Vitamin D Quantitative Test

A Rapid "Sandwich" Immunochromatographic Test for Quantitative Detection of total 25-OH Vitamin D in human finger-prick Blood or Serum

CE REF 711-25-DB

For in vitro Diagnostic use only ***Read Instructions before use***

INTENDED USE

Unimed's ***OnSight™- Vitamin D Quantitative Tes***t is an immunochromatography-based one step *in vitro* test. It is designed for the quantitative determination of total 25-hydroxy Vitamin D (25-OH Vitamin D) in human finger-prick blood or serum. This assay provides a preliminary diagnostic test result and can be used for screening of Vitamin D deficiency. The Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS) assays or other quantitative immunoassays are recommended to further confirm the diagnostic test results.

SUMMARY AND EXPLANATION

Vitamin D is a steroid hormone responsible for enhancing intestinal absorption of calcium and the regulation of its homeostasis. The two common forms of Vitamin D are Vitamin D2 and Vitamin D3. Vitamin D3 is naturally produced in the human skin through the exposure to ultraviolet light and Vitamin D2 is mainly obtained from foods. Vitamin D is transported to the liver where it is metabolized to 25-hydroxy Vitamin D. In medicine, a 25-hydroxy Vitamin D blood test is used to determine Vitamin D concentration in the body. The blood concentration of 25-hydroxy Vitamin D is considered the best indicator of Vitamin D status.

Vitamin D deficiency is now recognized as a global epidemic. Virtually every cell in our body has receptors for Vitamin D, meaning that they all require "Sufficient" Level of Vitamin D for adequate functioning. The health risks associated with Vitamin D deficiency are far more severe than previously thought. Vitamin D deficiency has been linked to various serious diseases: Osteoporosis, Osteomalacia, Multiple Sclerosis, Cardiovascular Diseases, Pregnancy Complications, Diabetes, Depression, Strokes, Autoimmune Diseases, Flu, COVID-19, Infectious Diseases, Different Cancers, Alzheimer, Obesity and Higher Mortality etc. Therefore, now detecting (25-OH) Vitamin D level is considered as ***"Medically Necessary Screening Test",*** and maintaining sufficient levels not just to improve bone health, but to improve overall health and well-being.

Multiple guidelines for Vitamin D deficiency have been published by various health organizations; but a common recommendation remained to be established. Recent literature has suggested the following ranges for the classification of Vitamin D status:

25-OH Vitamin D Level	Reference Range (ng/ml)	Reference Range (nmol/l)
Deficient	0 – 10	0 – 25
Insufficient	10 – 30	25 – 75
Sufficient	30 – 100	75 – 250
Excess, but not toxic	100 - 150	250 - 375
Toxicity	>150	>375

TEST PRINCIPLE

OnSight™- Vitamin D Quantitative Test utilizes the principle of Immunochromatography, a unique two-site "Sandwich" immunoassay on a membrane. The test employs a very "Exclusive" pair of anti-25-OH Vitamin D Monoclonal Antibodies; one conjugated with colloidal gold and another one immobilized on the solid phase. This will selectively detect Vitamin D with a high degree of sensitivity and specificity.

As the test sample flows through the membrane assembly within the test device, the colored anti-25-OH Vitamin D-colloidal gold conjugate complexes with 25-OH Vitamin D from the sample. This complex moves further on the membrane by the capillary action to the test region (T) where it is immobilized by another anti-25-OH Vitamin D coated on the membrane, leading to formation of a pink / purple colored band, which confirms a positive test results. The intensity of colored band in the test line region is 25-OH Vitamin D concentration-dependent, higher the concentration of 25-OH Vitamin D in the tested sample, the stronger the colored band is. A control line is present in the test window to work as procedural control. This colored band should always appear on the control line region (C) if the test device is stored in good condition and the test is performed appropriately.

MATERIALS PROVIDED

1. ***OnSight™- Vitamin D Quantitative Test*** device (Kit Size: 25 Tests/Box)
2. **UniSampler™** Collection Tube (sealed Sampler Buffer Tubes – 26 pieces)
3. **UniSampler™** Blood Collector (26 pieces)
4. Instructions for use – 1

MATERIALS REQUIRED BUT NOT PROVIDED

1. Timer or clock
2. Lancet
3. Alcohol Swab
4. **Igloo** Reader Pro – (CE Marked) To be purchased separately from Goodscare GmbH, Germany.
5. **Vitamin D Control** (CE Marked) (Optional – to be purchased separately from Unimed International, Inc. (REF: 711-VDC)

STORAGE AND STABILITY

The test device should be stored at 4ºC to 30ºC and will be effective until the expiration date stated on the package. The product is humidity-sensitive and should be used immediately after being open. Any improperly sealed product should be discarded.

PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. Do not use the product beyond the expiration date.
3. Handle all specimens as potentially infectious.
4. Humidity sensitive product, do not open foil pouch until it is ready to be tested.
5. ***OnSight™- Vitamin D Quantitative Test*** must be quantified with **Igloo** Reader Pro.

QUALITY CONTROL

Good Laboratory Practice recommends the frequent use of control materials to validate the reliability of test device. If control values do not fall within established range, assay results are invalid. A set of two "LC-MS/MS confirmed" Vitamin D Controls is provided with the kit (Optional).

The ***OnSight™- Vitamin D Quantitative Test*** provides a built-in process control with a different antigen/antibody reaction at the control region (C). This control line should always appear regardless the presence of Vitamin D. If the control line does not appear, the test device should be discarded, and the obtained result is invalid. The presence of this control band in the control region serves as 1) verification that sufficient sample volume is added, 2) that proper sample flow is obtained.

CAUTION!

OnSight™- Vitamin D Quantitative Test has been designed for "Decision-Point" Finger-prick Blood (or Serum) samples ONLY. NO Anticoagulated Blood or Plasma samples should be used for testing ***OnSight™- Vitamin D Quantitative Test*** as Anticoagulants may impact the test results.

SPECIMEN COLLECTION AND PREPARATION

1. Wash your hand thoroughly and dry completely.
2. Rub and Wipe your ring or middle finger of non-dominant hand.
3. Using safety lancet puncture the side of your finger.
4. Collect 10 µl blood using Blood Collector (See instructions below) and perform testing immediately.

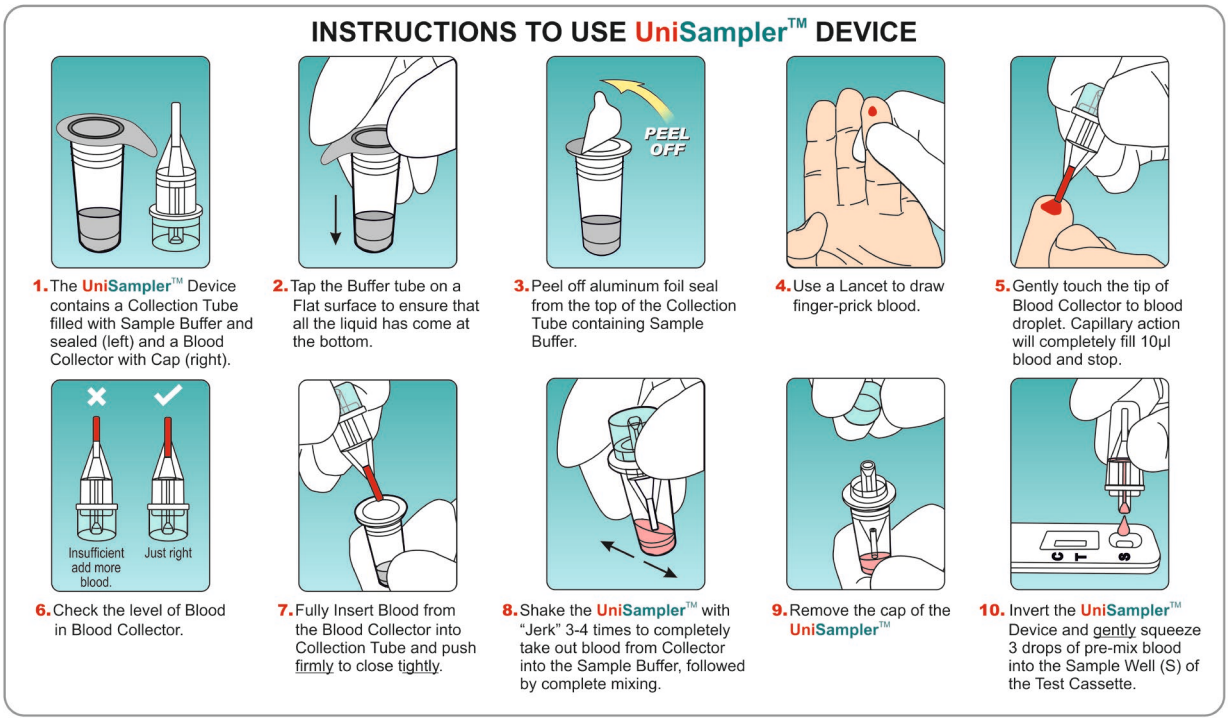
PROCEDURE:

1. Bring all materials and specimens to room temperature (between 21°C – 24°C).
2. Remove the test card from the sealed foil pouch and place it on a hard flat surface.
3. Follow Instructions to use **UniSampler™** Device.
4. After applying 3 drops of pre-mix blood into the sample well (S), read and record the results at 15 Minutes by **Igloo** Reader Pro.

VITAMIN D CONTROL / SERUM PROTOCOL:

OnSight™- Vitamin D Quantitative Test has been designed for human finger-prick blood. However, Vitamin D Control or Serum sample can be used for testing. Instead of taking finger prick blood with blood collector, apply 5µl of Vitamin D Control or Serum into the Collection Tube using Micropipette (not provided with the Kit) and follow "Instructions to Use **UniSampler™** Device".

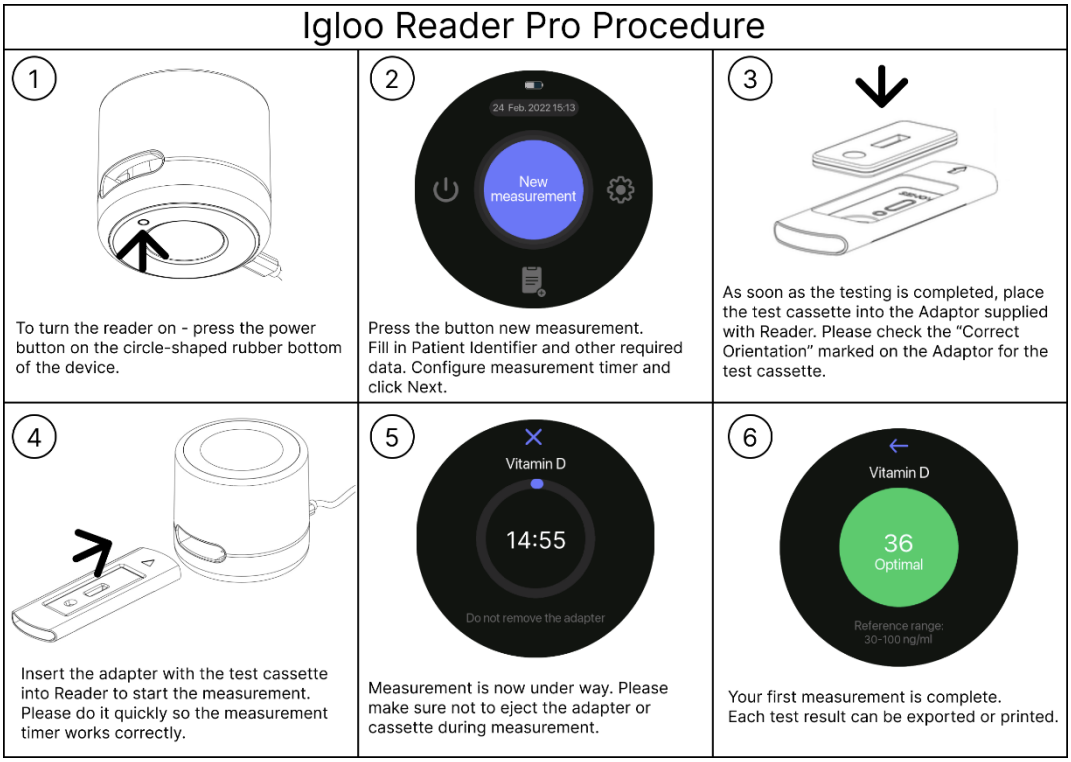
Important Note: ***Result after 15 minutes may not be accurate.***



CAUTION!

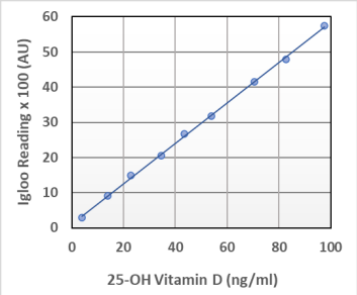
- Complete (100%) PRE-MIXING of finger-prick blood with sample buffer is "EXTREMELY" important and CRITICAL Step to get correct result. This can be determined by checking the UNIFORM red color of pre-mix blood in Collection Tube and Blood Collector.
- Incomplete mixing of Blood with Buffer means Sample Preparation has been compromised, and the test result is likely to show lower values.
- Pressing of **UniSampler™** should be "GENTLE" to get three full drops of pre-mix blood into the sample well (S).

Quantitative Vitamin D Detection using Igloo Reader Pro



STANDARD CURVE Using Igloo Reader Pro

A typical standard curve is illustrated on the right side. The reading AU is automatically converted into ng/ml in Igloo Reader Pro.



INTERPRETATION OF RESULTS

The Igloo Reader Pro automatically determines the final result by comparing the AU for each sample against a pre-established calibration curve. 25-OH Vitamin D levels are expressed in ng/ml. Results obtained with the ***OnSight™- Vitamin D Quantitative Test*** are interpreted with following Messages:

25-OH Vitamin D Level (ng/ml)	Result Message
< 10.0 ng/ml	DEFICIENT
≥ 10.0 – < 30.0 ng/ml	INSUFFICIENT
≥ 30.0 – < 100.0 ng/ml	SUFFICIENT
≥ 100.0 ng/ml	

PERFORMANCE CHARACTERISTICS:

Sensitivity:

The sensitivity of ***OnSight™- Vitamin D Quantitative Test*** is 3ng/ml (7.5nmol/l). The sensitivity was determined by calculating the mean plus 3.3 times of standard deviation of twenty Vitamin D-free serum tests.

Detection Range:

The Detection Range of ***OnSight™- Vitamin D Quantitative Test*** with Igloo Reader Pro is from 3ng/ml (7.5nmol/l) to 100ng/ml (250nmol/l).

Accuracy:

The accuracy of ***OnSight™- Vitamin D Quantitative Test*** was evaluated using human finger-prick blood samples in comparison with a reference 25-OH Vitamin D ELISA assay using corresponding serum samples. The comparison result showed a linear regression with slope of 1.02 and Correlation Coefficient of 92%. In conclusion, ***OnSight™*** test results of human blood samples showed good agreement with the ELISA results of corresponding serum samples.

The accuracy of ***OnSight™- Vitamin D Quantitative Test*** was also evaluated using 20 serum samples in comparison with LC-MS/MS Assay ("Gold Standard" for 25-OH Vitamin D measurement). The comparison result showed a linear regression with the slope of 0.98 and Correlation Coefficient of 98%. In conclusion, ***OnSight™- Vitamin D Quantitative Test*** results agree closely to the true values generated from LC-MS/MS assay.

Precision:

Intra Lot

Sample	No. of Replicates	Mean ng/ml	Standard Deviation	Coefficient Variation (CV)
Serum	20	40.4	3.1	7.6%
Blood -1	10	33.0	2.5	7.6%
Blood - 2	10	38.7	3.9	10.1%

Inter Lot

Sample	No. of Lot	No. of Replicates	Mean ng/ml	Standard Deviation	Coefficient Variation
Serum # 1	3	60	33.0	4.7	14.3%
Serum # 2	3	60	55.9	6.1	11.0%
Blood - 1	3	15	38.8	4.3	11.8%

Specificity:

30 Vitamin D free serum samples were tested and all showed negative results; suggesting 100% Specificity.
No interference and cross reactivity was observed with Bilirubin, Triglycerides, Cholesterol, Vitamin B12 and Vitamin C.

EXPECTED RESULTS

OnSight™ - Vitamin D Quantitative Test is a Rapid Quantitative assay. The test is intended to use for screening individuals to identify Vitamin D level. This assay provides only a preliminary analytical test result. The liquid chromatography with tandem mass spectrometry (LC-MS/MS) assays or quantitative immunoassays are recommended to confirm the analytical result.

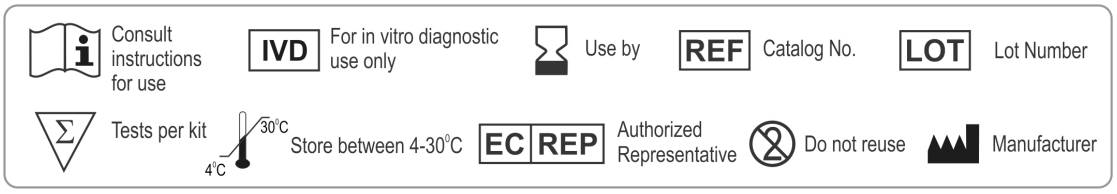
REFERENCES

1. Holick, MF. Vitamin D statues: Measurement, Interpretation and clinical application. Ann. Epidemoil. 2009, 19(2):73-78.
2. Morris HA. Vitamin D: A Hormone for All Seasons – How much is enough? Clin. Biochem. Rev., 2005, 26:21-32.
3. Moyad MA. Vitamin D: a rapid review. DermatolNurs. 2009, 21:25-30
4. Zerwekh JE. Blood biomarkers of vitamin D status. Am J. ClinNutr. 2008, 87:1087S-91S
5. Schöttker B, etal.Vitamin D and mortality: meta-analysis of individual participant data from a large consortium of cohort studies from Europe and the United States. BMJ. 2014, 348:g3656
6. Jenkinson C et al. High throughput LC-MS/MS method for the simultaneous analysis of multiple vitamin D analytes in serum. Chromatogr B AnalytTechnol Biomed Life Sci 2016 Mar 1;1014:56-63.

Issue Date: 2025-01, Version-3 (with Igloo Reader Pro)
ID # US711QR3

SHELF LIFE: 18 Months

INDEX OF CE SYMBOLS



Unimed International, Inc.
3556 Investment Boulevad
Hayward, California 94545, USA
Tel: (510) 398-8111
Fax: (510) 398-8132
Email: info@unimed.net
www.unimed.net

EC REP

Qarad EC-REP BV
Pas 257
2440 Geel
Belgium
www.qarad.com