en

Immunoassay

REF **CMS0501 / CMS0502 / CMS0503 / CMS0504 / CMS0505**

50 tests*1 / 100 tests*1 / 100 tests*2 / 100 tests*5 / 50 tests*2

AMH CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative detection of AMH (*Anti-Müllerian Hormone*) *in human serum.*

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		

EC REP

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CE IVD

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Contact your local dealers for all product-related questions in your local language.

Introduction

Anti-Müllerian hormone (AMH), also known as Müllerian-inhibiting hormone (MIH), is a dimeric glycoprotein with a molar mass of 140 kDa [1]. It is related to inhibin and activin from the transforming growth factor beta superfamily, whose key roles are in growth differentiation and folliculogenesis [2]. AMH is expressed by granulosa cells of the ovary during the reproductive years, and limits the formation of primary follicles by inhibiting excessive follicular recruitment by FSH[3]. The rise during childhood and adolescence is likely reflective of different stages of follicle development [4]. From 25 years of age AMH declines to undetectable levels at menopause [5]. The determination of AMH is used for general fertility assessment, as a biomarker of polycystic ovary syndrome, and for the assessment of the ovarian reserve prior to undergoing IVF treatment in order to identify poor responders and therefore reduce cancellation rate as well as to identify excessive responders and therefore reduce the risk of hyper-stimulation; also to predict ovarian response in a sample from a women undergoing ovulation induction.

Measurement Principle

This assay is based upon the one-step sandwich method. The sample, AMH antibodies coated microparticles and enzyme labeled anti-AMH are combined. During the incubation, AMH present in the sample is allowed to react simultaneously with the two antibodies, resulting in AMH being sandwiched between the microparticles and enzyme-linked antibodies. After washing, a complex is generated among the microparticles, the AMH within the sample and enzyme-linked antibodies by immunological reactions. The complex catalyzes substrate, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is proportional to the concentration of AMH in the patient sample.

Materials Provided

1. Calibrators

6 vials lyophilized calibrator A through F. The matrix is HEPES buffer containing BSA (bovine serum albumin). Contains a selection of preservatives.

Reconstitute each lyophilized calibrator with 1.0 mL of distilled water. Allow the reconstituted material to stand for at least 10 minutes. Then invert the calibrator to mix it completely.

2. Reagent pack

Reagent pack provided ready to use.

Σ	50*1	100*1	100*2	100*5	50*2
Microparticles Solution	1.2mL*1	2.3mL*1	2.3mL*2	2.3mL*5	1.2mL*2
Enzyme Conjugate	5.5mL*1	11.0mL*1	11.0mL*2	11.0mL*5	5.5mL*2

- Microparticles Solution

Contains polyclonal antibodies of AMH coated microparticles in BIS-TRIS propane buffer containing BSA. Contains a selection of preservatives.

- Enzyme Conjugate

Contains horseradish peroxidase labeled anti-AMH in a BIS-TRIS propane buffer containing BSA. Contains a selection of preservatives.

Assay Analyzers on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- AutoLumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzers which are AutoLumo A2000 Plus, AutoLumo A2000 Plus B and AutoLumo A1000.

Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample cup(s) or tube(s) for sample containing
4. Chemiluminescent Substrate
5. System Wash for washing the pipetting needles
6. Wash Buffer used in the washing procedure
7. Distilled water or deionized water

Metrological Traceability of Calibrators

The measurand (analyte) in the AMH Calibrators is traceable to the manufacturer's working calibrators. Traceability process is based on EN ISO 17511. The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials and wastes safely according to local requirement.
5. CAUTION: the calibrators contain material of human origin, which has been tested and found non-reactive for HBsAg, HIV-1 and HIV-2, HCV and syphilis. This assay contains materials of animal origin. Bovine components originate from countries where BSE has not been reported.
6. Some reagents contain ProClin 300® may cause sensitization by skin contact, which must be avoided to contact with skin. This material and its container must be disposed in a safe way. If swallowed, seek medical advice immediately.
7. Do not smoke, drink, eat or use cosmetics in the working area.
8. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
9. Conduct the assay away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
10. Do not use reagents beyond the labeled expiry date.
11. Do not mix or use components from kits with different batch codes.
12. Ensure the microparticles are re-suspended before loading it on the analyzer.
13. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
14. Do not substitute any reagent in this kit from other manufacturers or other lots.
15. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.

Storage

1. Store all components of the kit at 2-8 ℃. Do not freeze. Avoid strong

light. When stored as directed, all reagents are stable until the expiration date.
2. Refrigerate the reagent pack at 2-10 ℃ for a minimum of 2 hours prior to use.
3. Store the unsealed reagents pack upright on the analyzer or 2-10℃ for a maximum of 28 days. After 28 days, the reagent pack must be discarded. Once they are removed from the analyzer, store them at 2-10 ℃ in an upright position.
4. Seal and return the reconstituted calibrators at 2-8℃ immediately after the experiment, under which conditions the stability will be retained for 2 months.

Sample

1. Collect samples in accordance with correct medical practices.
2. Do not use heat-inactivated samples and samples with obvious microbial contamination. Do not use sodium azide preservative in samples.
3. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit in creased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
4. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
5. Insufficient processing of sample or disruption of the sample during transportation may cause depressed results.
6. Avoid grossly hemolytic, lipemic or turbid samples.
7. Cap and store the samples at 18-25℃ for no more than 8 hours, for longer use samples should be capped and stored at 2-8 ℃. Or freeze the samples that need to be transported for more than 5 days at -20℃. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortex or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
8. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy in appearance prior to use to ensure consistency in the results.
9. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
10. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
11. For optimal results, inspect all samples for bubbles. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

Measurement Procedure

1. Check the consumable materials
- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the Assay Analyzer's operation manual.

2. Load the kit
- Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the analyzer. Avoid foam formation in all reagents. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests
- Place the sample cups or tubes on the sample rack, 50 μL of samples are aspirated and transferred automatically before each test. But consider the sample container and 150 μL of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample rack and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
  - Moves the sample to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers sample into the reaction vessel
  - Adds Microparticles Solution and Enzyme Conjugate to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of AMH in the sample
  - Discards the used reaction vessel
  - Calculates the result
- Refer to the Assay Analyzer's operation manual.

4. Calibrate the curve
- Analyzer can read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the calibrators into the sample cups or tubes and place them on the sample rack. Conduct duplicate detection on the system.
- Load the sample rack and input calibrators' information on the system software interface.
- Select "run" to start the test and generate the calibration curve, calibration is required every 28 days.
- Once a calibration curve is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measurements
  - A reagent kit and substrate solution with new batch code is used
  - Beyond the expiry date of a calibration curve
  - Important parts of the analyzer are replaced or repaired.
- Refer to the Assay Analyzer's operation manual.

5. Dilute the sample

Samples with an AMH value exceeding 25 ng/mL may be diluted manually. Low concentration AMH sample is used to dilute the samples. After dilution, multiply the result by the dilution factor.
- The concentration of the sample after dilution should not be less than 0.6 ng/mL.

Measurement Results

The sample test results are determined automatically by the system software. The amount of AMH in the samples is determined from the measured light production by means of the stored calibration data. Refer to the Assay Analyzer's operation manual on reviewing sample results.

Control Procedure

Controls for the various concentration ranges should be run individually when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulin, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.
4. Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop HAMA (human anti-mouse antibodies). HAMA can produce either falsely high or falsely low values in immunoassays which use mouse monoclonal antibodies. Additional information may be required for diagnosis.
5. This test measures concentrations within the range of 0.02 ng/mL-25 ng/mL. If AMH concentrations above the measuring range to be expected, it is recommended to dilute samples with low-value sample. The recommended dilution is 1:4 of this test, allowing samples to be up approximately 125 ng/mL.

Biological Reference Interval

Normal ranges were obtained by testing serum samples from 120 males and 733 females defined as normal by clinician. It is recommended that each laboratory establish its own normal range which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.

Gender	Age	Number	Median (ng/mL)	95%RI (ng/mL)
Male	⩾18	120	4.86	1.49-11.68
Female	20-24	121	4.03	1.72-9.57
Female	25-29	125	3.40	1.24-9.23
Female	30-34	120	2.82	0.73-7.62
Female	35-39	120	2.11	0.84-5.31
Female	40-44	127	1.12	0.15-3.02
Female	45-50	120	0.28	0.10-2.12

*RI: Reference Ranges Interval

Performance Characteristics

1. Measurement Precision

3 samples were assayed in duplicate of 2, twice per day across 20 testing days. Data from this study are summarized in the following table.

Sample	n	Mean (ng/mL)	Within-run	Total
			%CV	%CV
1	80	1.19	2.77	3.05
2	80	6.25	1.94	3.57
3	80	16.45	2.30	2.96

*Representative data; results in individual laboratories may vary from these data.

2. Analytical Sensitivity

Limit of Blank: 0.01ng/mL.
Limit of Detection: 0.017 ng/mL.
Limit of Quantitation: 0.045 ng/mL with a coefficient of variation of 20%.

3. Analytical Specificity

Cross reaction: The following substances and concentrations were tested and found no cross reaction with the test.

Substances	Concentration tested
INH A	100 ng/mL
Activin A	15 μg/mL
LH	100 mIU/mL
FSH	115 mIU/mL
TGF β-1	65 ng/mL

Interference: No interference with 20 mg/dL of Bilirubin, 100 mg/dL of Hemoglobin, 3000mg/dL of Triglyceride.

4. Measurement Accuracy by Correlation

A study was performed where samples were tested using this assay and AMH Test which was already available on the market. Data were analyzed and are summarized in the following table.

Correlation Method	Number of Samples	Intercept	Slope	Correlation Coefficient
Linear Regression	214	-0.4799	0.9595	0.9939

5. High Dose Hook Effect

A sample spiked with AMH up to 2000 ng/mL was determined, the concentration result obtained was⩾25 ng/mL.

Literature References

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