Vitamin D FIA Rapid Test Device

(Whole Blood/Serum/Plasma)

Catalogue Number: FI2-VTD-001

A Fluorescence Immunoassay for the quantitative detection of Vitamin D levels in human whole blood, serum or plasma using the Biopanda Fluorescence Immunoassay Device (BR-FIA-2000).
For professional in vitro diagnostic use only.

INTENDED USE

The Biopanda Vitamin D FIA Rapid Test Device is based on Fluorescence Immunoassay for the *in vitro* quantitative determination of total Vitamin D (VTD) in human whole blood, serum or plasma. Measurement of total Vitamin D (D2+D3) is used as an aid in the assessment of Vitamin D levels.

BACKGROUND

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 (e.g., sunlight) and Vitamin D2 is mainly obtained from foods. Vitamin D is transported to the liver where it is metabolised to 25-hydroxy Vitamin D. 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 recognised as a global epidemic. Virtually every cell in the body has receptors for Vitamin D, meaning that they all require "Sufficient" levels 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's, Obesity and general higher mortality. Therefore, detecting (25-OH) Vitamin D levels is now considered a "Medically Necessary Screening Test", and maintaining sufficient levels not just to improve bone health, but to improve overall health and well-being.

TEST PRINCIPLE

The Biopanda Vitamin D FIA Rapid Test Device detects Vitamin D based on competitive fluorescence immunoassay technology. The specimen moves along the strip from the sample pad to the absorbent pad. If Vitamin D is present in the specimen, it will compete with the VTD antigen pre-coated on the strip for the anti-VTD antibodies conjugated to fluorescent microspheres. Therefore, the less VTD in the specimen, the more fluorescent microspheres will be captured by the VTD antigen coated on the strip. The concentration of VTD in the sample is inversely related to the intensity of the fluorescent signal captured on the T line. The analyser can then calculate the concentration of VTD in the sample from the intensity of the fluorescent signal and the standard curve.

REAGENTS

The test cassette contains Vitamin D antigen and anti-Vitamin D antibody.

PRECAUTIONS

1. For professional *in vitro* diagnostic use only.
2. Do not use after the expiration date indicated on the package.
3. Do not use the test if the foil pouch is damaged.
4. Do not reuse tests.
5. Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
6. Do not eat, drink or smoke in the area where the specimens and tests are handled.
7. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow standard procedures for proper disposal of specimens.
8. Wear protective clothing such as laboratory coats, disposable gloves and safety glasses when specimens are assayed.
9. Do not interchange or mix reagents from different lots.
10. Extremes in humidity and temperature can adversely affect results.
11. Used testing materials should be discarded in accordance with local regulations.
12. The Biopanda Vitamin D FIA Rapid Test Device should only be used with the Biopanda Fluorescence Immunoassay Device (BR-FIA-2000) by approved medical professionals.

STORAGE AND STABILITY

1. The kit should be stored at 4-30°C until the expiry date printed on the sealed pouch. Do not freeze.
2. The test must remain in the sealed pouch until use.
3. Care should be taken to protect the components of the kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

KIT CONTENTS

- 25 x Foil wrapped Vitamin D test devices
- 25 x Buffer tubes
- 25 x Capillary droppers (for finger-prick whole blood only)
- 25 x Disposable droppers (for finger-prick whole blood only)
- 1 x ID card (Vitamin D)
- 1 x Package insert

EQUIPMENT REQUIRED BUT NOT PROVIDED

- Timer
- Blood collection containers
- Centrifuge
- Pipette
- Biopanda Fluorescence Immunoassay Analyser

SPECIMEN COLLECTION AND PREPARATION

1. Collect the specimen according to standard procedures.
2. Separate the serum or plasma from blood as soon as possible to avoid haemolysis. Only clear, non-haemolysed specimens can be used.
3. Do not leave specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 3 days; for long term storage, store 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. Do not freeze whole blood specimens. Whole blood collected by finger-prick should be tested immediately.
4. Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Avoid repeat freeze-thaw cycles.
5. EDTA and Heparin Sodium can be used as the anticoagulant for collecting the blood specimen.

For Finger-pricked Whole Blood Specimens, please refer to the DIRECTIONS FOR USE for further information.

DIRECTIONS FOR USE

Refer to the Biopanda Fluorescence Immunoassay Device User Manual for the complete instructions on operating the device. The test should be conducted at room temperature. Cold buffer solution or moisture condensation on the membrane can lead to invalid test results.

Note: There are two test modes for the Biopanda Fluorescence Immunoassay Device: *Standard Test* mode and *Quick Test* mode.

***Standard Test* mode** is a 'set and forget' method where the test will automatically be read after 10 minutes.

***Quick Test* mode** provides an instant result but the user must monitor the 10 minute test time themselves. It is suitable when running multiple tests simultaneously.

Refer to the user manual for further details.

Allow the test cassette, specimen, buffer and/or controls to reach room temperature (15-30°C) prior to testing.

1. Turn on the analyzer and insert the ID card into the analyzer 'Code' port and wait for the 'ID card imported successfully' pop-up. Fill in the necessary details in the 'Test Info' section.
2. Remove the test device from the sealed foil pouch and place on a clean, level surface.
3. Follow the appropriate steps below for the chosen specimen type:

3.1 <u>For venipuncture whole blood/serum/plasma specimens:</u>

i. Pipette **10 µl of whole blood/serum/plasma** into the buffer tube.
ii. Close the tube cap and shake the tube for approximately **10**

seconds to mix the specimen and dilution buffer well.

iii. Let the diluted specimen homogenise for approximately 1 minute. Diluted specimens should be used as soon as possible.

iv. **Pipette 75 µl of diluted specimen** into the specimen well (S) of the test device.

3.2 For finger-prick whole blood specimens:

i. Wash hands with soap and warm water or clean finger with an alcohol pad. Allow to dry.

ii. Massage the hand without touching the puncture site by applying pressure down the hand towards the finger to be pricked. The middle or ring finger is recommended.

iii. Use a sterile lancet (not provided) to puncture the skin and wipe away the first sign of blood.

iv. Gently apply pressure from palm to the pricked finger so a rounded drop of blood forms over the puncture site.

v. Using the provided capillary dropper and ensuring the dropper is level, touch the open end to the rounded drop of blood without squeezing the dropper bulb. **The dropper will automatically collect the correct volume of blood (approx. 10 µl),** *see Figure 1 below*.

vi. Dispense the whole blood specimen into the buffer tube by squeezing the dropper bulb.

vii. Close the tube cap and shake the tube for approximately **10 seconds** to mix the specimen and dilution buffer well.

viii. Using a disposable dropper, **draw the diluted specimen to the fill line** marked on the dropper (approx. 75 µl), then add to the specimen well (S) of the test device.

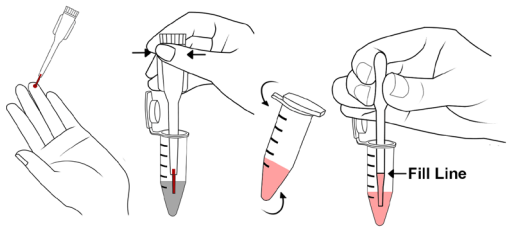

Figure 1

4. Insert the test device into the analyzer test device slot. According to user requirements, select either 'Standard test' or 'Quick test' mode.
5. Test results should be read at **15 minutes** with the use of the Biopanda Fluorescence Immunoassay Analyser.

INTERPRETATION OF RESULTS

The level of Vitamin D is calculated by the Biopanda Fluorescence Immunoassay Analyser with the result displayed on the screen in ng/ml and accompanied by an interpretation: 'Def/Insuf/Suf' (short for Deficient/Insufficient/Sufficient). For additional information, please refer to the analyser user manual.

The detection range of the test is 5-100 ng/ml.

QUALITY CONTROL

Each Biopanda Vitamin D FIA Rapid Test Device contains an internal control that satisfies routine quality control requirements. This internal control is performed each time a patient sample is tested. This control indicates that the test device was inserted and read properly by the Biopanda Fluorescence Immunoassay Device.

An invalid result from the internal control causes an error message on the Biopanda Fluorescence Immunoassay Device indicating that the test should be repeated. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control failure. Review the procedure and repeat the test with a new device. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

EXPECTED RESULTS

Concentrations	Clinical Reference
< 20 ng/ml	Deficient
20 - 30 ng/ml	Insufficient
> 30 ng/ml	Sufficient

PERFORMANCE CHARACTERISTICS

1. **METHOD COMPARISON**: The assay was compared with a commercially available CLIA test kit on 98 samples. The correlation coefficient(r) is 0.956.
2. **ACCURACY:** The test deviation ≤ ±15%.
3. **ASSAY RANGE:** 5 - 100 ng/ml
4. **PRECISION**:

Intra-lot precision

Within-run precision has been determined by using 10 replicates of 2 different concentrations of Vitamin D control; C.V. is ≤ 15%.

Inter-lot precision

Between-run precision has been determined by using 10 replicates for each of three lots using 2 different concentrations of Vitamin D control; C.V. is ≤15%.

5. **CROSS-REACTIVITY**

The following cross-reactants were tested for:

Cross-reactant	Concentration Tested	Cross-reactivity (%)
25-hydroxyvitamin D3	20 ng/ml	100
25-hydroxyvitamin D2	20 ng/ml	87
24,25-hydroxyvitamin D3	10 ng/ml	>100
24,25-hydroxyvitamin D2	10 ng/ml	>100
1,25-hydroxyvitamin D3	200 ng/ml	Not detected
1,25-hydroxyvitamin D2	200 ng/ml	Not detected
Vitamin D3	200 ng/ml	Not detected
Vitamin D2	200 ng/ml	Not detected
C3-epimer of 25-hydroxyvitamin D3	200 ng/ml	Not detected

LIMITATIONS OF THE TEST

1. The Biopanda Vitamin D FIA Rapid Test Cassette is for professional *in vitro* diagnostic use, and should be used for the quantitative detection of total Vitamin D in human whole blood, serum or plasma specimens only.
2. For optimal results, perform assay with freshly collected samples.
3. The test may yield abnormally low results due to Vitamin D epitopes being covered by unknown interfering components. Test results may also be affected by instability or degradation of Vitamin D in the sample due to time or temperature.
4. Other factors that can cause erroneous test results include technical/procedural errors, degradation of the test components, and presence of interfering substances in the test samples.
5. The test will only indicate the level of Vitamin D in the specimen and should not be used as the sole criteria for diagnosis or treatment.

INDEX OF SYMBOLS

	Manufacturer		Tests per kit		Do not reuse test
IVD	*In vitro* diagnostic medical device		Expiration date	REF	Catalogue number
	Store between 4-30°C	LOT	Lot Number		Consult instructions for use
	Do not use if package is damaged				

Thank you for purchasing Biopanda's Vitamin D FIA Rapid Test Device. Please read this manual carefully before operating to ensure proper use.

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