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Anti-Müllerian hormone (CLIA) PACKAGE INSERT

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INTENDED USE

Anti-Müllerian hormone (CLIA), is a chemiluminescence immunoassay test for the quantitative measurement of circulating anti-müllerian hormone (AMH) in human serum or plasma, which is intended as an aid to diagnosis of ovarian function or polycystic ovarian syndrome (PCOS), etc.The assay can be used for samples over the range of 0.01 ng/mL -23.00 ng/mL.
The test must be performed on the ACCRE series analyzers (including ACCRE 6, ACCRE 8, ACCRE 90, ACCRE 100, ACCRE 120 and other ACCRE series analyzers).

For *in vitro* diagnostic use only. For professional use only.

SUMMARY

Women's capacity to procreate depends largely on good mobilization of oocytes during their cycles, from a limited reserve of follicles constituted during fetal life, until exhaustion at menopause. This complex process is regulated by the gonadal axis hormones. In women, AMH is produced by the granulosa cells of small growing follicles. Its measurement in the circulation helps to estimate the number of antral and pre-antral follicles present in the ovaries and is cycle independent. Good correlation is observed between AMH measurement in peripheral blood, follicle count by transvaginal ultrasonography and age.
AMH is a dimeric glycoprotein, a member of the transforming growth factor β (TGF-β) superfamily. It is produced as a precursor protein consisting of two monomers linked by a disulfide bond. The cleaved complex then remains bound and can be measured in the blood with the ELISA technique by monoclonal antibodies giving sensitivity performance suited to monitoring changes in ovarian reserves from birth to menopause.
AMH measurement has proven to be useful in different domains for women. As an indicator of the natural decline of the ovarian reserve and therefore the risk of hypofertility, it is recommended as an aid for women who are considering parenthood. In the context of medically assisted procreation, AMH measurement helps to select the best strategy for the patient, by optimizing the controlled ovarian stimulation step whilst avoiding the risk of hyperstimulation. AMH measurement can also be used to monitor the ovarian reserve in young girls and women who, for example, have undergone gonadotropic treatment for cancer. For women with ovulation problems, the measurement of serum AMH levels enables better characterization of the type of ovarian dysfunction, particularly hypergonadotropic anovulation associated with ovarian insufficiency such as polycystic ovary syndrome.

ASSAY PRINCIPLES

The principle of Anti-Müllerian hormone (CLIA) hereinafter also referred to as the "AMH" combines a two-sites sandwich immunoassay method with enzyme catalytic chemiluminescence detection (CLIA). The principle is described as following:
Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent cartridges. All the assay steps are performed automatically by the ACCRE analyzer.
The sample is manually pipetted into the sample well of reagent cartridge, then sample, anti-AMH antibody labeled with alkaline phosphatase and anti-AMH antibody coated magnetic particles are mix thoroughly together, incubating at 37 °C. The antigen in the sample, label antibody and magnetic particles were bonded together as an antibody-antigen sandwich immuno-complex. The immuno-complex then washed in the magnetic field to eliminate the unbound components in the sample. The substrate (APS-5) was added to trigger the chemiluminescent reaction, and the resulting chemiluminescent reaction was measured by photomultiplier tube (PMT) as relative light units (RLUs). The concentration of the substance to be detected in the sample is proportional to the luminescence intensity. At the end, the results were automatically calculated by the ACCRE analyzer in relation to the calibration curve stored.

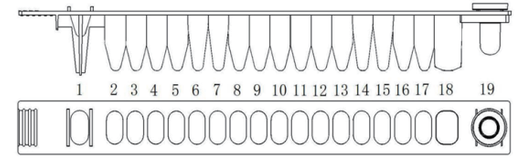
CONTENT OF THE KIT

Package: 60 tests/kit with quality controls, 36 tests/kit with quality controls, 60 tests/kit without quality controls, 36 tests/kit without quality controls.
The calibration of this kit has been traced to Roche Elecsys AMH for testing. For specific calibrator concentration values, see the Calibrator Barcode Sheet for each lot.

Contents	Quantities				Notice
	REF W117A	REF W117 C	REF W02A	REF W02C	
AMH Reagents Cartridges	60 strips	36 strips	60 strips	36 strips	Ready to Use
AMH Calibrator 1 (CAL1)	1.5 mL x 1 vial	1.5 mL x 1 vial	1.5 mL x 1 vial	1.5 mL x 1 vial	Borate buffer, 100mM; ProClin300, 0.5g/L; BND, 0.2g/L
AMH Calibrator 2 (CAL2)	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	Borate buffer, 100mM; ProClin300, 0.5g/L; BND, 0.2g/L; Purified AMH antigen, 0.1 ng/mL
AMH Calibrator3 (CAL3)	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	Borate buffer, 100mM; ProClin300, 0.5g/L; BND, 0.2g/L; Purified AMH antigen, 8 ng/mL
AMH Quality Control Level 1 (QC1)	1.0 mL x 1 vial	1.0 mL x 1 vial	/	/	Phosphate buffer, 50mM; ProClin300, 0.48 g/L; BND, 0.2 g/L; AMH antigen, 1nmol /L
AMH Quality Control Level 2(QC2)	1.0 mL x 1 vial	1.0 mL x 1 vial	/	/	Phosphate buffer, 50mM; ProClin300, 0.48 g/L; BND, 0.2 g/L; AMH antigen,5nmol/L
Instruction for Use	1	1	1	1	N/A
Reagent Registration Card	1	1	1	1	N/A
Calibrator Barcode Sheet	1	1	1	1	N/A
Quality Control Sheet	1	1	/	/	Only for the configurations with Quality Controls

Note: Refer to the target value list of the controls from Quality Control Sheet within the kit.

Reagent Cartridge
The strip consists of 19 wells. The 1st well is the sample well and the last well 19th is the reading well for chemiluminescent signal. From the 2nd to the 18th well are all covered with a labeled, aluminum foil seal. The label comprises a barcode which mainly indicates the reagent assay information, reagent lot number and expiration date.
All reagents components needed for the assay are ready-to-use and pre-dispensed in the cartridge. And please do not interchange integral component from different reagents or lots.



Description of The Reagent Cartridge

Well	Component	Content	Volume
5	Enzyme Label(R2)	ALP (alkaline phosphatase) labeled anti-AMH antibody (Rat), 2.0 mg/L; MES Buffer , 50mmol/L;	50μL
7	Magnetic Particles(R1)	Anti-AMH antibody coated magnetic particles 0.2g/L; Tris Buffer, 50mmol/L; ProClin300, 0.5g/L; BND, 0.2g/L	50μL
10, 11, 12	Wash Solutions	Tris Buffer, 50mmol/L; ProClin300, 0.5g/L; BND, 0.2g/L	350μL
16	Substrate	APS-5	200μL

Note: Well 1,2,3,4,6,8,9,13,14,15,17,18 are empty.

MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED

- Sample diluent (manufactured by Tisenc)
- Pipette with disposable tip to dispense 200 μL.
- Powderless, disposable gloves.
- For other specific materials and disposables, please refer to the Instrument User Manual.
- Instruments of the ACCRE series analyzer.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- For professional use only by qualified laboratory personnel.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest; do not inhale).
- Do not use reagents after the expiration date indicated on the box label.
- Do not use if the packaging of reagent cartridge is damaged or leaked.
- Do not use the reagents if the quantity is obviously insufficient.
- If any component adheres to inside wall of wells or aluminum film of the reagent cartridge, please gently switch the reagent cartridge to let the component back the wells.
- Do not reverse the reagent cartridge. Otherwise, the result will be unreliable.
- Do not mix reagents (or disposables) from different lots.
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.
- Use powderless gloves, as powder has been reported to cause false results for certain enzyme immunoassay tests.

STORAGE

- Store the Anti-Müllerian hormone (CLIA) kits including reagent cartridges, calibrators and quality controls **upright** at 2~8°C in dark place and the kit can be valid for 18 months from manufactured date.
- After the reagent cartridge is on-board in the ACCRE analyzer, the testing should be finished within 2 hours.
- When opened, the calibrators and quality controls will be stable for 30 days at 2~8°C, and for long term storage at -20°C and below -20°C up to 60 days. Calibrators and quality controls can only be frozen and thawed 3 times.
- Keep away from sunlight.
- Do not freeze reagent cartridges.

SAMPLES

The sample volume for Anti-Müllerian hormone (CLIA) assay is 50 μL, please note that at least 75 μL of sample must be pipetted manually into the reagent cartridge.

Sample Type and Sample Collection
Human serum or plasma (Heparin lithium and EDTA-K_2) are recommended.
Types of tubes:
- Plastic tube with clot activator,
- Plastic tube with clot activator and separation gel,
- Plastic tube with Heparin lithium,
- Plastic tube with EDTA-K_2.

It is recommended to validate collection tubes before use as some may contain substances which interfere with test results.
Note: Blood sampling tube results may vary from one manufacturer to another depending on the materials and additives used.
It is the responsibility of each laboratory to validate the type of sample tube used and to follow the manufacturer's recommendations for use.

Sample Preparation
The current revision of the WHO/DIL/LAB/99.1 document provides recommendations for the preparation of samples.
For use of the sample tubes, follow the tube manufacturer's instructions for use.
The pre-analytical step, including the preparation of blood samples, is an essential part of medical analyses. In accordance with Good Laboratory Practice, this step is performed under the responsibility of the laboratory manager.
Insufficient clot time can result in the formation of fibrin with micro-clots that are invisible to the naked eye. The presence of fibrin, red blood cells, or suspended particles can lead to erroneous results.
Samples containing suspended fibrin particles or erythrocyte stroma should be centrifuged before testing.
If there is a lipid layer over the sample after centrifuging, please transfer the clear sample into a new sample tube without transferring the lipid layer.
Severe hemolysis samples (hemoglobin >1000mg/dL) or heat-inactivated samples are not recommended for applying on the reagents.
Preparation of frozen-stored samples: after thawing, these samples must be thoroughly mixed before testing.
Mix using a vortex-type mixer. If necessary, clarify samples by centrifuging before testing.

Sample Stability
Samples (serum and plasma) can be stored at 15-30°C for up to 8 hours or at 2~8°C for up to 4 days; if longer storage is required, freeze the serum or plasma at -20°C or below -20°C for up to 6 months, without exceeding 1 freeze/thaw cycle.

INSTRUCTIONS FOR USE

For complete instructions, see the Instrument User Manual.
When using the assay for the first time. Register the assay using the registration code in the reagent kit before any assay is used in the ACCRE analyzer for the first time. Calibration, QC and sample test are only available after assay was registered successfully.
When opening a new lot of reagents. Each new lot of reagent must be registered by using the registration code in Reagent Registration Card within the reagent kit.

Calibration

Calibration must be performed with the reagents and calibrators from Manufacturer.
Calibration is necessary under the following conditions:
(1) New reagent lot number applied;
(2) Same lot of reagents used more than 4 weeks;
(3) Quality Control out of control;
(4) When necessary, for example: system maintenance or repair that may affect the analytic performance.

Test Procedure

1. Remove the kit from storage at 2-8°C and take out the required number of reagent cartridge. Allow the reagent cartridges, specimens, calibrators, and/or controls to equilibrate to room temperature (15-30°C) prior to testing.
2. Use each assay AMH strips for each sample, calibrator or control respectively.
3. Place the reagent cartridge into the reagent rack.
4. Manually pipette the Sample, Calibrator, or Controls into the sample well of each reagent cartridge.

Please note that at least 75μL of sample has to be pipetted manually into the reagent cartridge and 50μL sample is needed for each test.

** Before pipetting: Mix the samples using a vortex-type mixer if necessary, and ensure that the samples and sample diluent (if applicable) DO NOT have any bubbles. The same for calibrators and controls.*

5. Put the sample rack into the reagent chamber of ACCRE analyzer.
6. Place the assay tips into the reagent chamber of ACCRE analyzer.
7. Input Sample ID, sample type, dilution etc. into the software as directed by USER MANUAL.
8. Anti-Müllerian hormone (CLIA) assay will be completed within approximately 15 minutes. Assays will be performed automatically by the ACCRE analyzer.
9. After assay is completed, remove the reagent rack from the ACCRE analyzer. Dispose the used reagent cartridge into an appropriate recipient.

Dilution

Samples with concentrations above 23ng/mL can be diluted with negative serum or plasma manually. The recommended dilution is 1:2. And the concentration after dilution should be more than 10ng/mL. After manual dilution, multiply the result by the dilution factor. ACCRE analyzer cannot perform automatic dilution for samples.

Quality Control

Quality control can be performed in accordance with local regulations or requirements related to accreditation, as well as requirements defined in the laboratory's quality control procedure.

RESULTS AND INTERPRETATION

Calculation of Results

The results are automatically calculated by ACCRE analyzer using stored calibration curve and calculated according to a predefined mathematical model. The results are expressed in ng/mL. The results can be converted as the unit of ng/mL or pmol/L by manually through below conversion factors.
Conversion factors: ng/mL × 7.14 = pmol/L, pmol/L × 0.14 = ng/mL.

Interpretation of Results

The detection range is from 0.01ng/mL to 23ng/mL. For samples with AMH below the upper limit of detection, quantitative determination can be performed; if the sample is lower than the lower limit of detection, the reported result is <0.01ng/mL; if the sample is higher than the upper limit of detection, the reported result is >23ng/mL.
Results of Anti-Müllerian hormone (CLIA) assays are determined by testing serum specimens from a study in clinical centers with group of individuals (150 healthy adult males and 892 adult females in China). The 2.5th percentile value and 97.5th percentile value are shown as below:

Group	Cases	AGE	2.5th Percentile(ng/mL)	97.5th Percentile Value (ng/mL)
Healthy Males	150	/	0.782	14.84
Healthy Females	142	20-24	1.19	11.88
	158	25-29	0.892	9.77
	152	30-34	0.566	8.07
	150	35-39	0.141	7.52
	150	40-44	0.026	5.45
	140	45-50	0.01	2.67

** Please note that results may differ between laboratories due to variations in population and test method. If necessary, each laboratory should establish its own reference range.*

In interpreting the results, patient's clinical condition including symptoms, diseases history, and other relevant data and information should be considered.

LIMITATIONS

1. If the test results are inconsistent with clinical evidences, additional testing is suggested to confirm the result.
2. For diagnostic purposes, results should be used together with other information; e.g., symptoms, results of other tests, clinical expressions, etc.
3. Please note that heterophile antibody in human serum or plasma can interfere the test result.
4. High concentration of HAMA can also cause a false positive or false negative result.

PERFORMANCE CHARACTERISTICS

Detection limits

LOB (Limit of Blank) = 0.01ng/mL
LOB values were determined according to CLSI® EP17-A2 recommendations.

Measurement range

The measurement range is the range of values corresponding to the acceptable performance limits (precision and linearity).
The measurement range of the Anti-Müllerian hormone (CLIA) assay is 0.01 ng/mL to 23.00 ng/mL.

Linearity

Linearity was evaluated according to CLSI® EP06-A recommendations. The Anti-Müllerian hormone (CLIA) assay is linear between 0.01 ng/mL to 23.00 ng/mL. The absolute linear correlation coefficient (r) should be bigger than 0.9900.

Precision

A precision study was performed according to CLSI® EP05-A3 recommendations.
The within-lot precision CV% is not greater than 8%.
The between-lot precision CV% is not greater than 10%.

Interfering Substances

Potential interference substances were studied according to CLSI® EP07-A2 recommendations. No significant interference was detected up to the maximum concentrations tested.

Substance	Concentration
Hemoglobin	≤1000 mg/dL
Bilirubin	≤66 mg/dL
Triglyceride	≤1000 mg/dL
Total Protein	≤2.5 g/dL
Rheumatoid factors	≤1000 IU/mL

Hook

No hook effect appeared when the AMH concentration reached 1400ng/mL.

HAMA

Patient samples containing human anti-mouse antibodies (HAMA) may give falsely elevated or decreased values.
Although HAMA-neutralizing agents are added, extremely high HAMA serum concentrations may occasionally influence results.

Accuracy

The sample which is a control with the trueness traceability is used for the detection, and the relative deviation between the detection result and the calibration concentration is within ±10%.

WASTE DISPOSAL

Dispose of used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.
It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

INDEX OF SYMBOLS

Symbol	Meaning	Symbol	Meaning	Symbol	Meaning
	Consult instructions for use		Contains sufficient for <n> tests		Date of manufacture
IVD	*In vitro* diagnostic medical device		Use-by date		Do not re-use
	Temperature limit	LOT	Batch code	REF	Catalogue number
	Keep away from sunlight		Keep dry		Manufacturer
EC REP	Authorized representative in the European Community/ European Union		Do not use if package is damaged and consult instructions for use	BIO	Contains biological material of animal origin
	Biological risks		Caution	CE	CE marking
	Upright		Fragile, handle with care		Do not roll
CONTROL	Control				

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Shenzhen Tisenc Medical Devices Co., Ltd.
Address: 11F, 11G, Kechuang Building, Quanzhi Science and Innovation Park, Maozhoushan Industrial Park, Shajing Community, BaoAn District, Shenzhen, 518104, P.R.China
Phone: +86-0755-23225620
E-mail: globalmarketing@tisenc.com

EC REP

Shanghai International Holding Corp. GmbH (Europe)

Address: Eiffestrasse 80, 20537 Hamburg, Germany
Phone: +49-40-2513175
Fax: +49-40-255726
DIMDI No.: DE/0000040627
E-mail: shholding@hotmail.com

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