AnshLabs

FOR PRESCRIPTION USE ONLY

MenoCheck® picoAMH ELISA

IVD

AL-124

INTENDED USE

The picoAMH ELISA is an enzyme-linked immunosorbent assay (ELISA) for the *in vitro* quantitative measurement of anti-Müllerian hormone (AMH), also known as Müllerian Inhibiting Substance (MIS), concentrations in human serum. It is intended to be used as an aid in the determination of menopausal status in women between 42 and 62 years of age. This assay should only be used in conjunction with other clinical and laboratory findings and results from this test alone should not be used to make diagnostic or treatment decisions. It is intended for *in vitro* diagnostic use and for prescription use only.

SUMMARY AND EXPLANATION

Menopause is the cessation of menstrual cycling and fertility. Clinically it is recognized by a cessation of menstrual bleeding; menopause is a woman's status after her final menstrual period (FMP). Menstrual cycling and bleeding are driven by the dynamics of ovarian follicle maturation. Because a woman is born with a limited number of follicles (non-growing "primodial" follicles) that cannot be replaced and are slowly consumed by the monthly maturation of smaller groups of follicles (primary, secondary and antral growing follicles), the natural cause of menopause is the absence of follicles. However, many natural (e.g., certain diseases, starvation, etc.) or interventional processes (chemotherapy, surgical removal of the uterus, ovaries, or pituitary gland, etc.) can cause a cessation of menses that can be either irreversible (e.g., functionally the same as menopause) or reversible. Diagnosing menopause (e.g. the FMP) currently is only done unequivocally after cessation of menses for 12 months in a previously cycling woman.

Naturally occurring (i.e., as a result of aging) menopause is referred to as "spontaneous" and, on average, occurs at the age of 51 years, but there is a very large variance of approximately +/- 10 years with respect to the occurrence of menopause in healthy women. Ovarian function deteriorates gradually leading up to menopause and contributes to the variance observed among women with respect to menopausal status. Ovarian function affects, primarily via the steroid hormones produced by growing follicles, virtually every organ in a woman's body. Physiological responses to the gradual or abrupt loss of ovarian function include a multitude of menopausal "symptoms" and consequences that affect a woman's health and quality of life. [1,2]

During several years leading up to the FMP and several years immediately following the FMP is a time called the climacteric or menopausal transition, when a woman transitions from the reproductive to a non-reproductive state. The transition is highly variable among women with respect to duration and intensity of associated physiological changes, which affect her well being and level of disease risk. Thus, determining where a woman is in this process must be individualized and is clinically important, particularly during the menopausal transition and the 12 months following the FMP. Quantitative measurement of blood levels of Anti-Müllerian Hormone using the picoAMH assay can aid in determining a woman's menopausal status during the menopausal transition.

Blood levels of AMH represent one of the markers available to clinicians to determine where a woman is in her menopausal transition. Other clinical tests relevant in this context are estradiol which is produced only by follicles in their final stages of maturation, and thus only indirectly reflects the total number of follicles in the ovary, and FSH which reflects the negative feedback by estradiol on pituitary gland secretion (i.e., also an indirect marker of ovarian follicular pool based on large follicle function). In contrast AMH is produced by the majority of ovarian follicles (primary, secondary and antral).

Blood levels of AMH have been shown to be highly correlated with the number of primordial follicles in an ovary (i.e., true ovarian reserve).[3] The picoAMH assay was developed to allow more sensitive measurements. AMH measured using the picoAMH assay provides a significant new parameter to aid physicians in determining the status of women during the menopausal transition.

PRINCIPLE OF THE TEST

The picoAMH ELISA is a quantitative three-step sandwich type immunoassay that is designed to measure human AMH. In the first step Calibrators, Controls and Unknown Samples are added to AMH antibody-coated microtiter wells. After an incubation and washing, biotinylated AMH antibody solution is added. After a second incubation and washing, the wells are incubated with streptavidin horseradish peroxidase conjugate (SHRP) solution. Finally, after a third incubation and washing step, the wells are incubated with substrate solution (TMB) followed by an acidic stopping solution.

In principle, the AMH antibody-biotin conjugate binds to the solid phase antibody-antigen complex which in turn binds to the streptavidin-enzyme conjugate. The antibody-antigen-biotin conjugate-SHRP complex bound to the well is detected by enzyme-substrate reaction. The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 nm as primary test filter and 630 nm as reference filter. The absorbance measured is directly proportional to the concentration of AMH in the calibrators, controls, and specimens tested.

MATERIALS SUPPLIED

CAL-124A **picoAMH Calibrator A/Sample Diluent**

One vial, 10 mL, labeled AMH Cal A/Sample Diluent, containing 0 pg/mL AMH in serum with non-mercury preservative. Store unopened at 2-8°C until the expiration date.

CAL-124B - CAL-124F **picoAMH Calibrators B-F (Lyophilized)**

Five vials, labeled B-F, containing concentrations of approximately 10-1,100 pg/mL AMH in serum with non-mercury preservative. Refer to calibration card for exact concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute calibrators B-F with 1 mL deionized water. Solubilize, mix well and use after reconstitution. Aliquot and freeze immediately for multiple use. Avoid repeated freeze thaws.

Assay Calibration: The Ansh Labs picoAMH assay is traceable to internal reference standards (primary reference calibrators) which consist of recombinant human AMH preparation in a protein based matrix with non-mercury preservative.

CTR-124-I & CTR-124-II **picoAMH Controls I & II (Lyophilized)**

Two vials, labeled Levels I and II containing low and high AMH concentrations in serum with non-mercury preservative. Refer to calibration card for exact concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute control Levels I and II with 1 mL deionized water. Solubilize, mix well and use after reconstitution. Aliquot and freeze immediately for multiple use. Avoid repeated freeze thaws.

PLT-124 AMH/MIS Coated Microtitration strips

One strip-holder, containing 12 strips and 96 microtitration wells with AMH antibody immobilized to the inside wall of each well. Store at 2-8°C until expiration date (i.e., 2 years from date of manufacturing) in the resealable pouch with a desiccant to protect from moisture.

ASB-205 AMH/MIS Assay Buffer

One bottle, 12 mL, containing a protein-based (BSA)-buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

BCR-124 picoAMH Biotin Conjugate Ready-To-Use (RTU)

One bottle, 12 mL, containing biotinylated anti-AMH antibody in protein-based buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

SAR-124 picoAMH Streptavidin-Enzyme Conjugate-Ready-to-Use (RTU)

One amber bottle, 12 mL, containing streptavidin-HRP (horseradish peroxidase) in a protein-based buffer and a non-mercury preservative. Store undiluted at 2-8°C until expiration date.

TMB-100 TMB Chromogen Solution

One bottle, 12 mL, containing a solution of tetramethylbenzidine (TMB) in buffer with hydrogen peroxide. Store at 2-8°C until expiration date.

STP-100 Stopping Solution

One bottle, 12 mL, containing 0.2 M sulfuric acid. Store at 2 to 30°C until expiration date.

WSH-100 Wash Concentrate A

One bottle, 60 mL, containing buffered saline with a nonionic detergent. Store at 2-30°C until expiration date. Dilute 25-fold with deionized water prior to use.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of absorbance measurement at 450 nm, 405 nm and 630 nm.
2. Microplate orbital shaker.
3. Microplate washer.
4. Semi-automated/manual precision pipette to deliver 10–250 µL.
5. Repeater pipette.
6. Vortex mixer.
7. Deionized water Type 1 (>18 megohms resistivity).

WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

The following precautions should be observed:

a) Follow good laboratory practice. [4]

b) Use personal protective equipment. Wear lab coats and disposable gloves when handling immunoassay materials.

c) Handle and dispose of all reagents and material in compliance with applicable regulations.

d) If external package is damaged, inspect the components inside for any other damage. Do not use if the components are damaged.

WARNING: Potential Biohazardous Material

Samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment or prior certification.[5] Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.

WARNING: Potential Chemical Hazard

Some reagents in this kit contain Pro-Clean 400 and Sodium Azide[6] as a preservative. Pro-Clean 400 and Sodium Azide in concentrated amounts are irritants to skin and mucous membranes.

For further information regarding hazardous substances in the kit, please refer to the MSDS, either at AnshLabs.com or by request.

SAMPLE COLLECTION AND PREPARATION

a) Serum is the recommended sample type.

b) Sample handling, processing, and storage requirements depend on the brand of blood collection tube that used. Please reference the manufacturer's instructions for guidance. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products.

c) Within two hours after centrifugation, transfer at least 500 µL of cell free sample to a storage tube, vortex and tightly stopper the tube immediately.

d) Samples may be stored at 2 to 8°C if assayed within 24 hours; otherwise samples must be stored at -20°C or colder. Serum specimens are stable for up to 5 months at -20°C or colder.

e) Avoid repeated freezing and thawing of samples. Thaw samples no more than 3 times. No loss of activity greater than 15% has been observed for specimens up to three (3) freeze thaw cycles.

f) Avoid assaying lipemic, hemolyzed or icteric samples.

g) For shipping, place specimens in leak proof containers in biohazard specimen bags with appropriate specimen identification and test requisition information in the outside pocket of the biohazard specimen bag. Follow safe handling procedures (e.g., DOT and IATA requirements) when shipping specimens.[7]

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the picoAMH ELISA assay. It is the user's responsibility to validate the assay for their laboratory performance and for the purpose for which the assay is intended. Accurate results will only be obtained by using precise laboratory techniques and following the package insert.
2. Bring all kit reagents to room temperature before use. Thoroughly mix the reagents before use by gentle inversion. Do not mix various lots of any kit component and do not use any component beyond the expiration date.
3. A full calibration curve, low and high-level controls, should be included in each assay.
4. Use a clean disposable pipette tip for each reagent, calibrator, control or sample.
5. Use deionized water and avoid exposure of the reagents to excessive heat or direct sunlight. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies.
6. Avoid microbial contamination of reagents.
7. Incomplete washing will adversely affect the outcome and assay precision. Care should be taken to add TMB into the wells to minimize potential assay drift due to variation in the TMB incubation time.

PREPARATION OF REAGENTS

1. **picoAMH Calibrators B-F and picoAMH Controls I & II:** Tap and reconstitute picoAMH Calibrator B-F and picoAMH Controls I & II each with 1 mL deionized water. Solubilize, mix well and use after reconstitution.

2. **Wash Solution:** Dilute wash concentrate 25-fold with deionized water. The wash solution is stable for one month at room temperature when stored in a tightly sealed bottle.
3. **Microtitration Wells:** Select the number of coated wells required for the assay. The remaining unused wells should be placed in the re-sealable pouch with a desiccant. The pouch must be resealed to protect from moisture.

PREPARATION OF SAMPLES

1. Specimens producing absorbance readings above the measurable range (e.g., 6.0 to approximately 1,150 pg/mL) can be diluted with Calibrator A/Sample diluent prior to testing (maintaining a pipetting total volume of 100 µL for either neat specimen or diluted specimen).
2. The assay has been designed and validated for specimen dilutions of up to 20-fold. AMH concentrations of 6 to 23,000 pg/mL (0.006 to 23 ng/mL) to be measured quantitatively.
3. Diluted specimens must read > 160 pg/mL.
4. The read out (pg/mL) for diluted specimens must be corrected for the dilution factor. For example, a specimen that was diluted 10-fold prior to assay and reading 200 pg/mL will be reported as 2,000 pg/mL (i.e., 200 pg/mL x 10-fold dilution factor).
5. For specimens where the range of AMH concentrations can be estimated, an initial dilution protocol can be employed for efficient workflow and reagent use.

ASSAY PROCEDURE

Allow all specimens and reagents to reach room temperature and mix thoroughly by gentle inversion before use. Calibrators, controls, and unknowns should be assayed in duplicate.

1. Allow all specimens and reagents to reach room temperature and mix thoroughly by gentle inversion before use.
2. Calibrators, controls, and unknowns should be assayed in duplicate. Label the assay plate (96-well capture antibody coat plate supplied).
3. Add 50 µL of the AMH/MIS Assay Buffer to each microtiter well using a repeater pipette.
4. Pipette 100 µL of the reconstituted Calibrator and Controls to the appropriate wells.
5. Pipette 100 µL of the prepared samples using precision pipette to the sample designated wells.
6. Incubate the plate, shaking at a fast speed (600-800 rpm) on an orbital microplate shaker, for 3.0 hours (± 10 minutes) at room temperature (23 ± 2°C).
7. Aspirate and wash each strip 5 times with Wash Solution (350 µL/per well) using an automatic microplate washer.
8. Add 100 µL of the Antibody-Biotin Conjugate RTU (ready-to-use) to each well using a repeater pipette.
9. Incubate the plate, shaking at a fast speed (600-800 rpm) on an orbital microplate shaker, for 1.0 hour (± 5 minutes) at room temperature (23 ± 2°C).
10. Aspirate and wash each strip 5 times with the Wash Solution (350 µL/per well) using an automatic microplate washer.
11. Add 100 µL of the Streptavidin-Enzyme Conjugate-RTU to each well using a repeater pipette.
12. Incubate the plate, shaking at a fast speed (600-800 rpm) on an orbital microplate shaker, for 30 (± 5) minutes at room temperature (23 ± 2°C).
13. Aspirate and wash each strip 5 times with the Wash Solution (350 µL/per well) using an automatic microplate washer.
14. Add 100 µL of the TMB chromogen solution to each well using a repeater pipette. Avoid exposure to direct sunlight.
15. Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for 8-12 min at room temperature (23 ± 2°C).
16. Add 100 µL of the Stopping solution to each well using a repeater pipette.
17. Read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set for dual wavelength reading at 450 nm and a machine blank reference wavelength at 630 nm.

RESULTS

1. Calculate the mean optical density (OD) for each calibrator, control, or unknown specimen.
2. Plot the log of the mean OD readings for each of the Calibrators along the y-axis versus log of the AMH concentrations in pg/mL along the x-axis.
3. Determine the AMH concentrations of the Controls and Unknown specimens from the calibration curve by matching their mean OD readings with the corresponding AMH concentrations.

NOTE: *The results in this package insert were calculated by plotting the log optical density (OD) data on the Y-axis and log AMH concentration on X-axis using a cubic regression curve-fit.*

LIMITATIONS AND INTERFERENCES

1. AMH results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings when being interpreted for diagnostic purposes.
2. AMH test results < 10 pg/mL should be carefully evaluated in the context of a full clinical work up to ensure that the use of contraceptives is not discontinued in women who have not yet reached menopause.
3. AMH test results > 10 pg/mL should be carefully evaluated in the context of a full clinical work up to ensure that uterine bleeding due to endometrial cancer is not dismissed as a potential diagnosis.
4. This test should not be used to assess a woman's fertility status or for use in monitoring or predicting the ovarian response in women undergoing or planning to undergo fertility treatments.
5. The assay is unaffected by icterus (bilirubin ≤ 66mg/dL), hemolysis (Hb ≤ 1000mg/dL), lipemia (Triglyceride ≤2000 mg/dL) and biotin (200 ng/mL to ≤10,000 ng/mL). Criterion: Recovery within ± 10 % of initial value.
6. If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial.
7. As for any assay employing antibodies, the possibility exists for interference by heterophile antibodies in the samples.[8] Interference from heterophile antibodies has not been evaluated for this assay.

QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- picoAMH ELISA controls or other commercial controls should fall within established confidence limits.
- The confidence limits for picoAMH controls are printed on the Calibration card.
- A full calibration curve, low and high-level controls, should be included in each assay.
- TMB should be colorless. Development of any color may indicate reagent contamination or instability.

REPRESENTATIVE CALIBRATION CURVE DATA

Calibrator ID	Mean Absorbance (450 nm)	Concentration (pg/mL)	Concentration (pmoL/L)*
Calibrator A	0.022	0	0
Calibrator B	0.052	7.6	0.054
Calibrator C	0.134	31	0.22
Calibrator D	0.398	105	0.75
Calibrator E	1.17	360	2.57
Calibrator F	2.94	1091	7.79

CAUTION: *The above data must not be employed in lieu of data obtained by the user in the laboratory.*

PERFORMANCE CHARACTERISTICS

The performance characteristic results are reported in pg/mL and can be converted to pmol/L using the conversion factor below:

*** 1 pg/mL = 0.00714 pmol/L**

Limit of Blank, Limit of Detection and Limit of Quantitation:
The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI EP17-A2 guidelines.[9]

Limit of Blank (LOB): The Limit of Blank was 0.5 pg/mL, calculated as the 95th percentile value from a minimum of n = 324 measurements of 4-6 analyte-free samples in each of 4 reagent lots. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

Limit of Detection (LOD): The LOD was 1.3 pg/mL, calculated as the [LOB + (1.645 * SD)] of measurements of twelve (12) specimens containing AMH in the range of 2.8 to 10.0 pg/mL, 3 replicates each, in six independent runs and in 4 lots of reagents (n=862). The LOD is the lowest amount of AMH in a sample that can be detected (value above the LOB) with a probability of 90%.

Limit of Quantitation (LOQ): In the absence of a reference method, the LOQ determination was based on the CV of replicate testing of the 12 human serum pools evaluated to calculate the LOD. Serum was assayed in triplicate in each of 6 runs in each of 4 reagent lots. The LOQ of 3.2 pg/mL was calculated at an imprecision of 20% CV based on precision profiling.

Measuring Range:
The measuring range is 6.0 to 1,150 pg/mL (0.043 pmol/L to 8.21 pmol/L). Specimens may be pre-diluted up to 20-fold prior to assay, thus the extended measuring range is up to 23,000 pg/mL (23 ng/mL, 164 pmol/L).

Report values BELOW the measuring range as < 6 pg/mL (< 0.043 pmol/L). Values ABOVE the measuring range are to be tested at up to 20-fold dilution to report values between 1,150 and 23,000 pg/mL (8.21 and 164 pmol/L). Report specimens reading above 23,000 pg/mL as > 23,000 pg/mL (> 164 pmol/L).

Dilution:
Samples with AMH concentrations above the measuring range can be diluted with sample diluent. The maximum recommended dilution is 1:20 for a sample concentration > 160 pg/mL.

Imprecision:
Precision was determined using picoAMH reagents, using human specimens according to guidance from CLSI EP05-A3.[10] Each of 3 lots were tested using a protocol in which specimens were tested in quadruplicate (2 sets of duplicates in each assay run) in 2 runs per day over 20 days (n = 160 per sample per reagent lot) The following table summarizes results from all 3 reagent lots (n = 480 specimens).

Sample	Lot	Mean pg/mL	Repeatability		Intermediate Precision	
			SD (pg/mL)	% CV	SD (pg/mL)	% CV
Serum 1	Lot1	14.6	0.8	5.5%	1.2	8.1%
	Lot2	14.2	0.6	4.2%	0.8	5.9%
	Lot3	15.5	0.7	4.5%	1.0	6.7%
Serum 2	Lot1	80.1	2.2	2.8%	3.6	4.5%
	Lot2	80.0	2.0	2.5%	3.4	4.2%
	Lot3	80.8	2.8	3.5%	3.4	4.2%
Serum 3	Lot1	620.3	17.4	2.8%	22.9	3.7%
	Lot2	609.6	16.8	2.8%	24.2	4.0%
	Lot3	643.2	19.2	3.0%	24.0	3.7%
Serum 5	Lot1	942.8	28.9	3.1%	51.3	5.4%
	Lot2	924.1	23.7	2.6%	48.5	5.3%
	Lot3	935.2	36.8	3.9%	47.4	5.1%

Recovery:
Known amounts of recombinant human AMH were added to low analyte serum samples pool to generate fifteen specimens. The concentration of AMH was determined before and after the addition of exogenous AMH and the percent recovery was calculated on 3 lots of reagents run in triplicates. A representative data is summarized below.

Specimen Tested	Target AMH (pg/mL)	Mean AMH (pg/mL)	% Difference	Mean Recovery (%)
Specimen 1	6.75	7.4	106	110.0
Specimen 2	12.5	12.8	100	102.1
Specimen 3	24	21.7	88	90.6
Specimen 4	36	32.9	89	91.4
Specimen 5	60	58.2	99	97.0
Specimen 6	120	119.7	98	99.7
Specimen 7	240	259.4	108	108.1
Specimen 8	360	377.0	105	104.7
Specimen 9	480	494.2	100	103.0
Specimen 10	600	641.6	107	106.9
Specimen 11	720	739.5	103	102.7
Specimen 12	840	862.5	103	102.7
Specimen 13	960	981.8	102	102.3
Specimen 14	1080	1108.8	101	102.7
Specimen 15	1200	1164.1	100	97.0

Hook Effect:
There is no high-dose hook effect at AMH concentrations up to 256,000 pg/mL.

Linearity:
Linearity of picoAMH ELISA was evaluated according to CLSI EP6-A using 16 specimens in the measuring interval of 1-1200 pg/mL in replicates of 3 for each specimen. The allowable nonlinearity was calculated on 3 lots of reagents run. The results are summarized below.

Parameter	Reagent Lot		
	Lot 1	Lot 2	Lot 3
N	16	16	16
Linear Range (pg/mL)	6-1095	6-1091	7-1110
Slope	1.01	1.01	1.03
X- Intercept	5.1	2.8	-1.2
SE (least square linear regression)	19.7	16.9	27.0

Analytical Specificity:
The monoclonal antibody pair used in the assay is specific for human AMH. The table below summarizes results of cross-reactivity of proteins or compounds structurally and functionally related to AMH in the picoAMH assay.

Cross-Reactant	Concentration of Cross-Reactant Tested (pg/mL)	AMH Reported Value (pg/mL)	% Cross-reactivity
Activin B	50000	< 1.3	0.003%
Inhibin A	100000	< 1.3	0.001%
Inhibin B	100000	< 1.3	0.001%
alpha-2 Macroglobulin	65000	< 1.3	0.002%
Follistatin-288	50000	< 1.3	0.003%
Follistatin-315	50000	< 1.3	0.003%
hAMH, ProMature*	600	627.8	104.641%
hAMH, Mature	600	< 1.3	0.217%
Myostatin	50000	< 1.3	0.003%
FSH**	39683	< 1.3	0.003%
TSH***	869565	< 1.3	0.000%
LH****	9312	< 1.3	0.014%
Prolactin	211000	< 1.3	0.001%
Testosterone	100000	< 1.3	0.001%
Estrone Sulphate	100000	< 1.3	0.001%
DHEA	100000	< 1.3	0.001%
Progesterone	100000	< 1.3	0.001%
Estradiol	50000	< 1.3	0.003%

*Positive Control
**Based on 2nd International standard, human, bioassay: 08/282
***Based on 3rd international standard for immunoassay: 81/565
****Based on 1st international standard, human, recombinant: 96/602

Interference:
Interference was tested according to CLSI EP7-A2. Serum samples with AMH concentrations at 27 pg/mL and 300 pg/mL were evaluated as controls and tests with the doses of interferents specified in the table below. Interference was considered significant if the analyte recovery is ± 10% of the value of AMH measured. At the concentrations tested, none of the potential interferents tested showed more than ± 10% difference effect on picoAMH measurement in human sera.

Interferent	Highest Concentration Tested with no Significant Interference
Hemoglobin	1000 mg/dL
Ascorbic Acid	0.3 mg/mL
Bazedoxifene	1 µg/mL
Cefoxitin Na	2.5 mg/mL
Metformin	2 mg/mL
Triptorelin Acetate	15 µg/mL
Estradiol (beta)	1 ng/mL
Estrone Sulphate	1 ng/mL
Folic Acid	0.4 µg/mL
Levothyroxine	0.2 µg/mL
Medroxyprogesterone Acetate	1 µg/mL
Escitalopram Oxalate	0.1 mg/mL
Venlafaxine HCL	15 µg/mL
Doxycycline Hyclate	50 µg/mL
Bilirubin	0.66 mg/mL
Levodopa	30 µg/mL
Rifampicin	60 µg/mL
Heparin, Sodium Salt	30 U/mL
Intralipid	20 mg/mL
Acetaminophen	0.2 mg/mL
Ampicillin Na	1 mg/mL
Bisphosphonate	0.02 mg/mL
Cyclosporine	5 ug/mL
Estropipate	0.015 mg/mL
Fluoxetine HCl	3.5 µg/mL
Levonorgestrel	3 µg/mL
Metronidazole	0.2 mg/mL
Theophylline	0.1 mg/mL
Biotin	10,000 ng/mL
Acetylsalicyclic Acid	1 mg/mL
Citalopram	0.01 mg/mL
Ibuprofen	0.5 mg/mL
Phenylbutazone	0.1 mg/mL
Pregabalin	0.01 mg/mL
Raloxifene HCl	0.12 mg/mL
Paroxetine HCl	1 µg/mL
Gabapentin	0.09 mg/mL
Norethindrone	0.03 mg/mL
Cholesterol	5 mg/mL
Progesterone	0.4 mg/mL
Acetylcysteine	0.15 mg/mL
Cyproterone Acetate	0.3 mg/mL
Methyldopa	0.02 mg/mL

EXPECTED RESULTS

Age-stratified expected values for serum AMH is presented for ostensibly healthy women > 5 years from their final menstrual period (FMP), < 5 years from FMP, and at FMP or later in their menopausal transition in the following expected results data. AMH concentrations decrease relative to increasing age, and serum AMH measurements provide valuable additional information to support a physician's efforts to determine a woman's menopausal status.

The picoAMH reference ranges were determined in a longitudinal, multi-center study of SWAN (Study of Women's Health Across the Nation). The picoAMH expected values were generated from a cohort of 644 women ages 42-62 years. The picoAMH reference ranges are presented as 5th, 25th, 50th, 75th, and 95th percentiles. The lower end of the analytical measuring range for the Ansh picoAMH assay is 6 pg/mL. Women with values below the AMR are reported <6.0 pg/mL. As the categories of age increase, the median picoAMH values for a given centile is reduced. This vertical pattern is repeated for all the centiles where the results are 6 pg/mL or higher, providing laboratories age-specific reference ranges for AMH during ages associated with the menopausal transition.

Expected AMH concentrations by age-stratified picoAMH centiles.

		picoAMH centile					
Age	**n**	**5**	**25**	**50**	**75**	**95**	**CI[1]**
42.9 to 44.9	159	<6	100	390	1,200	2,900	2,200 -3,500
45.0 to 49.9	175	<6	<6	75	280	640	700 – 1,500
50.0 to 54.9	175	<6	<6	<6	16	98	160 – 350
55.0 to 62.4	135	<6	<6	<6	<6	28	6 – 110
Any	644						

[1] Confidence interval of the 95th centile

The final menstrual period (FMP) for each woman in the SWAN Study were assigned retrospectively after 12 months of amenorrhea (the clinical definition of natural menopause). Menopausal categories for assigning status were based on the approximate time to the final menstrual period (FMP). Three menopausal categories were defined based on the time to final menstrual period (TTFMP).

Years from FMP menopausal categories and AMH concentration.

Category#	Menopausal Category	picoAMH (pg/mL)
1 (HIGH)	>5 years from FMP	≥100 pg/mL
2 (MEDium)	< 5 years from FMP	10-99.9 pg/mL
3 (LOW)	at FMP or later	<10 pg/mL

We defined two cutoffs for AMH concentrations relative to the TTFMP. These were: LOW = less than 10 (<10) pg/mL, MEDium = between 10-99.9 pg/mL, and HIGH = greater than 100 (>100) pg/mL.

A separate cohort of the SWAN Study population was used to validate the picoAMH data stratified by menopausal category. This three-way classification is presented in the contingency table format as absolute values as well as the percent agreement of picoAMH test results with menopausal category. Only one sample was selected from each woman enrolled in the study, and the number of women defined in each these three categories are the same. Each category is comprised of women with a range of ages.

Contingency table stratifying 3 menopausal categories defined by TTFMP.

Healthy Women	Menopausal category			
picoAMH	**>5 years from FMP**	**<5 years from FMP**	**at FMP or later**	**Total**
<10 pg/mL (LOW)	19[1] (8.2%)[2]	89 (38.7%)	198 (86.1%)	**306**
10-99.9 pg/mL (MED)	22 (9.6%)	80 (34.8%)	28 (12.2%)	**130**
≥100 pg/mL (HIGH)	189 (82.2%)	61 (26.5%)	4 (1.7%)	**254**
Total	**230 (100%)**	**230 (100%)**	**230 (100%)**	**690**

[1] Number of women in each menopausal category by AMH concentration
[2] Percent agreement of picoAMH test result with menopausal category

Our studies calculated that approximately 12.2% of the time menopausal women presented with an AMH test result higher than 10 pg/mL, and 1.7% of the time menopausal women present with an AMH test result higher than 100 pg/mL. During the peri-menopausal transition, pituitary, gonadal, and sex steroid hormone levels will vary considerably. An AMH result that is high or low relative to the patient clinical presentation is recommended to be repeated.

In some women, an unexpectedly high AMH relative to the woman's age or clinical presentation can represent residual ovarian activity or presence of antral follicles despite the absence of a menstrual period due to amenorrhea or a physician's clinical determination of menopause.[11] The woman may still have some ovarian activity after her menopause was clinically defined by amenorrhea of >12 consecutive months. The absence of a menstrual cycle is not a confirmation that there are zero (0) primary follicles remaining in the ovary. Amenorrhea (at least three missed menstrual periods) may result even if there are still AMH-producing follicles. These follicles may not mature and may fail to produce oocytes such that there is no ovulation, preparation of the uterus for pregnancy, and no menstruation.[12-14]

These data were then used to compute sensitivity (Detection Rate) and specificity (1-False positive rate) for the cutoffs at <10 pg/mL and ≥100 pg/mL.

Clinical performance of picoAMH ELISA to identify menopausal category.

picoAMH cutoff level	Menopausal Category	Detection Rate (%) (95% CI)[1]
≥100 pg/mL	>5 years from FMP	82.2 (76.6 – 86.9)
<10 pg/mL	at FMP or later	86.1 (80.4 – 89.9)
10-99.9 pg/mL	<5 years from FMP	34.8 (28.6 – 41.3)

[1] 95% confidence interval in parentheses.

False positive rate of picoAMH ELISA to identify menopausal category.

picoAMH cutoff level	Classified Menopausal Category	True Menopausal Status	False Positive Rate (%) (95% CI)[1]
≥100 pg/mL	>5 years from FMP	<5 years from FMP	26.5 (20.9-32.7)
		at FMP or later	1.7 (0.4 – 4.4)
<10 pg/mL	at FMP or later	>5 years from FMP	8.2 (5.0-12.6)
		<5 years from FMP	38.7 (32.4 – 45.3)
10-99.9 pg/mL	<5 years from FMP	>5 years from FMP	9.6 (6.1-14.1)
		at FMP or later	12.2 (8.2-17.1)

[1] 95% confidence interval in parentheses.

This analysis shows that the picoAMH ELISA performs reasonably well in distinguishing women at FMP or later and women >5 years from FMP. Specifically, 86.1% of women who were at FMP or later also had a picoAMH level <10 pg/mL and 82.2% of women who were >5 years from FMP also had a picoAMH level > 100 pg/mL. However, the picoAMH ELISA test result has limited deterministic value when AMH values fall between 10 and 99.9 pg/mL, and test results falling into this range should be interpreted with caution. To aid in the interpretation of women in the menopausal transition, the following table provides the likelihood ratios (LR) for the comparison of women < 5 years away from their FMP vs. adjacent menopausal categories given the picoAMH concentrations obtained in the clinical study.

Likelihood ratios associated with menopausal categories.

Menopausal Category	Positive Likelihood Ratio (95% CI)	Negative Likelihood Ratio (95% CI)
<5 years from FMP vs. at FMP or later	2.86 (1.94-4.22)	0.74 (0.67-0.83)
<5 years from FMP vs. >5 years from FMP	3.64 (2.35-5.62)	0.72 (0.65-0.80)

As an example, the results above show that, comparing to any woman with an unknown AMH level who has reached her FMP or is < 5 years from her FMP, a woman with an AMH test result between 10 and 99.9 pg/mL is 2.86 times more likely to be < 5 years away from her FMP than to be at FMP or later. Likewise, comparing to any woman with an unknown AMH level who is < 5 years away from her FMP or > 5 years away from her FMP, a woman with an AMH test result between 10 and 99.9 pg/mL is 3.64 times more likely to be < 5 years away from her FMP than to be > 5 years away from her FMP. These data should be considered relative to other clinical findings and laboratory results, such as: patient age, self-reported menstrual history, vasomotor symptoms, family history (i.e., mother's reported age at menopause), history of fertility/infertility ovary or other reproductive surgery, FSH and estradiol determinations. AMH is not cycle day dependent. The menstrual cycle is expected to be irregular in women for whom there is a clinical need to assess menopausal status. Samples may be drawn at any time for assessment of AMH.[15-17]

Risks associated with falsely low or falsely high picoAMH test results:
Inaccurate test results in which the serum AMH concentration is reported higher than expected for age or relative to other clinical findings and laboratory results may indicate a woman is not post-menopausal. Physicians may counsel a patient to continue hormonal contraceptives when it is no longer necessary. Inaccurate test results in which the serum AMH concentration is reported lower than expected for age or relative to other clinical findings and laboratory results may lead a physician to recommend 1) discontinuing contraceptives when it is still necessary to prevent pregnancy, or 2) evaluating bone mineral density or 3) consider prescribing hormone replacement therapy.

A serum AMH concentration that is higher than expected relative to the patient age and clinical presentation may indicate residual ovarian activity in the absence of ovulation and a menstrual cycle. The determination of irregular or abnormal bleeding or vaginal discharge that is increasing in amount, occurring between anticipated periods, or occurring after the clinical assessment of menopause (i.e., > 12 consecutive months of amenorrhea) may indicate the presence of endometrial cancer. Abnormal bleeding episodes are independent of an AMH test result. Physicians should evaluate patients that report abnormal bleeding or unexpectedly high AMH test results for further evaluation including assessment of other risk factors of endometrial cancer and gynecological cancers. Physicians should counsel these patients regarding their options for diagnosis by endometrial biopsy and transvaginal ultrasound accordingly.

As with any single assay determination, it should be used in conjunction with other clinical and/or laboratory findings. Appropriate mitigations include obtaining a detailed clinical history and additional laboratory testing. Risk is mitigated by other clinical features which if disparate with a high AMH would indicate the need to re-examine the patient and re-test AMH after several months.

REFERENCES

1. Stuenkel CA, Davis SR, Gompel A, Lumsden MA, Murad MH, Pinkerton JV, Santen RJ 2015 Treatment of Symptoms of the Menopause: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 100:3975-4011
2. Su HI, Freeman EW 2009 Hormone changes associated with the menopausal transition. Minerva Ginecol 61:483-489
3. Dewailly D, Andersen CY, Balen A, Broekmans F, Dilaver N, Fanchin R, Griesinger G, Kelsey TW, La MA, Lambalk C, Mason H, Nelson SM, Visser JA, Wallace WH, Anderson RA 2014 The physiology and clinical utility of anti-Mullerian hormone in women. Hum Reprod Update 20:370-385
4. Clinical Laboratory Safety; Approved Guideline Second Edition, GP17-A2. 2004. Clinical and Laboratory Standards Institute.
5. HHS Publication, 5th ed., December 2009. Biosafety in Microbiological and Biomedical Laboratories. Available http://.cdc.gov/biosafety/publications/bmbl5.
6. DHHS (NIOSH) Publication No. 78–127, August 1976. Current Intelligence Bulletin 13 - Explosive Azide Hazard. Available http:// www.cdc.gov/niosh.
7. Approved Guideline – Procedures for the Handling and Processing of Blood Specimens, H18-A3. 2004. Clinical and Laboratory Standards Institute.
8. Kricka L. Interferences in immunoassays – still a threat. Clin Chem 2000; 46: 1037–1038
9. CLSI guideline EP17-A2 (Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline-Second Edition. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2012.
10. CLSI EP05-A3 (Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Third Edition. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2014.
11. J. van Disseldorp, M. J. Faddy, A. P. N. Themmen, F. H. de Jong, P. H. M. Peeters, Y. T. van der Schouw, F. J. M. Broekmans; Relationship of Serum Antimüllerian Hormone Concentration to Age at Menopause, The Journal of Clinical Endocrinology & Metabolism, Volume 93, Issue 6, 1 June 2008, Pages 2129–2134, https://doi.org/10.1210/jc.2007-2093
12. La Marca, A., Pati, M., Orvieto, R., Stabile, G., Artenisio, A., Volpe, A. [2006]. Serum anti-müllerian hormone levels in women with secondary amenorrhea. Fertility and Sterility , Volume 85 , Issue 5 , 1547 – 154.
13. Nerenz, RD., Jungheim, ES., Lockwood, CM. [2014]. Premenopausal Amenorrhea: What's in a Number? Clinical Chemistry 60:1 29–34 (2014).
14. Joop S. E. Laven, Annemarie G. M. G. J. Mulders, Jenny A. Visser, Axel P. Themmen, Frank H. de Jong, Bart C. J. M. Fauser; Anti-Müllerian Hormone Serum Concentrations in Normoovulatory and Anovulatory Women of Reproductive Age, The Journal of Clinical Endocrinology & Metabolism, Volume 89, Issue 1, 1 January 2004, Pages 318–323, https://doi.org/10.1210/jc.2003-030932
15. La MA, Stabile G, Artenisio AC, Volpe A 2006 Serum anti-Mullerian hormone throughout the human menstrual cycle. Hum Reprod 21:3103-3107.
16. Tsepelidis S, Devreker F, Demeestere I, Flahaut A, Gervy C, Englert Y 2007 Stable serum levels of anti-Mullerian hormone during the menstrual cycle: a prospective study in normo-ovulatory women. Hum Reprod 22:1837-1840.
17. Lambert-Messerlian G, Plante B, Eklund EE, Raker C, Moore RG 2016 Levels of antimullerian hormone in serum during the normal menstrual cycle. Fertil Steril 105:208-213.

Patents Pending:
Use of this kit is covered by one or more of the following pending U.S. and International Patents: US 14/888,739, PCT/US2013/069172, EP 13853068.8

This assay is intended for in vitro diagnostic use. **For Prescription Use Only.**

Caution: United States Federal law restricts this device to sale by or on the order of a physician.

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Symbol	English	Deutsch	Français	Español	Italiano
	Biohazard	Biogefahr	danger biologique	Riesgo biológico	rischio biologico
	Caution	Vorsicht	mise en garde	precaución	attenzione
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Date of Manufacture	Herstellungsdatum	date de fabrication	fecha de manufactura	Data di produzione
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità
	96-well plate	Platte mit 96 Vertiefungen	Plaque à 96 puits	placa de 96 pocillos	piastra a 96 pozzetti