Onestep Vitamin D RapiCard™ InstaTest (Whole Blood)

A rapid test for the semi-quantitative detection of total 25-OH Vitamin D in human Whole Blood. For professional in vitro diagnostic use only

TEST PRINCIPLE
The Vitamin D test is immunoassay based on the principle of competitive binding. During testing, the mixture migrates upward on the membrane chromatographically by capillary action. The membrane is pre-coated with 25-OH Vitamin D antigen on the test line region of the strip. During testing, if 25-OH Vitamin D is present in the specimen, it will compete with the 25-OH Vitamin D antibody for limited amount of particle coated 25-OH Vitamin D antibody. No line will form in the test region. And a visible colored line will form in the test region if there is no 25-OH Vitamin D in the specimen because all the antibody coated particles will be captured by the antigen coated in the test line region. To serve as a procedural control, a colored line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

REAGENTS
The test contains anti-25 OH Vitamin D antibody coated particles and 25-OH Vitamin D antigen coated on the membrane.

MATERIALS AND COMPONENTS
Materials provided with the test kit:
• Test Cassettes
• Buffer
• Color Card

Materials required but not provided:
• Specimen containers
• Timer
• Lancets (for fingerstick whole blood only)
• Heparinized capillary tubes and dispensing bulb (for fingerstick whole blood only)

PRECAUTIONS
Please read all the information in this package insert before performing the test.

1. For professional in vitro diagnostic use only. Do not use after the expiration date.
2. The test should remain in the sealed pouch until ready to use.
3. All specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
4. The used test should be discarded according to local regulations.

SPECIMEN COLLECTION AND PREPARATION
• For Whole Blood specimens
  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 dropper.
  • Touch the end of the capillary dropper to the blood, do not squeeze the bulb of the dropper, the blood migrates into the dropper through the capillarity to the line indicated on the dropper. Avoid air bubbles.
  • Squeeze the bulb to dispense the whole blood to the specimen area of the test Cassette.

• Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods. For long term storage, specimens should be kept below -20°C. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. 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 packaged in compliance with local regulations covering the transportation of etiologic agent.

INTENDED USE
The Cortez Diagnostic, Inc. Vitamin D RapiCard™ InstaTest (Whole Blood) is a rapid chromatographic immunoassay for the semi-quantitative detection of total 25-hydroxy Vitamin D (25-OH Vitamin D) in human blood at a cut-off concentration of 30 ± 4 ng/ml. This assay provides a preliminary diagnostic test result and can be used to screening for Vitamin D deficiency.

SUMMARY AND EXPLANATION
Vitamin D refers to a group of fat-soluble secosteroids responsible for increasing intestinal absorption of calcium, iron, magnesium, phosphate, and zinc. In humans, the most important compounds in this group are Vitamin D3 and Vitamin D2. 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 Comlications, 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.
ASSAY PROCEDURE
Allow the test, specimen, buffer and/or controls to reach room temperature (15-30°C) prior to testing

For whole blood specimen:
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.
2. Place the cassette on a clean and level surface.

For Venipuncture Whole Blood specimen:
- Hold the pipette vertically and transfer 20µl whole blood to the specimen well, then add 4 drops of buffer, and start the timer.
- See illustration below.

For Fingerstick Whole Blood specimen:
- To use a Capillary droppers: Fill the capillary tube to the specimen area of test cassette, then add 4 drops of buffer (approximately 160µl) and start the timer. See illustration below.
- 2. Wait for the colored line(s) to appear. Read results at 10 minutes. Do not interpret the result after 20 minutes.

RESULTS
(Please refer to the illustration and compare the test results with color card provided “Visual Interpretation of Results”)

Deficient: Two purple color lines appear. One is at the control region (C) and another should be in the region (T). Note: The shade of the purple color line at the test region may vary, as shown below “Visual Interpretation of Results”.

Sufficient: One purple color line appears. One purple color line appears at the control region (C) and no line appears at the test region (T).

INVALID: Control line fails 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. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

QUALITY CONTROL
A procedural control is included in the test. A colored line appearing in the control region (C) is the internal procedural control. It confirms sufficient specimen volume and correct procedural technique. 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 OF PROCEDURE
1. The Cortez Diagnostic, Inc. Vitamin D RapiCard™ InstaTest (Whole Blood) has been compared with ELISA. The following results were tabulated:

<table>
<thead>
<tr>
<th>Method</th>
<th>ELISA</th>
<th>Total Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rapid Test</td>
<td>Positive</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
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<tr>
<td>Cassette</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>81</td>
<td>91</td>
</tr>
</tbody>
</table>

   *Confidence Interval
   - Relative Sensitivity: 98.8% (95%CI*: 95.3%-99.9%)
   - Relatively Specificity: 96.0% (95%CI*: 89.1%-98.8%)
   - Accuracy: 97.1% (95%CI*: 93.3%-99.0%)

   Intra-Assay
   Within-run precision has been determined by using 15 replicates of three specimens: 15ng/ml, 30ng/ml and 45ng/ml specimens. The specimens were correctly identified >99% of the time.

   Inter-Assay
   Between-run precision has been determined by 15 independent assays on the same 3 specimens: 15ng/ml vitamin D, 30ng/ml vitamin D, 45ng/ml vitamin D standard sample. Three different lots of the Vitamin D RapiCard has been tested using these specimens. The specimens were correctly identified >99% of the time.

   Sensitivity and Cross-Reactivity
   The Cortez Diagnostic, Inc. Vitamin D RapiCard™ InstaTest (Whole Blood) can detect levels of Vitamin D in human whole blood as low as 30ng/ml. The addition of Vitamin A, B, C, E, K and M showed no cross-reactivity.

EXPECTED VALUES
- Intra-Assay
- Inter-Assay
- Sensitivity and Cross-Reactivity

COR CODE #44
There are differing views on cut-off levels for healthy individuals. However, with published reports, it has been categorized into three areas – Sufficient: >30 ng/ml; Insufficient: 21-30ng/ml and Deficient: <20 ng/ml. The Test is designed to distinguish levels at 20 ng/ml and 30 ng/ml.

**STORAGE**

Store as packaged at room temperature or refrigerated (2-30°C). The test is stable through the expiration date printed on the sealed pouch or label of the closed canister. The test must remain in the sealed pouch or closed canister until use. **DO NOT FREEZE.** Do not use beyond the expiration date.

**REFERENCE**