Rapid Response™

Vitamin D Test
(Whole Blood/Serum/Plasma)

REF VITD-13, VITD-13C25, VITD-13C40 Product Insert

A rapid test for the semi-quantitative detection of vitamin D in whole blood, serum or plasma.

For professional *in vitro* diagnostic use only.

Intended Use

The Rapid Response™ Vitamin D Test is a rapid chromatographic immunoassay for the semi-quantitative detection of vitamin D in Whole blood, serum or plasma.

Summary

Man is reported to have been aware since early antiquity of the substance we now know as vitamin D. The first scientific description of a vitamin D-deficiency, namely rickets, was provided in the 17th century by both Dr. Daniel Whistler (1645) and Professor Francis Glisson (1650). The major breakthrough in understanding the causative factors of rickets was the development in the period 1910 - 1930 of nutrition as an experimental science and the appreciation of the existence of vitamins.

Considering the fact that now we accept that the biologically active form of vitamin D, namely 1a,25(OH)2-vitamin D3, is a steroid hormone, it is somewhat ironic that vitamin D, through a historical accident, became classified as a vitamin. It was in 1919/20 that Sir Edward Mellanby, working with dogs raised exclusively indoors (in the absence of sunlight or ultraviolet light), devised a diet that allowed him to unequivocally establish that the bone disease, rickets was caused by a deficiency of a trace component present in the diet. In 1921 he wrote, "The action of fats in rickets is due to a vitamin or accessory food factor which they contain, probably identical with the fat-soluble vitamin." Furthermore, he established that cod liver oil was an excellent antirachitic agent.

Shortly thereafter E.V. McCollum and associates observed that by bubbling oxygen through a preparation of the "fat-soluble vitamin" they were able to distinguish between vitamin A (which was inactivated) and vitamin D (which retained activity). In 1923 Goldblatt and Soames clearly identified that when a precursor of vitamin D in the skin (7-dehydrocholesterol) was irradiated with sunlight or ultraviolet light, a substance equivalent to the fat-soluble vitamin was produced. Hess and Weinstock confirmed the dictum that "light equals vitamin D". They excised a small portion of skin, irradiated it with ultraviolet light, and then fed it to groups of rachitic rats. The skin that had been irradiated provided an absolute protection against rickets, whereas the unirradiated skin provided no protection whatsoever; clearly, these animals were able to produce by uv irradiation adequate quantities of "the fat-soluble vitamin", suggesting that it was not an essential dietary trace constituent. In parallel studies, Steenbock and Black at the Biochemistry Department of the University of Wisconsin found that rat food which was irradiated with ultra violet light also acquired the property of being antirachitic. However, because of the rapid rise of the science of nutrition -- and the discovery of the families of water-soluble and fat-soluble vitamins -- it rapidly became firmly established that the antirachitic factor was to be classified as a vitamin.

Principle

The Rapid Response™ Vitamin D Test is a semi-quantitative, membrane based immunoassay for the detection of vitamin D in whole blood, serum or plasma. The membrane is pre-coated with vitamin D antigen-BSA on the test line region of the cassette. During testing, the whole blood, serum or plasma specimen reacts with anti-VD antibody conjugated colloid gold. The mixture migrates upward on the membrane chromatographically by capillary action to react with vitamin D antigen-BSA on the membrane and generate a colored line. Presence of this colored line indicates a negative result, while its absence indicates a positive result. To serve as a procedural control, two colored lines will always appear at the reference line regions indicating that proper volume of specimen has been added and membrane wicking has occurred.

Reagents

The Rapid Response™ Vitamin D Test contains anti-VD antibody conjugated colloid gold and Vitamin D antigen-BSA coated on the membrane with PBS.

Precautions

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- The used tests, specimens and potentially contaminated materials should be discarded according to the local regulations.
- Humidity and temperature can adversely affect results.

Materials

Materials provided

- Test cassettes
- Droppers
- Buffer
- Product insert

Materials required but not provided

- Specimen collection containers
- Centrifuge (for plasma only)
- Timer

Storage and Stability

The kit can be stored at room temperature or refrigerated (35.6-86°F; 2-30°C). The test cassette is stable through the expiration date printed on the sealed pouch. The test cassette must remain in the sealed pouch until use. **DO NOT FREEZE.** Do not use beyond the expiration date.

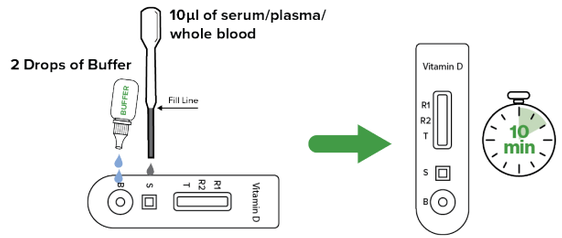
Collection and Storage of Specimens

- The Rapid Response™ Vitamin D Test can be performed using whole blood, serum or plasma.
- To collect Fingerstick Whole Blood specimens:
  - Wash the patient's hand with soap and warm water or clean with an alcohol swab. Allow to dry. Massage the hand without touching the puncture site by rubbing down the hand towards the fingertip of the middle or ring finger.
  - Puncture the skin with a sterile lancet. Wipe away the first sign of blood.
  - Gently rub the hand from wrist to palm to finger to form a rounded drop of blood over the puncture site.
- Add the Fingerstick Whole Blood specimen to the test by using a capillary tube:
  - Touch the end of the capillary tube to the blood until filled to approximately 10µL. Avoid air bubbles.
  - Place the bulb onto the top end of the capillary tube, then squeeze the bulb to dispense the whole blood to the specimen area of the test cassette.
- Separate serum or plasma from blood as soon as possible to avoid hemolysis. Use only clear non-hemolyzed specimens.
- Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 35.6-46.4°F (2-8°C) for up to 3 days. For long term storage, specimens should be kept below -4°F (-20°C). Whole blood collected by venipuncture should be stored at 35.6-46.4°F (2-8°C) if the test is to be run within 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by fingerstick should be tested immediately.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

Test Procedure

Allow test cassette, specimen, and/or controls to equilibrate to room temperature (59-86°F; 15-30°C) prior to testing.

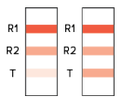
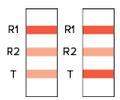
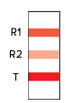
1. Bring the pouch to room temperature before opening it. Remove the test cassette from the sealed pouch and use it as soon as possible. Best results will be obtained if the assay is performed within one hour.
2. Place the test cassette on a clean and level surface. Hold the dropper vertically and draw the serum/plasma/Whole blood up to the Fill Line as shown in illustration below (approximately 10 µL) and 2 drops of buffer to each specimen well of the test cassette and start the timer. Avoid trapping air bubbles in the specimen well. See the illustration below.
3. Wait for the colored line(s) to appear. The test result should be read at 10 minutes. Do not interpret the result after 20 minutes.

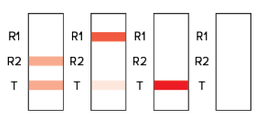


Results Interpretation

Valid Result: Two color lines appear in the Reference region 1 (R1) and Reference region 2 (R2). The result considers valid. The interpretation of result showed below.

	Concentration	Interpretation
R1 R2 T >100ng/ml >250nmol/l	**Conc.>100ng/ml**	Two color lines appear in the Reference region 1 (R1) and Reference region 2 (R2). Intensity of R1 line should be stronger than the intensity of R2 line(R1>R2). No apparent red or purple line appears in the test region (T).

 100ng/ml~30mg/ml 250nmol/l~75nmol/l	**100ng/ml ≥Conc.≥30ng/ml**	Two color lines appear in the Reference region 1 (R1) and Reference region 2 (R2). Intensity of R1 line should be stronger than the intensity of R2 line(R1>R2). Intensity of T line will be weaker than or equal to R2 line.
 30ng/ml~20ng/ml 75nmol/l~50nmol/l	**30ng/ml >Conc. ≥20ng/ml**	Two color lines appear in the Reference region 1 (R1) and Reference region 2 (R2). Intensity of R1 line should be stronger than the intensity of R2 line(R1>R2). Intensity of T line will be stronger than R2 line and weaker than or equal to R1 line.
 <20ng/ml <50nmol/l	**20ng/ml >Conc.**	Two color lines appear in the Reference region 1 (R1) and Reference region 2 (R2). Intensity of R1 line should be stronger than the intensity of R2 line (R1>R2). Intensity of T line will be stronger than R1 line.



Invalid

INVALID Result: Reference region 1 (R1) and/or Reference region 2 (R2) fail(s) to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test cassette. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

Quality Control

Internal procedural controls are included in the test. A color line appearing in the control region (C) is an internal valid procedural control. It confirms adequate membrane wicking.

Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

Limitations

1. The Rapid Response™ Vitamin D Test is for *in vitro* diagnostic use only. This test should be used for the detection of Vitamin D in serum or plasma or whole blood specimen.
2. As with all diagnostic tests, all results must be considered with other clinical information available to the physician.

Bibliography

1. Mason JB. Vitamins, trace minerals, and other micronutrients. In: Goldman L, Schafer AI, eds. Goldman-Cecil Medicine. 25th ed. Philadelphia, PA: Elsevier Saunders; 2016:chap 218.
2. National Osteoporosis Foundation website. Clinician's Guide to Prevention and Treatment of Osteoporosis. 2014 Issue, Version 1. www.iscd.org/documents/2014/10/nof-clinguidelines.pdf. Accessed May 5, 2017.

Glossary of Symbols

Consult instructions for use
Test per Kit
EC REP Authorized Representative
Store between 35.6°F to 86°F
Use by
Do Not Reuse
LOT Lot Number
IVD For *in vitro* diagnostic use only
REF Catalogue #
Do not use if package is damaged
Manufacturer

EC REP
MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany

CE

BTNX Inc.
722 Rosebank Road,
Pickering, ON L1W 4B2
Canada

Technical Support: 1-888-339-9964