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Hormone

AFIAS Vitamin D Neo

INTENDED USE

AFIAS Vitamin D Neo is a fluorescence immunoassay (FIA) for the quantitative determination of total 25(OH)D2/D3 level in human whole blood/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 seco-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-$(OH)_2$D3, a seco-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-$(OH)_2$D3 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-$(OH)_2$D3 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 sandwich immunodetection method.

The detector antibody-fluorescence conjugates in buffer bind to antigens in the sample, forming antigen-antibody complexes. The capture antibody-Biotin conjugates also in the buffer bind to previous antigen-antibody complex and formed new complexes migrate onto nitrocellulose matrix to be captured by the other immobilized-streptavidin on a test strip.

More antigens in the sample will form more antigen-antibody complexes which lead to stronger fluorescence signal by detector antibodies, which is processed by the instrument for AFIAS tests to show Vitamin D concentration in the sample.

COMPONENTS

AFIAS Vitamin D Neo consists of 'cartridges'.

- Each sealed aluminum pouch contains two cartridges.
- Each cartridge packaged in an aluminum pouch has three components including a cartridge part, a detector part, and an extraction buffer part.
- The cartridge part contains the membrane called a test strip which has streptavidin at the test line, and chicken IgY at the control line.
- The detector part has 2 granules containing antibody-fluorescence conjugate, antibody-biotin conjugate, anti-chicken IgY-fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide as a preservative in phosphate buffered saline (PBS).
- The extraction buffer part contains sodium chloride, sodium acetate, and sodium azide as a preservative in phosphate buffered saline (PBS).

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow instructions and procedures described in this 'Instructions 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 incorrect test result(s).
- Do not reuse cartridges. A cartridge should be used for testing one sample only.
- The cartridge should remain sealed in its original pouch until just before use. Do not use a cartridge, if the pouch is damaged or has already been opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with local regulations. Sample with severe hemolysis and/or hyperlipidemia must not be used.
- If test components and/or sample are stored in refrigerator, then allow cartridge and sample to be at room temperature for approximately 30 minutes before use.
- The instrument for AFIAS tests may generate slight vibration during use.
- Used cartridges, C-tips and pipette tips should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- The cartridge contains sodium azide (NaN_3), and they may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure. Avoid contact with skin, eyes, and clothing. In case of contact, rinse immediately with running water.
- No Biotin interference was observed in **AFIAS Vitamin D Neo** when biotin concentration in the sample was below 50 ng/mL. If a patient has been taking biotin at dosage of more than 0.03 mg a day, it is recommended to test again 24 hours after discontinuation of biotin intake.
- **AFIAS Vitamin D Neo** will provide accurate and reliable results subject to the below conditions.
  - **AFIAS Vitamin D Neo** should be used only in conjunction with the instrument for AFIAS tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
Sodium heparin, Sodium citrate, K_2 EDTA

- **C-tip should be used when the following conditions are met.**
  - C-tip provided with the kit is recommended to obtain correct test result.
  - Whole blood should be immediately tested after collection.
  - Do not perform a test with C-tip on General Mode. It might cause an erroneous result.
  - Excess whole blood around the C-tip should be wiped off.
  - In order to avoid cross-contamination, please do not re-use C-tip for multiple samples.
  - AFIAS cartridge should be inserted and positioned in the cartridge holder prior to the blood sample collection
  - While collecting blood, be careful not to create air bubbles in the C-tip.

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(s) due to the non-responsiveness of the antigen to the antibodies which is the most common if the epitope is masked by some unknown components, so therefore not being able to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may also cause false negative result as it makes the 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 in conjunction with clinical symptoms and other relevant test results.

STORAGE AND STABILITY

Storage condition			
Component	Storage Temperature	Shelf life	Note
Cartridge	2 - 30 °C	20 months	Unopened
		1 month	Resealed

- Return an unused cartridge to the spare cartridge zipper bag containing the desiccant pack. Reseal along entire edge of zip-seal.

MATERIALS SUPPLIED

REF SMFP-108

Components of **AFIAS Vitamin D Neo**

- Cartridge box:
  - Cartridge 24
  - Pipette tip (zipper bag) 24
  - Spare cartridge zipper bag 1
  - ID chip 1
  - Instructions for use 1
  - C-tip (30µL) 24

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **AFIAS Vitamin D** Neo

Please contact our sales division for more information.

- **Instrument for AFIAS tests**
  - **AFIAS-1** REF **FPRR019**
  - **AFIAS-3** REF **FPRR040**
  - **AFIAS-6** REF **FPRR020**
  - **AFIAS-10** REF **FPRR038**
  - **Boditech Vitamin D Control** REF **CFPO-102**

SAMPLE COLLECTION AND PROCESSING

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

- It is recommended to test the sample within 24 hours after collection.
- The samples (serum, plasma) should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- The samples (serum, plasma) may be stored for a week at 2-8 °C prior to being tested. If testing will be delayed more than a week, samples (serum, plasma) should be frozen at -20 °C.
- The samples (whole blood) may be stored for 3 days at 2 - 8 °C prior to being tested.
- The samples (serum, plasma) stored frozen at -20 °C for 3 months showed no performance difference.
- However, the whole blood sample should not be kept in a freezer in any case.
- As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen samples.
- Collection of whole blood sample using C-tip.
  - ① Clean the area with a pre-injection swab.
  - ② Pierce with a sterile lancet.
  - ③ Wipe away the first drop of blood.
  - ④ Gently massage the around the pricked fingertip for the second drop.
  - ⑤ Hold the C-tip horizontally and touch the blood drop with the tip of the C-tip.
  - ⑥ Capillary action will automatically draw the blood sample to C-tip and stop.
  - ⑦ Wipe off any excess blood around the tip.
  - ⑧ Double-check if whole blood is filled accurately in the C-tip and AFIAS reader is ready for a test on the 'C-tip mode'.

TEST SETUP

- Check the components of the **AFIAS Vitamin D Neo** as described below. : cartridges, pipette tips, C-tips , an ID chip, a spare cartridge zipper bag and an instructions for use.
- Ensure that the lot number of the cartridge matches that of the ID Chip.
- If the sealed cartridge has been stored in a refrigerator, place them on a clean and flat surface at room temperature for at least 30 minutes before testing
- 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-1, AFIAS-3, AFIAS-6**

General mode

1) Insert a cartridge into the cartridge holder.
2) Insert a tip into the tip hole of the cartridge.
3) Select the 'General Mode' in the instrument for AFIAS tests.
4) Take 100 µL of the sample (whole blood/serum/plasma/control) using a pipette and dispense it into the sample well of the cartridge.
5) Tap the 'Start' button on the screen.
6) The test result will be displayed on the screen after 12 minutes.

C-tip mode

1) Insert a cartridge into the cartridge holder.
2) Take 30 µL of whole blood using a C-tip.
3) Insert the whole blood-filled C-tip into the tip hole of the cartridge.
4) Select the 'C-tip mode' in the instrument for AFIAS tests.
5) Tap the 'Start' button on the screen.
6) The test result will be displayed on the screen after 12 minutes.

▶ **AFIAS-10**

Normal mode

1) Insert a cartridge into the cartridge holder.
2) Insert a tip into the tip hole of the cartridge.
3) Tap the 'Load' button of the bay that holds the cartridge with the tip to read the barcode of the cartridge and please confirm the item name written on the cartridge.
4) Insert the sample tube into the tube rack.
5) Insert the tube rack into the loading part of the sampling station.
6) Tap the 'Start' button on the screen.
7) The test result will be displayed on the screen after 12 minutes.

Emergency mode – General tip

1) The test procedure is same with the 'Normal mode 1) – 3)'.
2) Convert the 'Emergency mode' in AFIAS-10.
3) Select the tip type (general tip) on the screen.
4) Select the sample type (whole blood/serum/plasma) on the screen.
5) Take 100 µL of the sample using a pipette and dispense it into the sample well of the cartridge.
6) Tap the 'Start' button on the screen.
7) The test result will be displayed on the screen after 12 minutes.

Emergency mode – C-tip

1) Insert a cartridge into the cartridge holder.
2) Take 30 µL of whole blood using a C-tip.
3) Insert the C-tip with sample into the tip hole of the cartridge.
4) Tap the 'Load' button of the bay that holds the cartridge with a tip to read the barcode of the cartridge and please confirm the item name written on the cartridge.
5) Convert the 'Emergency mode' in AFIAS-10.
6) Select the tip type (C-tip) on the screen.
7) Tap the 'Start' button on the screen.
8) The test result will be displayed on the screen after 12 minutes.

INTERPRETATION OF TEST RESULT

- The 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.
- Working range: 5 - 100 ng/mL
- Conversion factor: 1 ng/mL = 2.5 nmol/L
- 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

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.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are provided on demand with **AFIAS Vitamin D Neo**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance.
  (Please refer to the instructions for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical sensitivity**
  - Limit of Blank (LoB) 1.888 ng/mL
  - Limit of Detection (LoD) 3.063 ng/mL
  - Limit of Quantitation (LoQ) 5.0 ng/mL
- **Analytical specificity**
  - Cross-reactivity
    Biomolecules such as below the ones in the table were added to the test sample(s) at concentrations much higher than their normal physiological levels in the blood. **AFIAS Vitamin D Neo** test results did not show any significant cross-reactivity with these biomolecules.

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

  - Interference
    Interferents listed in the following table were added to the test sample at the concentration mentioned below. **AFIAS Vitamin D Neo** test results did not show any significant interference with these materials.

Material	Concentration
D-glucose	600 mM
L-Ascorbic acid	2 mM
Bilirubin[unconjugated]	4 mM
Hemoglobin(human)	20 g/L
Cholesterol	130 mM
triglyceride	100 mg/ml
Biotin	50 ng/mL

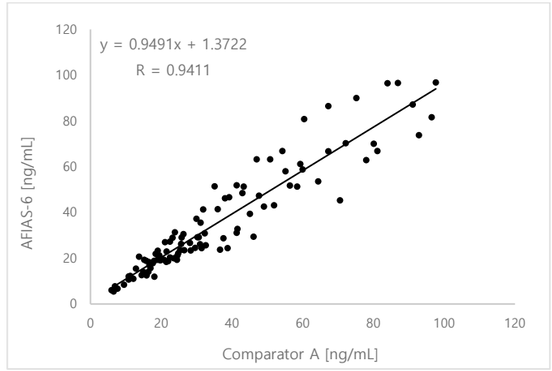
- **Precision**
  - Single-site study
    Repeatability (within-run precision)
    within-laboratory precision (Total precision)
    Lot to lot precision
    3 Lots of AFIAS Vitamin D Neo were tested for 20 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.
  - Multi-site study
    Reproducibility
    1 Lot of AFIAS Vitamin D Neo was tested for 5 days in 3 different sites (1 person per 1 site, 1 instrument per 1 site). Each standard material was tested 1 time per and 5 replicates per day.

Conc. [ng/mL]	Single-site study					
	Repeatability			Within-laboratory precision		
	Mean	SD	CV (%)	Mean	SD	CV (%)
10	9.99	1.18	11.79	9.88	1.16	11.76
30	30.14	3.77	12.52	30.09	3.80	12.63
50	48.85	6.30	12.90	48.82	5.77	11.83
Conc. [ng/mL]	Single-site study			Multi-site study		
	Lot to lot precision			Reproducibility		
	Mean	SD	CV (%)	Mean	SD	CV (%)
10	9.94	1.14	11.50	10.11	1.15	11.40
30	29.91	3.63	12.12	30.94	3.40	10.98
50	49.92	5.88	11.77	50.34	5.58	11.09

- **Accuracy**
  The accuracy was confirmed by testing 3 different lots of **AFIAS Vitamin D Neo**. The tests were repeated 10 times at each concentration of the control standard.

Expected value [ng/mL]	Lot 1	Lot 2	Lot 3	AVG	Recovery
6.06	6.04	6.15	6.05	6.08	100.4%
10	9.73	9.95	9.91	9.86	98.6%
20.83	20.63	20.55	21.84	21.01	100.8%
36.67	38.57	36.98	37.39	37.65	102.7%
52.5	55.81	57.68	52.6	55.36	105.5%
76.25	74.65	79.27	81.79	78.57	103.0%
91.36	90.28	85.84	93.69	89.94	98.4%

- **Comparability**
  Vitamin D concentration of 100 clinical samples were quantified independently with **AFIAS Vitamin D Neo (AFIAS-6)** and **comparator A** as per prescribed test procedures. Test results were compared, and their comparability was investigated with linear regression and correlation coefficient (R). The regression equation and correlation coefficient are as follows.



REFERENCES

1. Holick MF (March 2006). "High prevalence of vitamin D inadequacy and implications for health". Mayo Clin. Proc. 81 (3): 353–73.
2. Hollis BW (January 1996). "Assessment of vitamin D nutritional and hormonal status: what to measure and how to do it". Calcif. Tissue Int. 58 (1): 4–5.
3. Holick MF, Schnoes HK, DeLuca HF, Suda T, Cousins RJ (1971). "Isolation and identification of 1,25-dihydroxycholecalciferol. A metabolite of vitamin D active in intestine". Biochemistry 10 (14): 2799–804.
4. Bender, David A.; Mayes, Peter A (2006). "Micronutrients: Vitamins & Minerals". In Victor W. Rodwell; Murray, Robert F.; Harper, Harold W.; Granner, Darryl K.; Mayes, Peter A. Harper's Illustrated Biochemistry. New York: Lange/McGraw-Hill. pp. 492–3.
5. Institute of Medicine (1997). "Vitamin D". Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Washington, D.C: National Academy Press. p. 254.
6. "Dietary Supplement Fact Sheet: Vitamin D". Office of Dietary Supplements (ODS). National Institutes of Health (NIH). Retrieved April 11, 2010.
7. Wolf G (June 2004). "The discovery of vitamin D: the contribution of Adolf Windaus". J Nutr 134 (6): 1299–302.
8. Sattar, N.; Welsh, P.; Panarelli, M.; Forouhi, N. G. (2012). "Increasing requests for vitamin D measurement: Costly, confusing, and without credibility". The Lancet 379 (9811): 95–96.
9. Bilinski, K. L.; Boyages, S. C. (2012). "The rising cost of vitamin D testing in Australia: Time to establish guidelines for testing". The Medical Journal of Australia 197 (2): 90.
10. Lu, Chuanyi M. (May 2012). "Pathology consultation on vitamin D testing: Clinical indications for 25(OH) vitamin D measurement [Letter to the editor]". American Journal Clinical Pathology (American Society for Clinical Pathology) (137): 831–832.,

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

Σ	Sufficient for <n> tests
	Read instruction for use
	Use by Date
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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