Hormone

ichroma™ AMH

INTENDED USE

ichroma™ 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

The 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-antibody on test strip.

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

COMPONENTS

ichroma™ AMH consists of 'cartridges', 'detectors' and 'diluent'.

- The cartridge contains the membrane called a test strip which has streptavidin at the test line and chicken IgY at the control line. All cartridges are individually sealed in an aluminum foil pouch containing a desiccant in a box.
- The detector has 2 granules containing anti human AMH-fluorescence conjugate, anti-chicken IgY-fluorescence conjugate, anti-human AMH-biotin conjugate, bovine serum albumin (BSA) and sucrose as a stabilizer, MAB33 as a blocker in Tris-HCl buffer. All detectors are packed in a box.
- The diluent contains bovine serum albumin and NaCl as a stabilizer, tween 20 and CA-630 as a surfactant and sodium azide as a preservative in phosphate buffered saline (PBS), and it is pre-dispensed in a vial. The diluent is packed in a box.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow instructions and procedures described in this 'Instruction for use'.
- Use only fresh samples and avoid direct sunlight.
- It is possible to use frozen samples. Please refer to "SAMPLE COLLECTION AND PROCESSING."
- Lot numbers of all the test components (cartridge, detector, diluent, 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 or detectors. A cartridge should be used for testing one sample only. A detector should be used for processing of 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.
- Allow cartridge, detector tube, detector diluent, and sample to be at room temperature for approximately 30 minutes before use.
- The instrument for ichroma™ tests may generate slight vibration during use.
- Used cartridges, detectors, a diluent, and pipette tips should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- The detector and diluent contain NaN_3 as a preservative.
- 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.

- No Biotin interference was observed in **ichroma™ 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.
- **ichroma™ AMH** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ AMH** should be used only in conjunction with instrument for ichroma™ tests.
  - Have to use recommended anticoagulant sample.

Recommended anticoagulant
Lithium heparin

STORAGE AND STABILITY

Storage condition			
Component	Storage Temperature	Shelf life	Note
Cartridge	2 - 30 °C	20 months	Disposable
Detector	2 – 30 °C	20 months	Disposable
Diluent	2 - 30 °C	20 months	Unopened
		3 months	Opened

- After the cartridge pouch is opened, the test should be performed immediately.

LIMITATION 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 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.

MATERIALS SUPPLIED

REF CFPC-89

Components of **ichroma™ AMH**

- Cartridge Box:
  - Cartridge 25
  - ID chip 1
  - Instruction for Use 1
- Buffer Box
  - Detector (Capped with plastic lid) 25
  - Diluent 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **ichroma™ AMH**.

Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ II** REF FPRR021
- **i-Chamber** REF FPRR009
- **Boditech AMH Control** REF CFPO-214

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ AMH** is human serum/plasma.

- It is recommended to test the sample within 24 hours after collection.
- The serum or plasma should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- 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, serum or plasma sample should be frozen at -20 °C.
- Serum or plasma sample stored frozen at -20 °C for 2 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 contents of **ichroma™ AMH**: Sealed cartridges, detectors, a diluent, ID chip, and Instruction for use.
- Ensure that the lot number of the cartridge matches that of the detector, the diluent as well as an ID chip.
- If the sealed cartridge, the detector, and the diluent have been stored in a refrigerator, place them on a clean and flat surface at temperature for at least 30 minutes before testing.
- Turn on the i-Chamber and set temperature at 35 °C.
- Turn on the instrument for ichroma™ tests.
  (Please refer to the 'Instrument for ichroma™ tests Operation Manual' for complete information and operating instructions.)

TEST PROCEDURE

- **ichroma™ II**

1) Transfer 150 µL of diluent using a pipette to detector containing a granule.
2) Transfer 50 µL (Human serum/plasma/control) of sample using a pipette to the detector.
3) Close the lid of the detector and mix the sample thoroughly by shaking it about 20 times.
   (The sample mixture must be used immediately.)
4) Pipette out 75 µL of a sample mixture and load it into the sample well on the cartridge.
5) Insert the sample-loaded cartridge into the slot of the i-Chamber (35°C) and leave the cartridge in i-Chamber for 12 minutes.
   ⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inaccurate test result.*

6) To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow is marked on the cartridge especially for this purpose.
7) Tap the 'START' button on the instrument for ichroma™ tests to start the scanning process.
8) The instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
9) Read the test result on the display screen of the instrument for ichroma™ tests.
(Please refer to the ichroma™ II operation manual for complete information and operation instructions.)

INTERPRETATION OF TEST RESULT

- The instrument for ichroma™ tests calculates the test result automatically and displays AMH concentration of the test sample in terms of ng/mL.
- The 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.
- 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 provided on demand with **ichroma™ AMH**. 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):	0.014 ng/mL
Limit of Detection (LoD)	0.017 ng/mL
Limit of Quantitation (LoQ)	0.02 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. **ichroma™ 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**
  Interference materials such as below the ones in the table were added to the test sample(s) the same as the below concentrations. **ichroma™ AMH** test results did not show any significant interference with these materials except for EDTA and Sodium Citrate.

Interference material	Concentration
Heparin	100 U/mL
Sodium Citrate	25.8 mg/mL
EDTA	2 mg/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 μmol/L
Ascorbic acid	528 μmol/L

- **Precision**
  3 Lots of **ichroma™ AMH** were tested for 21days (7days per 1 Lot at 1 site by one operator). Each standard material was tested 2 times per day. For each test, each material was duplicated.
  - Repeatability (within-run precision)
  Repeatability of **ichroma™ AMH** was evaluated with results of 1 Lot.
  - Total precision (within-laboratory)
  Total precision (within-run, between-run, between-day) of **ichroma™ AMH** was evaluated with results of 1 Lot.
  - Lot to lot precision
  Lot to lot precision of **ichroma™ AMH** was evaluated with results of 3 Lots.

Standard material [ng/mL]	Repeatability		Total precision		Lot to lot precision	
	AVG	CV(%)	AVG	CV(%)	AVG	CV(%)
0.25	0.25	5.53	0.25	5.62	0.25	5.65
1.0	0.99	5.80	0.99	5.60	1.00	5.88
8.0	8.00	6.68	8.02	6.02	8.01	5.66

  - Between person
  Three different persons tested one lot of **ichroma™ AMH**, ten times at each concentration of the control standard.
  - Between site
  One person tested one lot of **ichroma™ AMH** at three different sites, ten times at each concentration of the control standard.

Standard material [ng/mL]	Between-person		Between-site	
	AVG	CV (%)	AVG	CV (%)
0.25	0.25	5.63	0.25	5.63
1.0	1.00	5.92	0.99	5.72
8.0	8.01	5.84	8.03	5.86

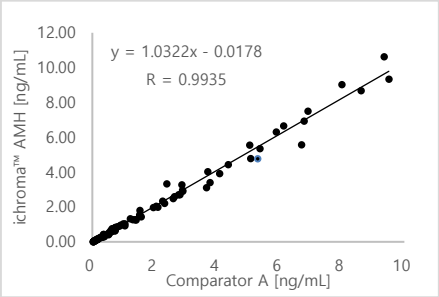
■ **Accuracy**

The accuracy was confirmed by testing with 3 different lots of **ichroma™ AMH**. The tests are repeated 10 times in each different concentration.

Expected value [ng/mL]	Lot 1	Lot 2	Lot 3	AVG	Bias (%)
0.05	0.05	0.05	0.05	0.05	101
0.85	0.85	0.83	0.87	0.85	100
1.65	1.62	1.60	1.67	1.63	99
4.84	4.88	4.78	4.96	4.87	101
6.43	6.52	6.38	6.60	6.50	101
9.62	9.13	9.55	9.28	9.32	97

■ **Comparability**

AMH concentration of 90 clinical samples were independently with **ichroma™ AMH (ichroma™ II)** and Comparator A as per prescribed test procedures. Test results were compared, and their comparability was investigated with linear regression and coefficient of correlation (R). Linear regression and coefficient of correlation between the two tests were Y=1.0322X - 0.0178 and R = 0.9935 respectively.



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 Biochemistry. 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

Boditech Med Incorporated