Care. 2006 Mar;29(3):650-6.

7. YIN L, et al. Meta-analysis: longitudinal studies of serum vitamin D and colorectal cancer risk. Aliment Pharmacol Ther. 2009 Jul 1;30(2):113-25.

8. CHEN P, et al. Meta-analysis of vitamin D, calcium and the prevention of breast cancer. Hu P, Xie D, Qin Y, Wang F, Wang H. Breast Cancer Res Treat. 2010 Jun;121(2):469- 77.

9. GRANT WB, HOLICK MF. Benefits and requirements of vitamin D for optimal health: a review. Altern Med Rev. 2005 Jun;10(2):94-111.

10. HOLICK MF. Vitamin D status: measurement, interpretation, and clinical application. Ann Epidemiol. 2009 Feb;19(2):73-8.

11. INSTITUTE OF MEDICINE. Dietary Reference Intakes for Calcium and Vitamin D. Washington, D.C.: National Academies Press, 2010.

12. NIST. Development of a Standard Reference Material for Vitamin D in Human Serum. http://www.nist.gov/mml/csd/organic/vitamindinserum.cfm

13. BAILEY D, VELJKOVIC K, YAZDANPANAH M, ADELI K: Analytical measurement and clinical relevance of vitamin D3 C3-epimer, Clinical Biochemistry 46, 2013, 190–196.

14. GLENVILLE JONES : Pharmacokinetics of vitamin D toxicity - Am J Clin Nutr 2008;88(suppl):582S– 6S

15. Clinical and Laboratory Standards Institute. Interference Testing in Clinical Chemistry; Approved Guideline— Second Edition. CLSI document EP7-A2: CLSI, 2005.

16. AU LAUREN, TUFTS University: Vitamin D Intakes & Status among US Children Aged 1-18 Years: Do Obese and Racially/Ethnically Diverse Youth Need More Vitamin D?. 2012, Selected characteristics by vitamin D status in children ages 1-18 in NHANES 2005-2006

17. 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).