Hormone

AFIAS Testosterone

INTENDED USE

AFIAS Testosterone is a fluorescence immunoassay (FIA) for the quantitative determination of Testosterone in serum/plasma. It is useful as an aid in management and monitoring of androgen level.

For *in vitro* diagnostic use only.

INTRODUCTION

Testosterone (17ß-hydroxyandrost-4-en-3-one) is an anabolic steroid synthesized primarily by Leydig cells in the testes of male, the ovary of female, and adrenal glands of both sexes1. It is synthesized from cholesterol, androstenediol, Dehydro-epiandrosterone (DHEA), progesterone, and pregnenolone acting as some of the intermediate substrates. Testosterone level in male increase 10 to 20-fold during puberty, driving the physiological changes associated with male puberty. It also exerts a powerful, wide-ranging influence over emotional well-being, sexual function, muscle mass and strength, energy, cardiovascular health, bone integrity, and cognitive ability throughout a man's entire life. In the blood only 1 to 15 % of testosterone is in its unbound or biologically active form. The remaining testosterone is bound to serum proteins.

PRINCIPLE

The test uses a competitive immunodetection method.

The antigens in the sample binds to the fluorescence-labeled detector antibodies in buffer, forming the complexes as a sample mixture. They will migrate onto nitrocellulose matrix, which will interfere with the binding of the free fluorescence-labeled detector antibodies to the immobilized antigen on the test strip.

More antigens in the sample will result in less free detection antibodies to accumulate, which lead to less fluorescence signal by the free fluorescence-labeled detector antibodies. This signal is processed by the instrument for AFIAS tests to show testosterone concentration in the sample.

COMPONENTS

AFIAS Testosterone 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 a diluent part.
- The cartridge part contains the membrane called a test strip which has testosterone-BSA conjugate at the test line, rabbit IgG at the control line and anti-mouse IgG at the antigen line.
- The detector part has a granule containing anti-testosterone-fluorescence conjugate, anti-rabbit IgG-fluorescence conjugate and sodium azide as preservative in phosphate buffered saline (PBS).
- The diluent part contains bovine serum albumin (BSA) as a stabilizer 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 a 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 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 it may cause certain health issues like convulsions, low blood pressure, low 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.
- **AFIAS Testosterone** will provide accurate and reliable results subject to the below conditions.
  - **AFIAS Testosterone** should be used only in conjunction with the instrument for AFIAS tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
K_2 EDTA

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 antigens 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 antigens with time and/or temperature may also cause false negative result as it makes the antigens 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-29

Components of **AFIAS Testosterone**

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

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately with **AFIAS Testosterone**.

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 Hormone Control** REF CFPO-95
- **Boditech Hormone Calibrator** REF CFPO-107
- **Boditech Testosterone Control** REF CFPO-239
- **Boditech Testosterone Calibrator** REF CFPO-265

SAMPLE COLLECTION AND PROCESSING

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

- It is recommended to test the sample within 24 hours after collection when collected sample is stored at room temperature.
- 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 should be frozen at -20°C.
- The samples (serum, plasma) stored frozen at -20°C for 2 months showed no performance difference.
- As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen samples.

TEST SETUP

- Check the components of the **AFIAS Testosterone** as described below.: Cartridges, pipette 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 200 µL of sample (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 15 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 15 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 (serum/plasma) on the screen.
5) Take 200 µL of 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 15 minutes.

INTERPRETATION OF TEST RESULT

- The instrument for AFIAS tests calculates the test result automatically and displays testosterone concentration of the test sample in terms of ng/mL.
- Working range: 0.5 – 12.0 ng/mL.

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 Testosterone**. 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) 0.181 ng/mL
  - Limit of Detection (LoD) 0.293 ng/mL
  - Limit of Quantitation (LoQ) 0.500 ng/mL
- **Analytical Specificity**
  - Cross-reactivity
    Biomolecules listed in the following table were added to the test sample(s) at concentrations much higher than their normal physiological levels in the blood. **AFIAS Testosterone** test results did not show any significant cross-reactivity with these biomolecules.

Cross reactants	Concentration
Androstenedione	1,000 ng/mL
Androsterone	100,000 ng/mL
Cortisol	8,000 ng/mL
Estradiol	1,000 ng/mL
Danazol	1,000 ng/mL
5-a-DHT	50 ng/mL
DHEA	10,000 ng/mL
Oxymetholone	100 ng/mL
Estrone	500 ng/mL
Corticosterone	5,000 ng/mL
Methyltestosterone	100 ng/mL
11-Deoxycortisol	1,000 ng/mL
Progesterone	1,000 ng/mL
19-Nor Testo	1,000 ng/mL

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

Interferents	Concentration
Bilirubin [unconjugated]	40 mg/dL
Triglycerides	1,500 mg/dL
Albumin	5,200 mg/dL
Ascorbic acid	1,000 mg/dL

- **Precision**
  - **Single-site study**
    Repeatability (within-run precision)
    Within-laboratory precision (Total precision)
    Lot to lot precision
    3 Lots of **AFIAS Testosterone** were tested for 21 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

Single-site study						
Testosterone [ng/mL]	Repeatability		within-laboratory precision		Lot to lot precision	
	AVG [ng/mL]	CV (%)	AVG [ng/mL]	CV (%)	AVG [ng/mL]	CV (%)
3.0	3.06	7.5	3.07	7.8	3.05	8.0
6.0	6.00	5.1	5.97	5.4	6.00	6.0
9.0	9.07	5.4	9.04	5.9	9.06	5.9

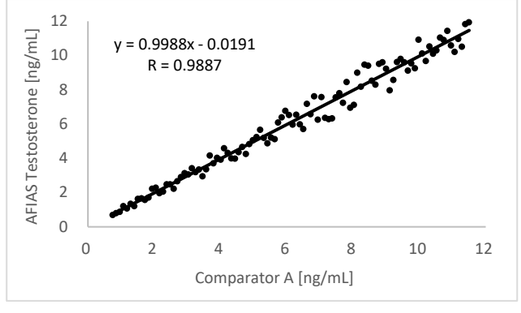
  - **Multi-site study**
    Reproducibility
    1 Lot of AFIAS Testosterone was tested for 5 days in 3 different sites (1 person per 1 site, 1 instrument per 1 site). Each standard material was tested 1 time per and 5 replicates per day.

Multi-site study		
Testosterone [ng/mL]	Reproducibility	
	AVG [ng/mL]	CV (%)
3.0	3.05	7.7
6.0	6.08	6.4
9.0	8.96	6.4

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

Testosterone [ng/mL]	Lot 1	Lot 2	Lot 3	AVG [ng/mL]	Recovery (%)
12.00	11.69	11.87	11.97	11.85	99
9.70	9.66	9.65	9.58	9.63	99
7.40	7.55	7.61	7.53	7.56	102
5.10	5.18	5.29	5.15	5.20	102
2.80	2.76	2.78	2.76	2.77	99
0.50	0.51	0.50	0.49	0.50	100

- **Comparability**
  Testosterone concentration of 100 clinical samples were quantified independently with **AFIAS Testosterone (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 follow.



REFERENCES

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2. Vining, R.F., and McGinley, R.A. The measurement of hormones in saliva: Possibilities and pitfalls. Journal of Steroid Biochemistry, 1987, 27: 81-94.
3. Tulsidas G. Shrivastav. Matrix interference in direct total Testosterone enzyme immunoassay and It's elimination with the use of non-cross reactivity steroids in serum based standards. Health and Population Perspectives and Issues, 2002,25(2):55-64

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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