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#### INSTRUCTION FOR USE

REF: VIT-GDB 7120

## 25 OH Vitamin D Rapid test

### *Rapid Whole Blood Vitamin D Test*

#### ***A Rapid “Sandwich” Immunochromatographic Test for the Quantitative Detection of total 25-OH Vitamin D in human Whole Blood***

***For Research purposes only***

***Read Instructions before use***

#### **INTENDED USE**

Global Diagnostics B's *25 OH Vitamin D Rapid Test* is an immunochromatography-based one step *in vitro* test. It is designed for quantitative determination of total 25-hydroxy Vitamin D (25-OH Vitamin D) in human blood. This assay provides a preliminary diagnostic test result and can be used to screening for 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 (including D2 and D3) 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 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)
Deficient	0 – 10
Insufficient	10 – 30
Sufficient	30 – 100
Toxicity	>100

### TEST PRINCIPLE

*25 OH Vitamin D Rapid 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. *25 OH Vitamin D Rapid Test* device (Kit Size: 25 Tests/Box, 10 Tests/Box)
2. Sample Buffer
3. Capillary Pipette 10 µl (included inside each Pouch)
4. Instructions for use
5. *RapiRead™* CUBE Reader (to be purchased separately)

### MATERIALS REQUIRED BUT NOT PROVIDED

1. Timer or clock
2. Lancet
3. Alcohol Swab

### 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 daily use of control materials to validate the reliability of device. If control values do not fall within established range, assay results are invalid. Control materials which are not provided with this test kit are commercially available.

The *25 OH Vitamin D Rapid 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.

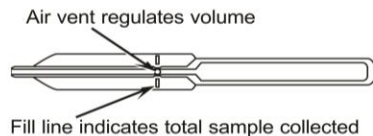
### SPECIMEN COLLECTION AND PREPARATION

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  $\mu$ l blood using Microsafe plastic capillary tube (See instructions below) and perform testing immediately

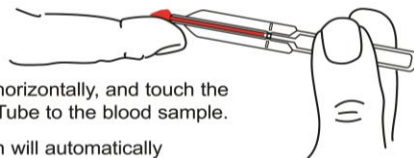
### INSTRUCTIONS TO USE MICROSAFE CAPILLARY TUBE:

## MICROSAFE<sup>®</sup> Tube

An air vent is strategically positioned on the sidewall of the tube to provide automatic air venting and sample volume control.

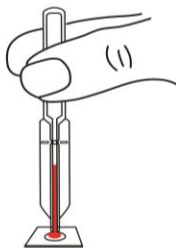


**CAUTION!** Filling is automatic: Never squeeze the tube while sampling.



Step 1: Hold the tube horizontally, and touch the tip of the MICROSAFE Tube to the blood sample.

Capillary action will automatically draw the sample to the fill line and stop.



Step 2: To expel the sample, align the tip of the tube with the sample target and squeeze the bulb.

If a sample won't expel, you probably didn't allow the tube to fill all the way. Touch the tip to the blood sample again and allow it to fill completely. Then align the tip with the sample target and squeeze the bulb.

### 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. Using Microsafe plastic capillary tube, apply 10  $\mu$ l of whole blood in the center of the sample well (S).
4. Apply immediately three drops of the Sample Buffer to the sample well (S).
5. Read and record the results at 15 Minutes by *RapiRead*<sup>™</sup> CUBE Reader.

### Serum Protocol:

*25 OH Vitamin D Rapid Test* has been designed for finger-prick capillary blood. However, Serum sample can be used for testing. Apply 5µl of Serum in Sample well (S) using Micropipette (Not provided with the Kit) and follow the above steps.

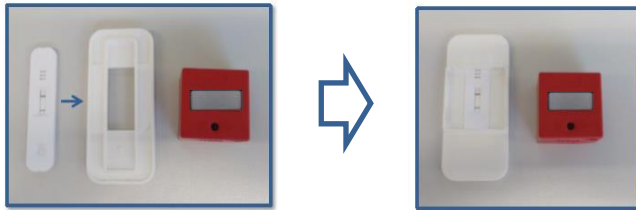
**Important Note: Result after 15 minutes will not be accurate.**

## QUANTITATIVE DETECTION

### MEASUREMENT USING *RapiRead*<sup>™</sup> CUBE READER

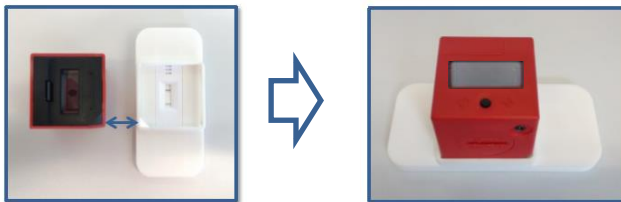
#### 1. Place test device into adaptor

Place the adapter on top of the test device to insert the test device into the adaptor. Please ensure that little plastic knob at the back of the adaptor goes into the Sample well (S) for correct orientation.



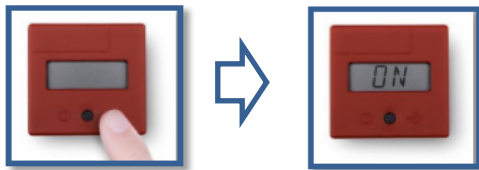
#### 2. Place reader on top of the adaptor

Place reader on top of the adaptor/test device. The slanted reader corner and an according counterpart inside the adapter help to find the correct position of the reader



#### 3. Turn-on the reader

Press the black button. Reader runs a self-test, during the self-test 'WAIT' is displayed. After an audible beep signal, 'ON' is displayed.



#### 4. Upload Vitamin D test specific configuration from RFID card to reader

Press the black button, the display will show 'RFID'. Put the lot specific RFID card onto the top side of the reader. Following an audible beep signal, 'TEST' is displayed.



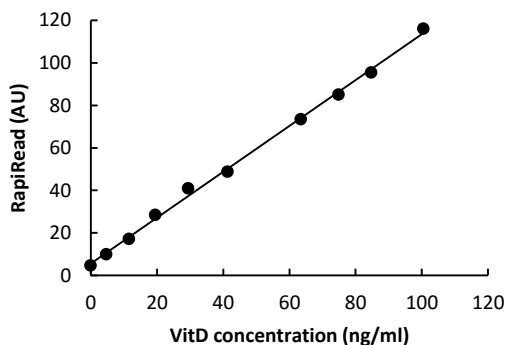
**5. Start measurement**

Press the black button, the reader displays 'RUN' first, then Vitamin D (VD) concentration in ng/ml with an audible beep signal.



**STANDARD CURVE USING *RapiRead*™ CUBE READER**

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



**PERFORMANCE CHARACTERISTICS**

**Sensitivity:**

The sensitivity of *25 OH Vitamin D Rapid Test* is 2.9 ng/ml. The sensitivity was determined by calculating the mean plus 3.3 times of standard deviation of twenty Vitamin D-free serum tests.

**Accuracy:**

The accuracy of *25 OH Vitamin D Rapid Test* was evaluated in comparison to Siemens' ADVIA Centaur Vitamin D Total Assay (US FDA Approved) on Serum Samples. *25 OH Vitamin D Rapid Test* was tested with 102 commercially available serum samples and found to be in 95% Agreement with Siemens' ADVIA Centaur Vitamin D Total Assay.

**Detection Range:**

The Detection Range of *25 OH Vitamin D Rapid Test* with *RapiRead*™ CUBE Reader is from 3 to 100 ng/ml.

**Precision:**

	Serum	No. of Replicates	Mean ng/ml	Standard Deviation	Coefficient Variation
Intra-Assay	1	20	31.1	4.5	14.6%
Inter-Assay	1	30	32.8	5.3	16.1%

**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 and Vitamin B12.

**EXPECTED RESULTS**

*25 OH Vitamin D Rapid 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**

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