



Quantitative Vitamin D Test

A Rapid “Sandwich” Immunochromatographic Test for Quantitative Detection of total 25-OH Vitamin D in human finger-prick blood

[REF] 1155Q-25 / 1155Q-10

For In Vitro Diagnostic use only

Read Instructions before use

INTENDED USE

Affimedix's **Rapi-D™** Quantitative Vitamin D Test 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. 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, Different Cancers, Infectious Diseases, 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
Toxicity	>100	>250

TEST PRINCIPLE

Rapi-D™ Quantitative Vitamin D 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. **Rapi-D™** Quantitative Vitamin D Test device (Kit Size: 25 Tests/Box, 10 Tests/Box)
2. Sample Buffer (One Bottle of 6ml in 25 Tests/Box; and 1 Bottle of 3ml in 10 Tests/Box)
3. **UniSampler™** Device (25 Devices in 25 Tests/Box; and 10 Devices in 10 Tests/Box)
4. RFID Card - 1
5. Instructions for use - 1

MATERIALS REQUIRED BUT NOT PROVIDED

1. Timer or clock
2. Lancet
3. Alcohol Swab
4. **RapiRead™** CUBE Reader (to be purchased separately)

STORAGE AND STABILITY

The test device should be stored at 4°C to 30°C 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.

QUALITY CONTROL

Good Laboratory Practice recommends the frequent use of control materials to validate the reliability of test device. The control values should fall within established range. A set of two “LC-MS/MS confirmed” Vitamin D QC Serum Controls is provided with the kit (optional).

The **Rapi-D™ Quantitative Vitamin D 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 serve as 1) verification that sufficient volume is added, 2) that proper flow is obtained.

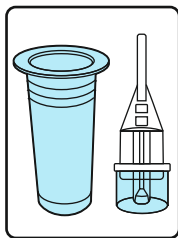
CAUTION!

Rapi-D™ Quantitative Vitamin D 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 **Rapi-D™ Quantitative Vitamin D Test** as Anticoagulants will 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.

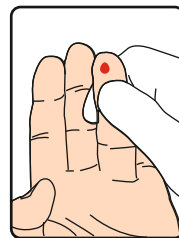
INSTRUCTIONS TO USE UniSampler™ DEVICE



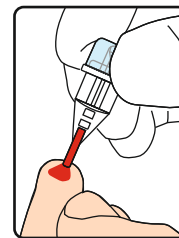
1. UniSampler™ Device contains Collection Tube (1) and Blood Collector with Cap (1)



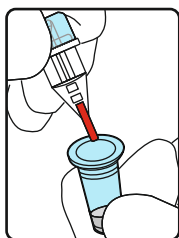
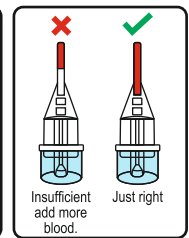
2. Add 5 drops of Sample Buffer from bottle into the Collection Tube.



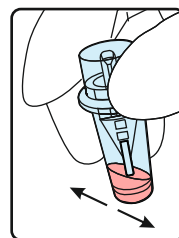
3. Use your own Lancet device to draw finger-prick blood.



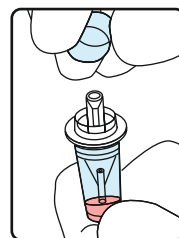
4. Gently touch the tip of Blood Collector to blood droplet. Capillary action will completely fill 10 µl of blood and stop.



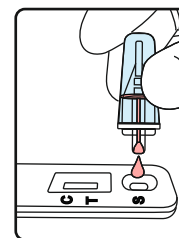
5. Fully Insert Blood Collector into Collection Tube and **push firmly** to close TIGHTLY.



6. Shake the UniSampler™ with “Jerk” 3-4 times to completely take out blood from Blood Collector into the Sample Buffer, followed by complete mixing.



7. Remove the Cap of UniSampler™



8. Invert UniSampler™ Device and GENTLY squeeze 3 drop of pre-mix blood into the Sample Well (S) of the Test Cassette.

CAUTION!

- Through mixing of blood with Sample Buffer is “Extremely” important to get correct result. This can be determined by checking the uniform red color of pre-mix blood in Collection Tube and Blood Collector.
- Pressing of UniSampler™ should be “GENTLE” to get three full drops of pre-mix blood into the sample well (S).

PROCEDURE:

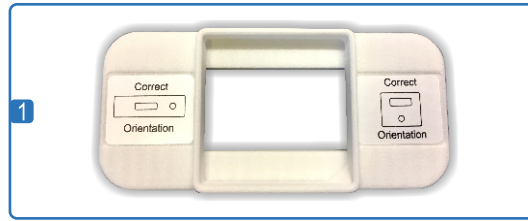
1. Bring all materials and specimens to room temperature.
2. Remove the test card from the sealed foil pouch and place it on a hard flat surface.
3. Follow Instructions to use UniSampler™ Device.
4. After applying 3 drops of pre-mix blood into the sample well (S), read and record the results at 15 Minutes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

Serum Protocol:

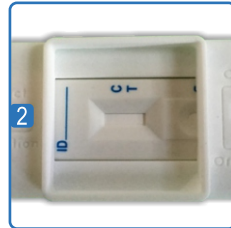
Rapi-D™ Quantitative Vitamin D Test has been designed for human finger-prick blood. However, Serum sample can be used for testing. Instead of taking finger prick blood with blood collector, apply 5µl of serum into the Collection Tube using Micropipette (not provided with the Kit) and follow “Instructions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

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

QUANTITATIVE DETECTION USING *RapiRead*[™] CUBE READER



1. Check the "Correct Orientation" shown on the Adaptor for the Test Device and *RapiRead*[™] CUBE Reader.

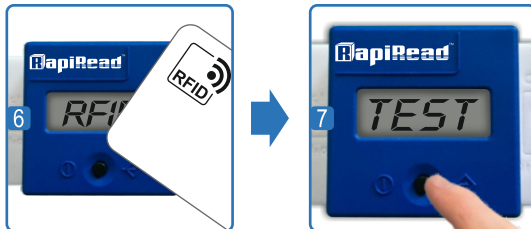


2. Place the Adaptor on top of the Test Device correctly.
3. Place the *RapiRead*[™] on top of the Adaptor correctly.

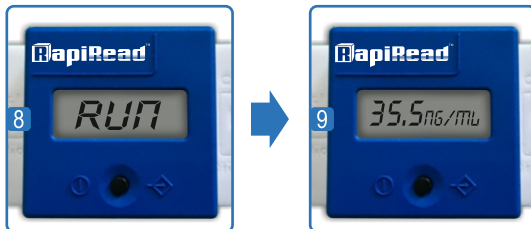
NON-TIMER PROTOCOL



4. Turn-on the *RapiRead*[™] by pressing the black button. Reader runs a self-test, during the self-test "WAIT" is displayed. After an audible beep signal, "ON" is displayed. To perform a reading, press the black button again once for 1 second.
5. The display will show "RFID".



6. Place the Lot specific RFID Card provided with the Kit onto the top side of the *RapiRead*[™]. This will upload Vitamin D test specific Calibration data from RFID Card to *RapiRead*[™].
7. Following an audible beep signal, "TEST" is displayed. Press the black button, the Reader displays "RUN".

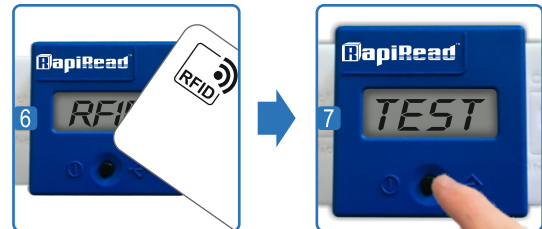


8. After successful data transmission the measurement will start.
9. Vitamin D (Vit D) concentration is displayed in ng/ml with an audible beep signal.

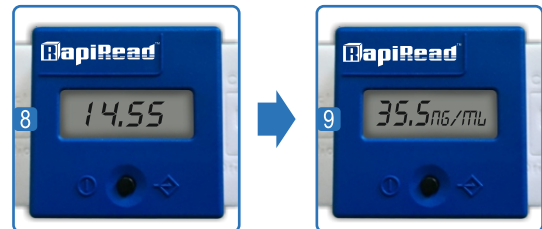
TIMER PROTOCOL



4. Turn-on the *RapiRead*[™] by pressing the black button. Reader runs a self-test, during the self-test "WAIT" is displayed. After an audible beep signal, "ON" is displayed. Keep pressing black button till display shows RFID.
5. The display will show "RFID"



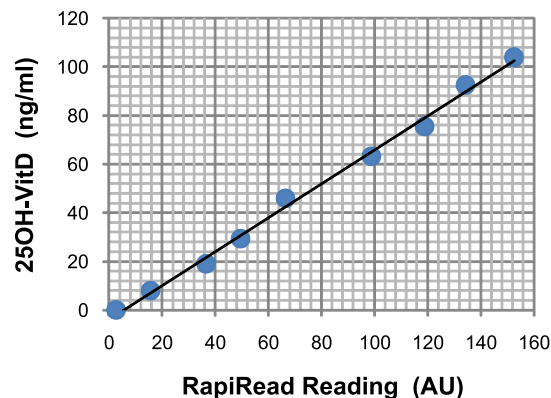
6. Place the Lot specific RFID Card provided with the Kit onto the top side of the *RapiRead*[™]. This will upload Vitamin D test specific Calibration data from RFID Card to *RapiRead*[™].
7. Following an audible beep signal, "TEST" is displayed. Press the black button, the countdown timer will start.



8. Countdown timer display will start.
9. After 15 minutes Vitamin D (Vit D) concentration is displayed in ng/ml with an audible beep signal.

STANDARD CURVE USING RapiRead™ CUBE READER

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



PERFORMANCE CHARACTERISTICS:

Sensitivity:

The sensitivity of Rapi-D™ Quantitative Vitamin D 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 Rapi-D™ Quantitative Vitamin D Test with RapiRead™ CUBE Reader is from 3ng/ml (7.5nmol/l) to 100ng/ml (250nmol/l).

Accuracy:

The accuracy of Rapi-D™ Quantitative Vitamin D 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, Rapi-D™ test results of human blood samples showed good agreement with the ELISA results of corresponding serum samples.

The accuracy of Rapi-D™ Quantitative Vitamin D 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, Rapi-D™ Quantitative Vitamin D Test results agree closely to the true values generated from LC-MS/MS assay.

Precision:

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%

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

Rapi-D™ Quantitative Vitamin D 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

- Holick, MF. Vitamin D statuses: Measurement, Interpretation and clinical application. Ann. Epidemiol. 2009, 19(2):73-78.
- Morris HA. Vitamin D: A Hormone for All Seasons – How much is enough? Clin. Biochem. Rev., 2005, 26:21-32.
- Moyad MA. Vitamin D: a rapid review. Dermatol Nurs. 2009, 21:25-30
- Zerwekh JE. Blood biomarkers of vitamin D status. Am J. Clin Nutr. 2008, 87:1087S-91S
- Schöttker B, et al. 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



Ver-5/19-7-17(Intl)
(Rapi-D™ Quantitative Vitamin D Test)



510 Myrtle Avenue, Suite # 101
South San Francisco, CA 94080, USA
Tel: +1-650-634-0300
Fax: +1-650-634-0301
Email: info@affimedix.com
www.affimedix.com
www.Rapi-D.com

EC	REP
----	-----

Qarad B.V.
Flight Forum 40
5657 DB Eindhoven
The Netherlands
www.qarad.com