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Hormone

AFIAS AMH

INTENDED USE

AFIAS AMH is a fluorescence immunoassay (FIA) for the quantitative determination of AMH (Anti-müllerian hormone) in human serum/plasma. It is useful as an aid in management and monitoring of premature ovarian insufficiency, menopause and ovarian reserve.

For *in vitro* diagnostic use only.

INTRODUCTION

AMH is a dimeric glycoprotein, also called müllerian inhibiting substance (MIS). AMH is a member of the transforming growth factor b (TGF-b) family of growth and differentiation factors.[1,2] In males, the major function of AMH is accountable for regression of the müllerian structures in utero. AMH is produced in the testicles until puberty and then slowly declines after puberty.[3] Release of AMH from the granulosa cells of antral follicles leads to measurable serum levels, and these concentrations have shown to be proportional to the number of developing follicles in the ovaries. Therefore, AMH was considered to be a marker for the process of ovarian ageing. [1]

AMH is an ideal marker for ovarian functional reserve because it is formed only by the primary follicles, which are potentially capable of maturation, and the secondary follicles. There is thus a very good correlation between the serum AMH level and the number of follicles potentially capable of maturation and thus also the ovarian functional reserve.[2] In women over 30 and particularly those over 35 years of age, AMH can be used as a screening test to assess fertility status.[3] As regards the rate of response to ovarian stimulation, AMH is of much greater value than inhibin B.[2] In addition, AMH is not subject to the same cycle-dependent fluctuations as inhibin B and FSH in the assessment of ovarian functional reserve. AMH can thus be used at any point during the menstrual cycle, whereas days 3-5 of the cycle should be selected when testing FSH and inhibin B.[4]

PRINCIPLE

This test uses a sandwich immunodetection method.

The detector antibodies in buffer bind to antigens in the sample, forming antigen-antibody complexes, and 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 AMH concentration in the sample.

COMPONENTS

AFIAS AMH 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 streptavidin at the test line, and chicken IgY at the control line.
- The detector part has a granule containing mouse monoclonal anti-human AMH-fluorescence conjugate, mouse monoclonal anti-human AMH-biotin conjugate, mouse monoclonal anti-chicken IgY-fluorescence conjugate, MAB-33-IgG as a blocker, sucrose and bovine serum albumin (BSA) as a stabilizer in Tris-HCl.
- The diluent part contains BSA, tween 20, sodium chloride, and sodium azide as a preservative in phosphate buffered saline.

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 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 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) 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 AMH** when biotin concentration in the sample was below 2 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 AMH** will provide accurate and reliable results subject to the below conditions.
  - **AFIAS AMH** should be used only in conjunction with the instrument for AFIAS tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
Lithium 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 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-62

Components of **AFIAS AMH**

- Cartridge Box Contains
  - 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 AMH.** 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 AMH Control** REF CFPO-214
- **Boditech AMH Calibrator** REF CFPO-215

SAMPLE COLLECTION AND PROCESSING

The sample type for **AFIAS AMH** is human 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 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 AMH** as described below. : cartridges, pipette tips, an ID chip and an instructions for use.
- 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 (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.

▶ **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 (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.

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 AMH concentration of the test sample in terms of ng/mL.
- Working range : 0.02 – 15 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 AMH**. 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): 0.015 ng/mL
Limit of Detection (LoD): 0.020 ng/mL
Limit of Quantitation (LoQ): 0.020 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 AMH** test results did not show any significant cross-reactivity with these biomolecules.

Cross-reactivity material	Concentration
Activin A	100 ng/mL
Activin B	100 ng/mL
Inhibin A	50 ng/mL
Inhibin B	50 ng/mL
FSH	500 IU/L
LH	500 IU/L

  - **Interference**

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

Interference material	Concentration
Heparin	100 U/mL
Hemoglobin	5 g/L
Triglyceride	35 g/L
Bilirubin	300 mg/L
HAMA	2 µg/L
Albumin	65 g/L
Acetaminophen	1655 µmol/L
Ibuprofen	2425 µmol/L
Ampicillin	152 µmol/L
Acetylsalicylic acid	3.62 mmol/L
Ascorbic acid	528 µmol/L
Biotin	2 ng/mL

- **Precision**
  - **Single-site study**

Repeatability (within-run precision)
Within-laboratory precision (Total precision)
Lot to lot precision

3 Lots of **AFIAS AMH** were tested for 21 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

  - **Between person**

Three different persons tested three lots of **AFIAS AMH**, ten times at each concentration of the control standard.

  - **Between site**

One lot of **AFIAS AMH** was tested at three different sites; ten times at each concentration of the control standard.

  - **Between reader**

One lot of **AFIAS AMH** was tested with three different instruments; five times at each concentration of the control standard.

Conc.	Repeatability (within-run)		Total precision (within-laboratory precision)	
[ng/mL]	AVG	CV (%)	AVG	CV (%)
0.25	0.25	5.8	0.25	5.7
1.00	0.99	6.6	0.99	6.0
8.00	8.08	6.0	8.04	6.0
Conc.	Lot to lot precision		Between-person	
[ng/mL]	AVG	CV (%)	AVG	CV (%)
0.25	0.25	5.7	0.25	6.1
1.00	0.99	6.0	1.00	6.1
8.00	8.03	5.9	7.9	6.0
Conc.	Between-site		Between-reader	
[ng/mL]	AVG	CV (%)	AVG	CV (%)
0.25	0.25	5.1	0.25	4.9
1.00	1.00	5.8	1.01	5.6
8.00	7.97	6.4	8.00	6.2

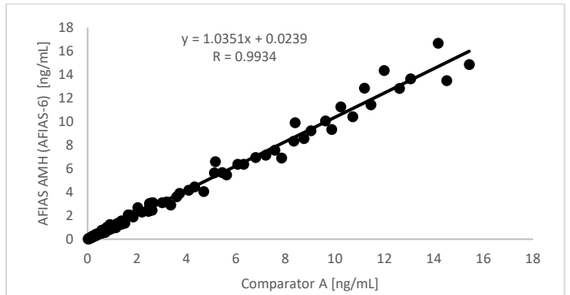
- **Accuracy**

The accuracy was confirmed by testing with 3 different lots of **AFIAS AMH**. 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 (%)
0.05	0.05	0.05	0.05	0.05	102.7%
0.85	0.83	0.83	0.84	0.84	98.3%
1.65	1.65	1.66	1.66	1.66	100.5%
3.24	3.20	3.32	3.26	3.26	100.6%
5.37	5.34	5.49	5.30	5.38	100.1%
8.03	8.05	8.02	8.08	8.05	100.2%

- **Comparability**

AMH concentrations of 100 clinical samples were quantified independently with **AFIAS AMH (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. Didier Dewailly et al. The physiology and clinical utility of anti-Mullerian hormone in women, Hum Reprod 20(3):370-385., 2014.
2. de Vet A et al. Antimüllerian hormone serum levels: a putative marker for ovarian aging, Fertil Steril 77(2): 357–62., 2002.
3. Taguchi, Osamu et al. Timing and irreversibility of Müllerian duct inhibition in the embryonic reproductive tract of the human male. Developmental Biology. 106 (2): 394–398., 1984
4. Rey R et al. AMH/MIS: what we know already about the gene, the protein and its regulation. Molecular and Cellular Endocrinology. 211 (1-2): 21–31., 2003
5. Behringer RR. The in vivo roles of müllerian-inhibiting substance. Current Topics in Developmental Biology. Current Topics in Developmental Biology. 29: 171–87., 1994
6. Gnoth C et al.: Relevance of anti-Mullerian hormone measurement in a routine IVF program. Human Reprod. 23: 1359–65., 2008.
7. Tsepelidis S et al.: Stable serum levels of anti-Müllerian hormone during the menstrual cycle: a prospective study in normo-ovulatory women. Human Reprod 22: 1837–1840., 2007.
8. E. Anckaert et al. Multicenter analytical performance evaluation of a fully automated anti-Mullerian hormone assay and reference interval determination. Clinical Biochrmistry. 49:260-267, 2016

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

For technical assistance, please contact:
Boditech Med Inc.'s Technical Services
Tel: +(82) -33-243-1400
E-mail: sales@boditech.co.kr

Boditech Med Inc.
43, Geodudanji 1-gil, Dongnae-myeon, Chuncheon-si, Gang-won-do, 24398, Republic of Korea
Tel: +(82) -33-243-1400
Fax: +(82) -33-243-9373
www.boditech.co.kr

EC REP **Obelis s.a**
Bd. Général Wahis 53, 1030 Brussels, Belgium
Tel: +(32) -2-732-59-54
Fax: +(32) -2-732-60-03
E-Mail: mail@obelis.net

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