Quantitative Vitamin D Test

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

REF 2009-Q1

For In Vitro Diagnostic use only ***Read Instructions before use***

INTENDED USE

Spark Diagnostics SparkD Quantitative Vitamin D Testis 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 prevalent in high percentage of individuals. 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, 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 status1,2.

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

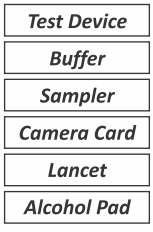
TEST PRINCIPLE

SparkD 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 result. The intensity of colored band in the test line region is 25-OH Vitamin D concentration-dependent, proportional to the level of the 25-OH Vitamin D in the sample. 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.SparkD Quantitative Vitamin D Test (1 Test)
2.Buffer tube (1 tube with buffer)
3.Capillary tube(1 capillary tube)
4.Camera Test Card(1 card)
5.Lancet(1 Lancet)
6.Alcohol pad (1 alcohol pad)
7.Instructions for use



Note: Alcohol pad and Lancet are medical devices meeting the provisions of Directive 93/42/EEC. Lancet is sterile and bears CE0197.

MATERIALS REQUIRED BUT NOT PROVIDED

1.Timer or clock 2. Spark-D Mobile Reader App 3. Smart phone

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.

CAUTION! 5. Do not use if (a) Test device seal is broken, (b) Box contents are missing, or (c) inside contents are damaged or broken.

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 SparkD 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.

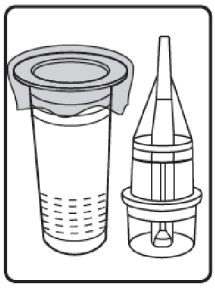
CAUTION!

SparkD 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 SparkD Quantitative Vitamin D Test as Anticoagulants will impact the test results.

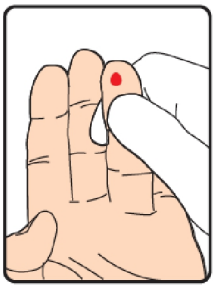
SPECIMEN COLLECTION AND PREPARATION

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.

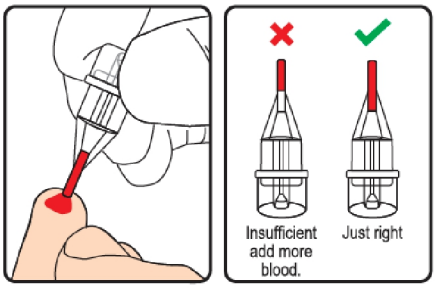
INSTRUCTIONS TO USE SAMPLING DEVICE



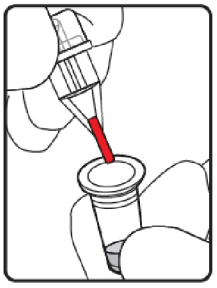
1. The **SAMPLER** Device contains a Collection Tube (left) and a Blood Collector with Cap (right).



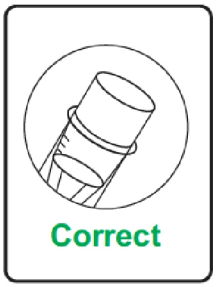
2. Use a Lancet to draw finger-prick blood.

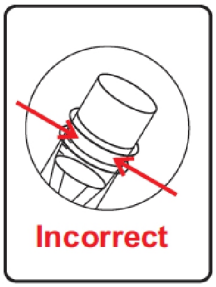


3. Gently touch the tip of Blood Collector to blood droplet. Capillary action will completely fill 10 µl of blood and stop.

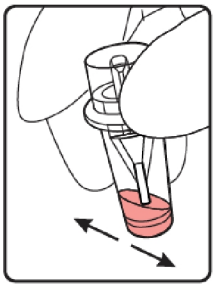


4. Fully Insert the Blood from the Blood Collector into the Collection Tube and push firmly to close tightly.

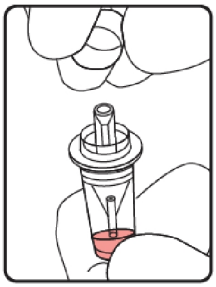




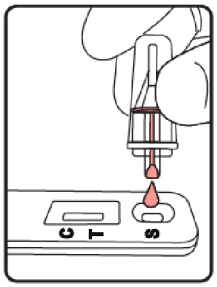
5. Correct compression of Buffer and Sampler



6. Shake the **SAMPLER** 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 the **SAMPLER**



8. Invert the **SAMPLER** Device and gently squeeze 3 drop of pre-mix blood into the Sample Well (S) of the Test Cassette.

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 **Sampler** 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 (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 **Sampling** Device.
4. Follow the Instruction on how to use the Spark Dx app to get the results.

Disposal

After use, dispose all components of the SparkD Quantitative Vitamin D Test as biohazard waste.

Serum Protocol:

SparkD 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 **Sampler** Device".

Same Protocol is also used for testing Vitamin D Controls.

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

Instructions on how to use the SPARK Dx App (REF 8002-RF) :

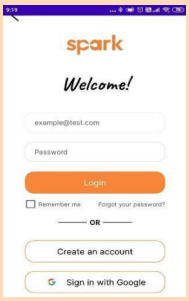
INTENDED USE OF APP:

The SPARK Dx mobile app is designed to read the SPARK D, quantitative Vitamin D test (Ref 2009 Q-1 and 2009-Q25). The mobile app scans the completed SPARK D test using the camera function of a mobile phone (or smartphone) to determine quantitative readings of Vitamin D levels of the test specimen and store the results in the phone.
Open the app and follow the below instruction to perform the test after preparing the test cassette.

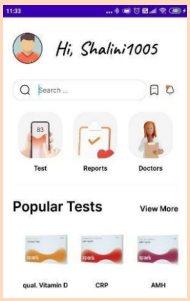
1. About App: SparkDx App is a universal app that enables its user to run different Spark tests on a single platform.

INSTRUCTIONS TO USE THE APP:

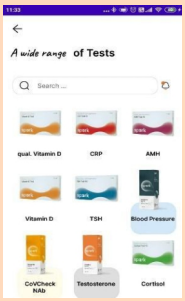
1. **Open SPARKDx Reader App (One-Time):** By using the App, the user, can either create their own Spark account or Sign-in with their Google account (1a). Once logged in, the home screens appear as shown in the figure below (1b). Upon using 'Test', the user can navigate the wide range of tests offered by Spark (1c).



1a



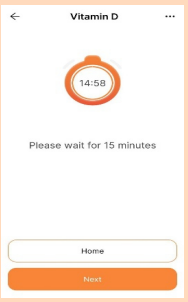
1b



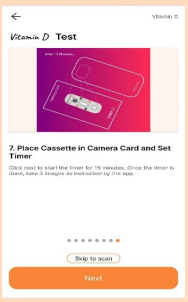
1c

2. **Start Test Instructions and Timer SPARK ReaderApp:** After selecting a particular test, the user can see the test instructions by clicking on "Start test" (2a). The procedure for preparation of the test cassette is given step by step with an image guide on the screen. Follow all the steps until step 7 (2b). At the end of the instructions, the user will receive a prompt to set a 15 minutes timer (2c) for the test completion (before the next steps). Alternatively, the user can skip the timer to directly scan the test cassettes after the completion of 15 min (The user should make sure that 15 min. has been completed after the sample application).

2a



2b



2c

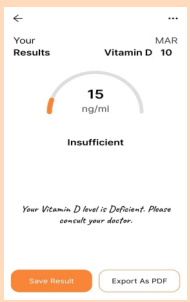
3. **Camera Permissions SPARK DX App (One-Time):** In order to perform the test, grant permission to access the Camera function of the phone and to store the image files in your storage, when needed (see privacy policy).

4. **Scan Test Cassette in SPARK Dx App:** After 15 minutes timer completion (in Step 3), the camera will start to take images (4a). Important: Use the "CAMERA CARD" provided with the package as background as shown in the screen below (4b). Keep the test cassette as shown in the picture below on the Camera Card before taking the image. Click "Next" to take a total of 3 images.

4a

4b

5. **Display Result SPARK Dx App:** After the 3 Scans are completed the result will be displayed on the screen as shown (5). Click "Save report" to save the results in the history screen or Export them as pdf.



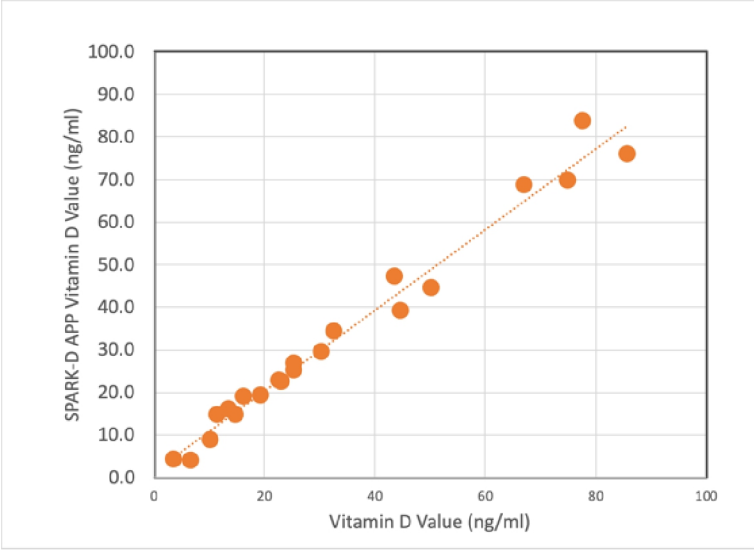
5

PRECAUTIONS FOR APP:

1. Use Camera Scan functions only after completion of a 5 minutes timer.
2. Use Camera Scan immediately.
3. Always use a camera card to take pictures.

STANDARD CURVE USING SPARK Dx APP

A typical standard curve with respect to the control values obtained with SPARK-D App is illustrated on right side.



PERFORMANCE CHARACTERISTICS:

Sensitivity:

The limit of detection (LOD) of SPARK D Quantitative Vitamin D Test is 4 ng/ml (10 nmol/l).

Detection Range:

The Detection Range of SPARK D Quantitative Vitamin D Test with SPARK D Reader is from 4 ng/ml (10 nmol/l) to 100 ng/ml (250 nmol/l).

Accuracy:

The accuracy of SPARK 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, SPARK D test results of human blood samples showed good agreement with the ELISA results of corresponding serum samples.
The accuracy of SPARK 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 0.97 and Correlation Coefficient of 98%. In conclusion, SPARK D *Quantitative Vitamin D Test* results agree closely to the true values generated from LC-MS/MS assay.

Specificity:

30 Vitamin D free serum samples were tested and all showed negative results; suggesting 100% Specificity.

Limitations:

No interference and cross reactivity were observed with Bilirubin, Triglycerides, Cholesterol, Vitamin B12 and Vitamin C.

EXPECTED RESULTS

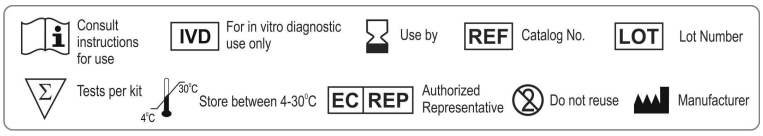
SPARK D Quantitative Vitamin D Test is a Rapid Quantitative assay. The test is intended to use for screening individuals to identify Vitamin D level. This assay provides only a preliminary analytical test result. The liquid chromatography with tandem mass spectrometry (LC-MS/MS) assays or quantitative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

REFERENCES

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. Dermatol Nurs. 2009, 21:25-30
4. Zerwekh JE. Blood biomarkers of vitamin D status. Am J. Clin Nutr. 2008, 87:1087S-91S
5. 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

SHELF LIFE: 18 Months

INDEX OF CE SYMBOLS



SPARK DIAGNOSTICS LLC
7820 Eldorado Pkwy, Suite#150,
Mckinney, TX 75070

USA
Tel: +1-415-326-4177
support@sparkdiagnostics.com
www.sparkdiagnostics.com

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