Velo™ Vitamin D Rapid Test

(Whole Blood/Serum/Plasma)

CARBONTECHNOLOGIES

REF	Σ
VE242006	1
VE242005	25

Intended use

Velo™ Vitamin D Rapid Test is a lateral flow immunoassay intended for the qualitative detection of Vitamin-D in human whole blood, serum or plasma specimens. It is intended to be used by healthcare professionals as an aid in the assessment and evaluation of patients with vitamin D deficiency related diseases. The test only provides preliminary analysis results but not critical diagnosis criteria. Any use or interpretation of the test must be analyzed and confirmed with alternative testing method(s) and clinical findings based on professional judgment of healthcare providers.

Summary

Vitamin D is a steroid hormone that is important in the management of calcium and phosphorus concentrations required in the mineralization of bone. Vitamin D has two important forms: cholecalciferol (D3) formed in the skin from ultraviolet light and ergocalciferol (D2) found in dairy products. However, these forms do not have significant biological activity.

The hormonal active form, 1-25-dihydroxylcholecalciferol, is produced through transformations in the liver and kidney. The first step in this conversion is an enzymatic reaction of D2 or D3 into 25OH-D2 or 25OH-D3. These 25 (OH) D forms are not freely circulating in blood, but are primarily bound to vitamin D binding protein (VDBP).

The high binding affinity of the 25OH D (2 or 3) compared to other derivatives of vitamin D leads to a long half-life in blood and its use as an accurate indicator of Vitamin D status. Vitamin D deficiency has been associated to diseases related to bone damage such as osteomalacia and rickets. Vitamin D can be dietarily supplemented via use of Vitamin D2 or Vitamin D3. The sum of the 25OH D (2 or 3) in serum or plasma is referred to as total 25OH Vitamin D. The accurate measurement of total Vitamin D is necessary in monitoring deficient Vitamin D patients to achieve the optimum dosage and avoid excessive levels, which are considered toxic.

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	< 20	< 50
Insufficient	20 to < 30	50 to <75
Sufficient	30 – 100	75 – 250
Upper Safety Limit	> 100 – 150	> 250

The detection limit of Velo Vitamin-D Rapid Test is determined as 20ng/mL. The test can be performed without cumbersome laboratory equipment, and the results are available at 15-20 minutes.

Principle

Velo™ Vitamin D Rapid Test is an immunoassay based on the principle of sandwich method. During testing, the mixture migrates upward on the membrane chromatographically by capillary action. The membrane is pre-coated with 25 (OH) D antigens on the test line region (T). During testing, the specimen reacts with the particle coated with 25 (OH) D antigens. The mixture migrates upward on the membrane chromatographically by capillary action to react with 25 (OH) D antigens on the membrane and generates a colored line(s). The presence of this colored line in the test line region (T), indicates a positive result, while its absence indicates a negative result.

An internal quality control is included in the test, in the form of a colored line appearing in the control line region (C), indicating that the test is functional, and proper and sufficient volume of specimen has been applied to enable migration through the test and control line, regardless of whether there is a test line or not. If the control line (C) does not appear within the testing time, test result is invalid and the test should be repeated with a new test device.

Kit Content

Components	1 Test/Kit	25 Tests/Kit
Test cassette individually foil pouched with a desiccant	1	25
Buffer (vial)	1	25
Safety Lancet	1	25
Alcohol Pad	1	25
Dropper (30µL)	1	25
Package insert	1	25

Materials required but not provided

- Laboratory Pipettes
- Timer (for incubation on bench)
- Specimen Collection Containers

- Centrifuge (for Plasma/Serum only)

Warnings & Precautions

- For in vitro diagnostic use only. Do not reuse the test.
- Do not freeze the test kit or its components.
- These instructions must be carefully read and strictly followed by a trained healthcare professional to achieve accurate results. All users should read the instructions before performing test.
- The test is only for the detection of Vitamin-D, not for any other viruses or pathogens.
- Inadequate or inappropriate specimen collection, storage, and transportation are likely to result in false negative test results.
- Do not use hemolyzed blood specimens for testing.
- Do not eat, drink or smoke in the area where handling specimens or performing the test.
- Do not use the test kit beyond its expiration date.
- Do not mix components from different kit lots.
- Leave test cassette sealed in its foil pouch until just before use. Do not use the test cassette if the pouch is damaged or the seal is broken.
- To avoid contamination or inaccurate test result, do not touch the reaction area of test cassette when performing the test.
- Wear appropriate personal protection equipment and gloves when performing the test, collecting and handling patient specimens.
- Dispose of all used test cassettes and potentially contaminated materials in a biohazard container as if they were infectious waste and dispose according to applicable local laws and regulations.

Storage & Stability

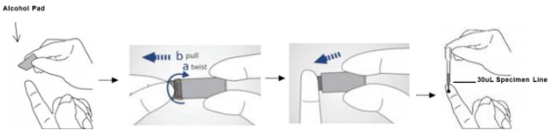
- The test kit should be stored either at room temperature or refrigerated (2°C-30°C), away from direct sunlight. Do not freeze the kit or expose the kit to temperatures over 30°C.
- The shelf life of the kit is as indicated on the outer package (24 months from date of manufacture).
- This test kit is stable until the expiration date marked on the outer package and foil pouch. Ensure all test components are at room temperature (15-30°C) before use.
- Perform the test within one hour after taking out the test cassette from the foil pouch.

Sample Collection & Preparation

Consider any materials of human origin as infectious and handle them using standard biosafety procedures. The test can be performed using whole blood (from venipuncture or fingerstick), serum or plasma specimens. Follow standard laboratory procedures to collect specimens.

For Whole Blood Collected by Fingerstick Using a Safety Sterile Blood Lancet (see illustrations below):

1. Tear off to open the Alcohol Prep Pad. Clean the area to be lanced with an alcohol swab.
2. Carefully twist off the protective cap until it is separated from the device.
3. Pace the lancet firmly against the puncture site to activate. Do not remove the device until an audible click is heard.
4. Squeeze the end of the fingertip, using a disposable dropper (30µL) provided, dip the tip end of the dropper into the blood specimen to collect fingerstick whole blood until reaching the 30µL specimen line, automatically.



NOTE:

- Use a new disposable dropper for each specimen in order to avoid cross contamination of specimens, which could produce erroneous results.
- Discard the lancet or alcohol swab if package is pierced or damaged. The item may no longer be sterile; there is risk of infection if used.

For Whole Blood Collected by Venipuncture:

1- Using standard phlebotomy procedure, collect a venipuncture whole blood specimen using a blood collection tube with suitable anticoagulant (EDTA recommended).

2- It is recommended that specimens should be tested immediately. Do not leave the specimens at room temperature for prolonged periods. If the specimens are not tested immediately, they may be stored at 2°C-8°C.

3- It's not recommended to test the whole blood specimens storing at 2°C-8°C for more than 2 days.

NOTE:

- Do not freeze a whole blood specimen, otherwise the red blood cell will break, which may cause hemolysis.
- Whole blood specimens should be stored in refrigeration (2-8°C) if not tested immediately. The specimens must be tested within 24 hours after collection.
- Anticoagulants including heparin, EDTA and sodium citrate do not affect the test results. Use of other anticoagulants have not been validated. Their use may affect the test result.

Plasma/Serum

1- Collect blood specimen into collection tube containing EDTA, citrate or heparin for plasma or collection tube containing no anticoagulants for serum by venipuncture.

2- To make plasma specimen, centrifuge collected specimens and carefully withdraw the plasma into a new pre-labeled tube.

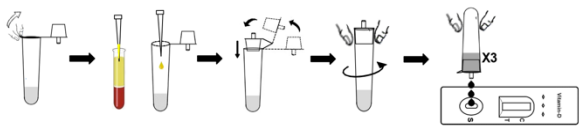
3- To make serum specimen, allow blood to clot, then centrifuge collected specimens and carefully withdraw the serum into a new pre-labeled tube.

Test the specimens as soon as possible after collecting. Store specimens at 2-8°C if not tested immediately. Specimens can be stored at 2-8°C for up to 3 days, and should be frozen at -20°C for longer storage.

NOTE:
- Avoid multiple freeze-thaw cycles (no more than 3 times). Prior to testing, equilibrate frozen specimens to room temperature slowly and mix gently.
- Specimens containing visible particulate matter should be clarified by centrifugation before testing.
- Do not use samples demonstrating gross lipemia, gross hemolysis or turbidity so as to avoid interference on result interpretation.

Preparation
Before testing, open the package and equilibrate the test cassette, buffer, specimens and/or controls to room temperature, and shake the buffer gently before use. The most suitable temperature condition to perform the test is room temperature (15-30°C). If the test kit is stored at room temperature, it can be opened and used immediately.

Test Procedures
1- Take out the test device from sealed foil pouch and place on a dry, clean and level surface.
2- Be sure to label the device with specimen's ID number.
3- Uncap the buffer tube. Draw 20µL serum or plasma specimen, or 30µL whole blood specimen, with a laboratory transfer pipette or fingerstick dropper (30uL) as needed, and add to the buffer tube. Screw on the cap and tighten securely to close the buffer tube. Shake the buffer tube vigorously so as to mix well the specimen with buffer. Then add three drops of mixed specimen to the specimen well (S) on the test cassette. See illustrations below.



4- Start the timer.
5- Wait for the colored line(s) to appear. Read test results at 15 minutes. Do not interpret the result after 20 minutes.

Interpretation of Test Results
(Please refer to the illustrations below)

- Positive: Two lines appear. One colored line should be in the control line region (C) and another apparent colored line(s) should be in the test line region (T).

NOTE: The intensity of the color in the test line region will vary depending on the concentration of Vitamin D present in the specimen. Therefore, the presence of any test line, no matter how faint, within the designated observation time, indicates a positive result.

- Negative: One colored line appears in the control line region (C). No line appears in the test line region.
- 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 device. If the problem persists, stop using the test kit immediately and contact your local distributor.

Positive	Very Weak Positive	Negative	Invalid
(＞30ng/mL)	(20~30ng/mL)	(＜20ng/mL)	

Quality Control
Internal Control: An internal quality control is included in the test, in the form of a colored line appearing in the control line region (C), indicating that the test is functional, and proper and sufficient volume of specimen has been applied to enable migration through the test and control line, regardless of whether there is a test line or not. If the control line (C) does not appear within the testing time, test result is invalid and the test should be repeated with a new test device.
External Control: 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.

Limits
This test has been developed for testing human whole blood, serum, or plasma specimens only. The results of Velo™ Vitamin D Rapid Test should be evaluated with all clinical and laboratory data available. If test results do not agree with the clinical evaluation, additional tests should be performed. Other factors may interfere with Velo™ Vitamin D Rapid Test and may cause erroneous results. These include technical or procedural errors, as well as additional substances in blood specimens.

Performance Characteristics
- Clinical performance

Velo™ Vitamin D Rapid Test been has correctly identified specimens of a performance panel and has been evaluated with a reference commercial enzyme-linked immunosorbent assay (ELISA) test using clinical specimens. Test results are presented in the table below.

Clinical performance compared to ELISA

Velo™ Vitamin D Rapid Test	ELISA		
	Positive	Negative	Total
Positive	170	2	172
Negative	2	268	270
Total	172	270	442

Sensitivity (Positive Percent Agreement): 98.83% = 170/172 (95% CI: 95.86%~99.68%)
Specificity (Negative Percent Agreement): 99.25% = 268/270 (95% CI: 97.34%~99.80%)
Accuracy (Overall Percent Agreement): 99.09% = (170+268)/442 (95% CI: 97.70%~99.65%)

- Precision

Within-run and between-run precisions have been determined by testing 10 replicates on the same four specimens: a negative, a weak positive, a medium positive and a strong positive. The negative, weak positive, medium positive and strong positive specimens were correctly identified in all of the tests performed during each run.

- Cross-reactivity

No cross-reactivity was observed by testing the following positive specimens respectively: HAMA, HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, HIV, HCV, H. pylori, MONO, CMV, Rubella and TOXO.

- Interference

The following potentially interfering substances were added to Vitamin D negative and positive specimens. Test results demonstrate that performance of Velo™ Vitamin D Rapid Test was not affected by the listed potentially interfering substances at the concentrations tested.

Acetaminophen	20 mg/dl	Caffeine	20 mg/dl
Ascorbic acid	20 mg/dl	Creatinine	200 mg/dl
Acetylsalicylic acid	20 mg/dl	Gentistic acid	20 mg/dl
Albumin	10.5 g/dl	Hemoglobin	1000 mg/dl
Bilirubin	1,000 mg/dl	Oxilic acid	600 mg/dl
Cholesterol	800 mg/dl	Triglycerides	1,600 mg/dl

References

1- Bikle D. D. Vitamin D and the skin. J. Bone Miner. Metab., 2010, 28, 117-30.
2- Holick, MF. Vitamin D Status: Measurement, Interpretation and Clinical Application. Ann Epidermoil. 2009, 19(2):73-78.
3- Morris H. A. Vitamin D: A Hormone for All seasons-How Much is enough? Clin. Biochem. Rev., 2005, 26, 21-32.
4- Zerwekh J. E. Blood biomarkers of vitamin D statues. Am. J. Clin. Nutr. 2008, 87, 1087S-91S.
5- Schöttker B, etal. 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.

Symbols

	Do not reuse	IVD	For in vitro diagnostic use only
	Temperature limit		Consult instruction for use
	Caution	LOT	Lot Number
	Keep away from sunlight	REF	Catalogue Number
	Use by	Σ	Contains sufficient for <n> tests
	Do not use if package is damaged		Keep dry
CE	CE Marking		Manufacturer
EC REP	EU Representative		Date of manufacture

Contact Information

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CE
IVD

Release Date: ... ... Date of Manufacture: ... ...