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Hormone

AFIAS T3

INTENDED USE

AFIAS T3 is a fluorescence immunoassay (FIA) for the quantitative determination of total T3 (total Triiodothyronine) in human serum/plasma. It is useful as an aid in management and monitoring of thyroid disorders.

For *in vitro* diagnostic use only.

INTRODUCTION

3,5,3' Triiodothyronine (T3) is a thyroid hormone with a molecular weight of 651 daltons.[1]

T3 circulates in the blood as an equilibrium mixture of free and protein bound hormone.[2]

T3 is bound to thyroxin binding globulin (TBG), prealbumin, and albumin. The actual distribution of T3 among these binding proteins is controversial as estimates range from 38-80% for TBG, 9-27% for prealbumin, and 11-35% for albumin.[3]

T3 plays an important role in the maintenance of the euthyroid state. T3 measurements can be a valuable component in diagnosing certain disorders of thyroid function.[4]

Most reports indicate that T3 levels distinguish clearly between euthyroid and hyperthyroid subjects, but provide a less clear-cut separation between hypothyroid and euthyroid subjects.[5]

Total T3 measurements may be valuable when hyperthyroidism is suspected and the free T4 is normal.[6]

For example, one recognized type of thyroid dysfunction is T3 thyrotoxicosis, associated with a decrease in serum thyroid stimulating hormone (TSH), increased T3 level, normal T4, normal free T4, and normal to increase in vitro Uptake results.[7-11]

T3 levels are affected by conditions which affect TBG concentration.[12-14] Slightly elevated T3 levels may occur in pregnancy or during estrogen therapy, while depressed levels may occur during severe illness, renal failure, myocardial infarction, alcoholism, inadequate nutritional intake, and during therapy with some medications such as dopamine, glucocorticoids, methimazone, propranolol, propylthiouracil, and salicylates.[6,15,16]

Numerous conditions unrelated to thyroid disease may cause abnormal T3 values.[5, 17-20] Consequently, total T3 values should not be used on their own in establishing the thyroid status of an individual. The level of serum T4, TSH and other clinical findings must be considered as well.

PRINCIPLE

The test uses a competitive immunodetection method.

The antigens in the sample bind 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 antigens on a 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 T3 concentration in the sample.

COMPONENTS

AFIAS T3 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 T3-BSA conjugate at the test line, and chicken IgY at the control line.
- The detector part has a granule containing anti-T3-fluorescence conjugate and anti-chicken IgY-fluorescence conjugate, and sodium azide as a preservative in sodium phosphate buffer.
- The diluent part contains 8-anilinonaphthalene-1-sulfonic acid (ANS) and tween 20, and sodium azide as a preservative in sodium hydroxide solution.

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 the cartridge, if the pouch is damaged or have 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 the test components and/or samples 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 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.
- No Biotin interference was observed in **AFIAS T3** when biotin concentration in the sample was below 3,600 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 T3** will provide accurate and reliable results subject to the below conditions.
  - **AFIAS T3** should be used only in conjunction with the instrument for AFIAS tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
Sodium heparin

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-18

Components of **AFIAS T3**

- 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 from **AFIAS T3**. 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 T3 Control** REF CFPO-240
- **Boditech T3 Calibrator** REF CFPO-266

SAMPLE COLLECTION AND PROCESSING

The sample type for **AFIAS T3** 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 or below.
- The samples (serum, plasma) stored frozen at -20°C for 3 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 T3** 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 the cartridge into the cartridge holder.
2) Insert a tip into the tip hole of the cartridge.

※ **AFIAS-3) When tested with two or more cartridges, insert additional 2 tips per test into the extra tip station.**

3) Select the 'General mode' in the instrument for AFIAS tests.
4) Take 150 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 10 minutes.

▶ **AFIAS-10**

Normal mode

1) Insert a cartridge into the cartridge holder.
2) Insert a tip into the tip hole of the cartridge.

※ **When tested with two or more cartridges, insert additional 2 tips per test into the extra tip station.**

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 10 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 150 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 10 minutes.

INTERPRETATION OF TEST RESULT

- The instrument for AFIAS tests calculates the test result automatically and displays T3 concentration of the test sample in terms of ng/mL and nmol/L.
- Working range: 0.5-5.0 ng/mL (0.77-7.7 nmol/L)
- Conversion factor as unit of nmol/L
  - nmol/L (SI unit) = 1.54 × ng/mL
  - ng/dL = 100 × ng/mL
- Reference range[21]

Subject	ng/mL	nmol/L (SI unit)
Adult	0.8-2.0	1.23-3.08

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 T3**. 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) 0.25 nmol/L
  - Limit of Detection (LoD) 0.40 nmol/L
  - Limit of Quantitation (LoQ) 0.77 nmol/L
- **Hook effect**
  No high-dose effect is observed in this assay at T3 concentrations up to 46.2 nmol/L.
- **Analytical Specificity**
  - Cross reactivity
    Biomolecules listed such as below the ones in the table were added to the test sample at the concentration mentioned below. **AFIAS T3** test results did not show any significant cross-reactivity with these biomolecules.

Cross-reactants	Concentration
D-Thyroxine	300 ng/mL
L-Thyroxine	300 ng/mL
Reverse T3	500 ng/mL
Salicylic Acid	1,000,000 ng/mL
Monoiodotyrosine	50,000 ng/mL

  - **Interference**
    Interferents listed in the following table were added to the test sample(s) the same as the below concentrations listed below. **AFIAS T3** test results did not show any significant interference with these materials except for K_2EDTA, Sodium citrate and Cholesterol.
    - K_2EDTA and sodium citrate as an anticoagulant are not recommended on **AFIAS T3**.
    - **AFIAS T3** does not recommend the use of lipid-rich samples.

Interferents	Concentration
D-glucose	60 mM/L
L-Ascorbic acid	0.3 mM/L
Bilirubin(conjugated)	0.7 mM/L
Hemoglobin	1,000 mg/dL
Triglyceride	50 g/L
Sodium heparin	54 mg/mL
Biotin	3,600 ng/mL
K_2EDTA	10.8 mg/mL
Sodium citrate	40 mg/mL
Cholesterol	13 mM/L

- **Precision**
  - **Single site study**
    Repeatability (within-run precision)
    Total precision (within-laboratory precision)
    Lot to lot precision
    3 Lots of **AFIAS T3** were tested for 21 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

T3 [nmol/L]	Repeatability		Total precision		Lot to lot precision	
	AVG [nmol/L]	CV (%)	AVG [nmol/L]	CV (%)	AVG [nmol/L]	CV (%)
1.08	1.07	5.6	1.08	6.1	1.09	6.3
2.31	2.26	6.3	2.28	6.3	2.30	6.4
6.16	6.08	6.3	6.15	6.5	6.15	6.2

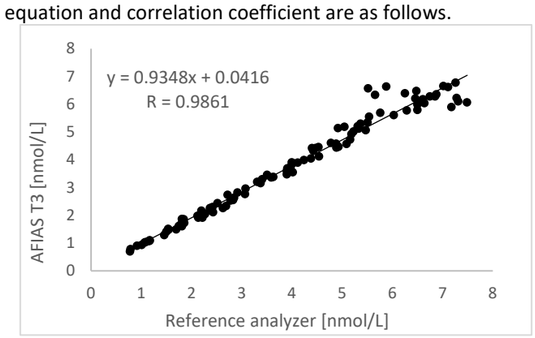
  - Between-person
    Three different persons tested one lot of **AFIAS T3**, ten times at each concentration of the control standard.
  - Between-site
    One person tested **AFIAS T3** at three different sites, ten times at each concentration of the control standard.
  - Between-reader
    One person tested **AFIAS T3** with three different readers, ten times at each concentration of the control standard.

T3 [nmol/L]	Between-person		Between-site		Between-reader	
	AVG [nmol/L]	CV (%)	AVG [nmol/L]	CV (%)	AVG [nmol/L]	CV (%)
1.08	1.08	5.5	1.06	6.7	1.07	5.9
2.31	2.29	5.7	2.26	5.9	2.36	5.5
6.16	6.11	5.8	6.14	5.8	6.21	4.8

- **Accuracy**
  The accuracy was confirmed by testing with 3 different lots of **AFIAS T3**. The tests were repeated 10 times at each concentration of the control standard.

T3 [nmol/L]	Lot 1	Lot 2	Lot 3	AVG [nmol/L]	Recovery (%)
6.16	6.35	6.01	6.25	6.20	100.7
5.14	5.22	5.03	5.05	5.10	99.2
4.13	4.22	4.30	4.27	4.26	103.2
3.11	3.12	3.12	3.06	3.10	99.7
2.09	2.18	2.13	2.11	2.14	102.4
1.08	1.05	1.04	1.06	1.05	97.2

- **Comparability**
  T3 concentrations of 100 clinical samples were quantified independently with **AFIAS T3 (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.



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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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