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Hormone

AFIAS Vitamin D

INTENDED USE

AFIAS Vitamin D is a fluorescence Immunoassay (FIA) for the quantitative determination of total 25(OH)D2/D3 level in human serum/plasma. It is useful as an aid in management and monitoring of regulating the concentration of calcium and phosphate in the bloodstream and promoting the healthy growth and remodeling of bone.

For *in vitro* diagnostic use only.

INTRODUCTION

Vitamin D from the diet or dermal synthesis from sunlight is biologically inactive and is a fat soluble steroid hormone involved in the active intestinal absorption of calcium and in the regulation of its homeostasis. In humans, the most important compounds in this group are vitamin D3 (also known as cholecalciferol) and vitamin D2 (ergocalciferol). [1] In the liver, cholecalciferol (vitamin D3) is converted to calcidiol, 25-hydroxycholecalciferol (abbreviated 25(OH)D3). Ergocalciferol (vitamin D2) is converted in the liver to 25-hydroxyergocalciferol (25(OH)D2). It is widely known that circulating 25(OH)D is the best indicator of vitamin D status.[2,3] 25(OH)D3 is then converted in the kidneys (by the enzyme 25(OH)D-1α-hydroxylase) into $1,25\text{-}(OH)_2D3$, a steroid hormone that is the active form of vitamin D. It can also be converted into 24-hydroxycalcidiol in the kidneys via 24-hydroxylation.[4,5] $1,25\text{-}(OH)_2D3$ circulates as a hormone in the blood, regulating the concentration of calcium and phosphate in the bloodstream and promoting the healthy growth and remodeling of bone. $1,25\text{-}(OH)_2D3$ also affects neuromuscular and immune function.[6] Vitamin D has a significant role in calcium homeostasis and metabolism. Its discovery was due to effort to find the dietary substance lacking in rickets (the childhood form of osteomalacia).[7]

This test can be used to diagnose vitamin D deficiency, and it is indicated in patients with high risk for vitamin D deficiency and when the results of the test would be used as supporting evidence for beginning aggressive therapies.[8] Patients with osteoporosis, chronic kidney disease, malabsorption, obesity, and some other infections may be high risk and thus have greater indication for this test.[9,10]

PRINCIPLE

The test uses a competitive immunodetection method. In this method, the target material in the sample binds to the fluorescence (FL)-labeled detection antibody in detection buffer, to form the complex as sample mixture. This complex is loaded to migrate onto the nitrocellulose matrix, where the covalent couple of 25(OH)D3 and bovine serum albumin (BSA) is immobilized on a test strip, and interferes with the binding of target material and FL-labeled antibody. If the more target material exists in blood, the less detection antibody is accumulated, resulting in the less fluorescence signal.

COMPONENTS

AFIAS Vitamin D consists of 'Cartridge', 'Pipette tip', 'ID chip' and 'Instruction for use'.

- Each cartridge packaged in an aluminum pouch has two components, a detector part and cartridge part.
- Cartridge part contains a test strip, the membrane which has BSA-25(OH)D3 conjugate at the test line, while rabbit IgG at the control line.
- The releasing part contains NaOH and DMSO.
- The detection part contains anti 25(OH)D2/3-fluorescence conjugate, anti rabbit IgG-fluorescence conjugate, gelatin as a stabilizer and sodium azide in Tris-HCl buffer as a preservative.

WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use only.
- Carefully follow the instructions and procedures described in this 'Instruction for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge and ID chip) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield misleading of test result(s).
- The cartridge should remain sealed in its aluminum pouch until use. Do not use the cartridge if that is damaged or already opened.
- For shipping, samples must be packed in accordance with the regulations. Sample with severe hemolytic and hyperlipidemia cannot be used and should be recollected.
- Just before use, allow the cartridge and sample to be at room temperature for approximately 30 minutes.
- **AFIAS Vitamin D** as well as the instrument for AFIAS tests should be used away from vibration and/or magnetic field. During normal usage, it can be noted that the instrument for AFIAS tests may produce minor vibration.
- Used pipette tips, and cartridges should be handled carefully and disposed by an appropriate method in accordance with relevant local regulations.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.
- **AFIAS Vitamin D** will provide accurate and reliable results subject to the following conditions.
  - Use **AFIAS Vitamin D** should be used only in conjunction with the instrument for AFIAS tests.
  - Any anticoagulants other than **EDTA, Heparin, and Sodium Citrate** should be avoided.

LIMITATIONS OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result. The non-responsiveness of the antigen to the antibodies is most common where the epitope is masked by some unknown components, so as not to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may cause the false negative as it makes antigen unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician including clinical symptoms and other relevant test results.

STORAGE AND STABILITY

- The cartridge is stable for 20 months (while sealed in an aluminum pouch) if stored at 2-8 °C.
- Return an unused cartridge to the spare cartridge zipperbag containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.

MATERIALS SUPPLIED

REF SMFP-63

Components of **AFIAS Vitamin D**

- Cartridge Box Contains
  - Cartridge 24
  - Pipette Tip (Zipperbag) 24
  - ID Chip 1
  - Instruction For Use 1
  - Spare Cartridge Zipperbag 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **AFIAS Vitamin D**. Please contact our sales division for more information.

- **AFIAS-1 REF FPRR019**
- **AFIAS-6 REF FPRR020**
- **Boditech Vitamin D Control REF CFPO-102**

SAMPLE COLLECTION AND PROCESSING

The sample type for **AFIAS Vitamin D** is human serum/plasma.

- It is recommended to test the sample within 24 hours after collection.
- Samples may be stored for up to a week at 2-8 °C prior to being tested. If testing will be delayed more than a week, samples should be frozen at -20 °C.
- Samples stored frozen at -20 °C for 6 months showed no performance difference.
- Once the sample was frozen, it should be used one time only for test, because repeated freezing and thawing can result in the change of test values.

TEST SETUP

- Check the components of the **AFIAS Vitamin D** as described below. : Cartridge, pipette tip, ID chip and instruction for use
- Keep the sealed cartridge (if stored in refrigerator) at room temperature for at least 30 minutes just prior to the test. Place the cartridge on a clean, dust-free and flat surface.
- Turn on the instrument for AFIAS tests.
- Empty the tip box.
- Insert the ID chip into the "ID chip port".
- Please refer to the instrument for AFIAS tests 'Operation Manual' for complete information and operating instructions.

TEST PROCEDURE

※ **AFIAS Vitamin D** measurement is not possible with other items simultaneously in AFIAS-6. Use only vitamin D cartridges.

1) Take 150 µL of sample with a pipette and dispense it into the sample well on the cartridge.
2) Insert the cartridge into the cartridge holder
3) Insert a tip into the tip hole of the cartridge.
4) Tap the 'START' icon on the screen.
5) The test result will be displayed on the screen after 28 minutes.

※ Note: Refer to the instrument for AFIAS tests Operation Manual to select sample type.

INTERPRETATION OF TEST RESULT

- Instrument for AFIAS tests calculates the test result automatically and displays total 25(OH)D2/D3 concentration of the test sample in terms of ng/mL.
- The cut-off (reference range)

25(OH)D		status
<10 ng/mL	<25 nmol/L	Deficiency
10-30 ng/mL	25-75 nmol/L	Insufficiency
30-100 ng/mL	75-250 nmol/L	Sufficiency

- Working range: 8.0-70 ng/mL
- Conversion factor: ng/mL = 2.5 x nmol/L

QUALITY CONTROL

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- The control tests should be performed immediately after opening a new test lot to ensure the test performance is not altered.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are not provided with **AFIAS Vitamin D**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance.
  (Please refer to the instruction for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical sensitivity**

Limit of Blank (LoB)	6.38 ng/mL
Limit of Detection (LoD)	7.35 ng/mL
Limit of Quantification (LoQ)	7.92 ng/mL

- **Analytical specificity**
  - **Cross-reactivity**
    There was no significant cross-reactivity from these materials with the **AFIAS Vitamin D** test measurements.

material	Concentration (ng/mL)
Vitamin D2	300 ng/mL
Vitamin D3	300 ng/mL

  - **Interference**
    There was no significant interference from these materials with the **AFIAS Vitamin D** test measurements.

material	Concentration
L-Ascorbic acid	300 µg/mL
Urea	2.6 mg/mL
EDTA	2.0 mg/mL
Heparin	200 U/mL
Sodium citrate	38 mg/mL

- **Precision**
  [Intra assay] For testing intra-assay precision, one person tested three different lots of **AFIAS Vitamin D**, ten times at each concentration of the control standard.

Vitamin D [ng/mL]	Lot 1	Lot 2	Lot 3	AVG.	CV%
9.33	9.42	9.44	9.37	9.41	4.35
26.15	26.11	25.65	25.26	25.67	5.35
63.83	62.65	65.26	63.33	63.75	5.26

  [Inter-assay] For testing inter-assay precision under the same conditions, three persons tested three different lots of **AFIAS Vitamin D**; three times at each concentration of the control standard.

Vitamin D [ng/mL]	Person			AVG.	CV%
	A	B	C		
9.33	9.19	9.23	9.60	9.34	5.89
26.15	26.75	26.04	25.87	26.22	6.40
63.83	63.65	63.56	64.62	63.95	6.18

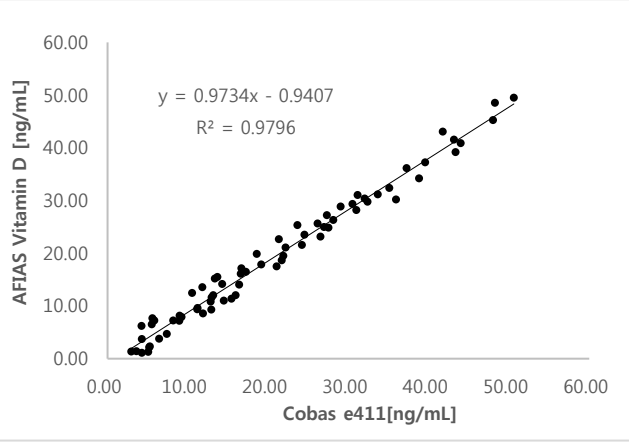
- **Accuracy**

The accuracy was confirmed by 3 different lots testing ten times each different concentrations.

Vitamin D [ng/mL]	Lot 1	Lot 2	Lot 3	Mean	CV(%)	Bias (%)
9.33	9.40	9.32	9.44	9.38	5.54	0.58
26.15	25.88	26.66	25.82	26.12	5.98	-0.13
63.83	62.24	66.18	61.37	63.26	5.80	-0.89

- **Comparability**

Using Cobas e411 as a comparison machine for **AFIAS Vitamin D**, 75 clinical samples were independently tested for its vitamin D concentration following each instrument's procedure. Results of both the test methods were analyzed and their comparability was investigated with linear regression and coefficient of correlation (R). The coefficient of correlation between the two methods was found to be 0.9796.



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Note: Please refer to the table below to identify various symbols.

	Sufficient for <n> tests
	Read instruction for use
	Use by
LOT	Batch code
REF	Catalog number
	Caution
	Manufacturer
EC REP	Authorized representative of the European Community
IVD	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
CE	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices

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