Elecsys AMH Plus

cobas®

REF		Σ	SYSTEM
07957246190	07957246500	100	**cobas e** 801

English

System information

Short name	ACN (application code number)
AMHP	10158

Please note

Results that are intended to be used for the dosing of the follitropin delta of Ferring must be accompanied by the following statement:
This AMH value in pmol/L was generated with the Elecsys AMH Plus assay and is suitable for the individualized dosing of follitropin delta of Ferring.

Intended use

Immunoassay for the in vitro quantitative determination of anti-Müllerian hormone (AMH) in human serum and plasma. The determination of AMH is used for the assessment of the ovarian reserve and the prediction of response to controlled ovarian stimulation (COS) in conjunction with other clinical and laboratory findings.

In addition, the determination of AMH (in pmol/L) in combination with body weight is used for the establishment of the individual daily dose of the human recombinant follicle-stimulating hormone (rFSH) follitropin delta of Ferring (in accordance with the current prescribing information of the Ferring follitropin delta) in controlled ovarian stimulation for the development of multiple follicles in women undergoing an assisted reproductive technology program.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on the **cobas e** 801 immunoassay analyzer.

Summary

The anti-Müllerian hormone is a homodimeric glycoprotein belonging to the transforming growth factor β (TGF β) family. All members of this superfamily are involved in the regulation of tissue growth and differentiation. Prior to secretion, the hormone undergoes glycosylation and dimerization to produce an approximately 140 kDa precursor of two identical disulfide-linked 70 kDa subunits. Each monomer contains a large N-terminal pro-region and a much smaller C-terminal mature domain. In contrast to other TGF β family members, AMH is thought to require the N-terminal domain to potentiate activity of the C-terminal domain to attain full bioactivity.[1,2]

A part of AMH is then cleaved at a specific site between the pro-region and the mature region during cytoplasmic transit to generate biologically active 110 kDa N-terminal and 25 kDa C-terminal homodimers which remain associated in a non-covalent complex. The AMH type II receptor (AMH RII) has the capacity of binding only the biologically active form of AMH.[2]

In males, AMH is secreted by the Sertoli cells of the testes. During embryonic development in males, secretion of AMH from testicular Sertoli cells is responsible for the regression of the Müllerian duct and the normal development of the male reproductive tract. The secretion of AMH by the Sertoli cells starts during the embryogenesis and continues throughout life. AMH is continuously produced by the testicles until puberty and then decreases slowly to post-puberty values.[3]

In females AMH plays an important role in the ovarian folliculogenesis.[4] Follicle development in the ovaries comprises two distinct stages: initial recruitment, by which primordial follicles start to mature, and cyclic recruitment, which leads to the growth of a cohort of small antral follicles, among which the dominant follicle (destined to ovulate) is subsequently selected. FSH (follicle-stimulating hormone) directs the cyclic recruitment. AMH expression in granulosa cells starts in primary follicles and is maximal in granulosa cells of preantral and small antral follicles up to approximately 6 mm in diameter. When follicle growth becomes FSH-dependent, AMH expression diminishes and becomes undetectable. This pattern of AMH expression supports the inhibitory role of AMH at two distinct stages of folliculogenesis. First, AMH inhibits the transition of follicles from primordial into maturation stages and thereby has an important role in regulating the number of follicles remaining in the primordial pool. Second, AMH has inhibitory effects on follicular sensitivity to FSH and therefore has a role in the process of follicular selection.[5,6]

Serum levels of AMH are barely detectable at birth in females, reach their highest levels after puberty, decrease progressively thereafter with age, and become undetectable at menopause.[7,8] Serum AMH levels have been shown to be relatively stable during the menstrual cycle with substantial fluctuations being observed in younger women.[9,10,11] AMH levels further demonstrate lower intra- and inter-cyclic variation than baseline FSH.[10] Serum AMH levels decrease significantly during the use of combined contraceptives.[12] Clinical applications of AMH measurements have been proposed for a variety of indications.[13,14,15] Measurement of serum AMH is clinically mainly used for assessment of ovarian reserve reflecting the number of antral and pre-antral follicles, the so-called antral follicle count (AFC), and for the prediction of response to controlled ovarian stimulation.[13,15,16] Further clinical applications of AMH are diagnosis of disorders of sex development in children[17,18] and monitoring of granulosa cell tumors to detect residual or recurrent disease.[19,20] AMH has been suggested as a surrogate biomarker for AFC in the diagnosis of polycystic ovary syndrome (PCOS)[21,22] and for the prediction of time to menopause.[23,24]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 30 µL of sample, a biotinylated monoclonal AMH-specific antibody, and a monoclonal AMH-specific antibody labeled with a ruthenium complex[a)] form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the **cobas** link.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The **cobas e** pack is labeled as AMHP.

M Streptavidin-coated microparticles, 1 bottle, 5.8 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-AMH-Ab~biotin, 1 bottle, 7.2 mL:
Biotinylated monoclonal anti-AMH antibody (mouse) 1.0 mg/L; phosphate buffer 50 mmol/L, pH 7.5; preservative.

R2 Anti-AMH-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 7.2 mL:
Monoclonal anti-AMH antibody (mouse) labeled with ruthenium complex 1.0 mg/L; phosphate buffer 50 mmol/L, pH 7.5; preservative.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261	Avoid breathing mist or vapours.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves.

Response:

P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
on the **cobas e** 801 analyzer	16 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin plasma. Do not use EDTA plasma.

Criterion: Recovery within ± 30 % of serum value ≥ 3.57 pmol/L (≥ 0.5 ng/mL); recovery within ± 1.43 pmol/L (± 0.2 ng/mL) for serum value < 3.57 pmol/L (< 0.5 ng/mL) and slope of 0.9-1.1 + intercept within ± 0.714 pmol/L (± 0.1 ng/mL) + coefficient of correlation ≥ 0.95.

Stable for 3 days at 20-25 °C, 5 days at 2-8 °C, 6 months at -20 °C (± 5 °C). Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 07957203190, CalSet AMH Plus, for 4 x 1.0 mL
- REF 07957211190, PreciControl AMH Plus, for 4 x 2.0 mL
- REF 07299001190, Diluent Universal, 36 mL sample diluent
- General laboratory equipment
- **cobas e** 801 analyzer

Additional materials for the **cobas e** 801 analyzer:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

Traceability: This method has been standardized against the Beckman Coulter AMH Gen II ELISA (unmodified version without predilution) assay.

The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl AMH Plus.

Additional suitable quality control material may also be used in combination with PreciControl AMH Plus.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, and following each calibration.

Special care needs to be taken to ensure that the accuracy and precision of the testing stays within acceptable limits. Besides meeting the PreciControl AMH Plus target ranges provided, the user needs to ensure that the systematic bias with respect to the assigned target value is within ± 12 %, the intermediate precision CV is ≤ 8 % and the maximal total error is within ± 25 % (TE = |bias| + 1.65*CV). It is recommended to use quality control rule software.

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.

If necessary, repeat the measurement of the samples concerned.

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

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Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in pmol/L or in ng/mL).

Conversion factors: pmol/L x 0.14 = ng/mL

ng/mL x 7.14 = pmol/L

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1129 µmol/L or ≤ 66 mg/dL
Hemoglobin	≤ 0.62 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 1000 mg/dL
Biotin	≤ 123 nmol/L or ≤ 30 ng/mL
IgG	≤ 2.5 g/dL
IgA	≤ 1.8 g/dL
IgM	≤ 0.5 g/dL

Criterion: Recovery within ± 10 % of initial value.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1000 IU/mL.

There is no high-dose hook effect at AMH concentrations up to 9996 pmol/L (1400 ng/mL).

Pharmaceutical substances

In vitro tests were performed on 20 commonly used pharmaceuticals. No interference with the assay was found up to the concentrations indicated within the below table.

Active agent	Concentration tested mg/L
Acetylcysteine	1660
Ampicillin-Na	1000
Ascorbic acid	300
Cyclosporine	5
Cefoxitin	2500
Heparin	5000 U
Levodopa	20
Methyldopa	20
Metronidazole	200
Phenylbutazone	400
Doxycycline	50
Acetylsalicylic acid	1000
Rifampicin	60
Acetaminophen	200
Ibuprofen	500
Theophylline	100
Triptorelin acetate	0.1
Metformin	2000
Folic Acid	0.4
Levothyroxine	0.2

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

0.07-164 pmol/L (0.01-23 ng/mL) (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.07 pmol/L (< 0.01 ng/mL). Values above the measuring range are reported as > 164 pmol/L (> 23 ng/mL) or up to 328 pmol/L (46 ng/mL) for 2-fold diluted samples.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.049 pmol/L (0.007 ng/mL)

Limit of Detection = 0.07 pmol/L (0.01 ng/mL)

Limit of Quantitation = 0.214 pmol/L (0.03 ng/mL)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

Dilution

Samples with AMH concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:2 (either automatically by the analyzer or manually). The concentration of the diluted sample must be > 71.4 pmol/L (> 10 ng/mL).

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzer, the software automatically takes the dilution into account when calculating the sample concentration.

Expected values

A study in a Caucasian population with the Elecsys AMH Plus assay on samples from apparently healthy adults (148 males, 887 females not taking contraceptives) and 149 women with PCOS yielded the following results (Roche study No. RD001727):

	N	2.5th perc. ng/mL (95 % CI[b])	5th perc. ng/mL (95 % CI)	Median ng/mL (95 % CI)	95th perc. ng/mL (95 % CI)	97.5th perc. ng/mL (95 % CI)
Healthy men						
	148	0.77 (0.17-1.58)	1.43 (0.256-1.97)	4.79 (4.35-5.35)	11.6 (10.3-17.0)	14.5 (10.9-17.6)
Healthy women (years)						
• 20-24	150	1.22 (0.478-1.67)	1.52 (0.758-1.81)	4.00 (3.60-4.44)	9.95 (7.87-13.6)	11.7 (9.11-15.7)
• 25-29	150	0.890 (0.493-1.21)	1.20 (0.797-1.75)	3.31 (3.00-3.89)	9.05 (7.59-10.3)	9.85 (8.91-11.3)
• 30-34	138	0.576 (0.256-0.958)	0.711 (0.256-1.12)	2.81 (2.35-3.47)	7.59 (6.84-9.52)	8.13 (7.27-9.72)

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	N	2.5th perc. ng/mL (95 % CI[b)])	5th perc. ng/mL (95 % CI)	Median ng/mL (95 % CI)	95th perc. ng/mL (95 % CI)	97.5th perc. ng/mL (95 % CI)
• 35-39	138	0.147 (0.053-0.474)	0.405 (0.053-0.496)	2.00 (1.73-2.36)	6.96 (5.31-9.37)	7.49 (6.49-10.9)
• 40-44	142	0.027 (0.010-0.063)	0.059 (0.017-0.119)	0.882 (0.726-1.13)	4.44 (2.94-5.56)	5.47 (3.92-6.76)
• 45-50	169	0.010 (0.010-0.010)	0.010 (0.010-0.010)	0.194 (0.144-0.269)	1.79 (1.43-2.99)	2.71 (1.79-4.16)
PCOS women*						
	149	1.86 (1.54-2.50)	2.41 (1.67-3.01)	6.81 (6.30-7.42)	17.1 (13.3-20.3)	18.9 (16.0-21.1)

b) CI = confidence interval

	N	2.5th perc. pmol/L (95 % CI)	5th perc. pmol/L (95 % CI)	Median pmol/L (95 % CI)	95th perc. pmol/L (95 % CI)	97.5th perc. pmol/L (95 % CI)
Healthy men						
	148	5.5 (1.2-11.3)	10.2 (1.8-14.1)	34.2 (31.1-38.2)	82.8 (73.5-121)	103 (78.1-125)
Healthy women (years)						
• 20-24	150	8.71 (3.41-11.9)	10.9 (5.41-12.9)	28.6 (25.7-31.7)	71.0 (56.2-97.1)	83.6 (65.0-112)
• 25-29	150	6.35 (3.52-8.64)	8.57 (5.69-12.5)	23.6 (21.4-27.8)	64.6 (54.2-73.5)	70.3 (63.6-81.0)
• 30-34	138	4.11 (1.83-6.84)	5.08 (1.83-8.00)	20.0 (16.8-24.8)	54.2 (48.8-68.0)	58.0 (51.9-69.4)
• 35-39	138	1.05 (0.378-3.38)	2.89 (0.378-3.54)	14.2 (12.4-16.9)	49.7 (37.9-66.9)	53.5 (46.3-77.9)
• 40-44	142	0.193 (0.071-0.450)	0.421 (0.121-0.850)	6.29 (5.18-8.07)	31.7 (21.0-39.7)	39.1 (28.0-48.3)
• 45-50	169	0.071 (0.071-0.071)	0.071 (0.071-0.071)	1.39 (1.03-1.92)	12.8 (10.2-21.3)	19.3 (12.8-29.7)
PCOS women*						
	149	13.3 (11.0-17.8)	17.2 (11.9-21.5)	48.6 (45.0-53.0)	122 (95.0-145)	135 (114-151)

* According to the revised diagnostic criteria of PCOS defined by the Rotterdam ESHRE/ASRM-sponsored (ESHRE = European Society of Human Reproduction and Embryology; ASRM = American Society of Reproductive Medicine) PCOS consensus workshop group.[25]

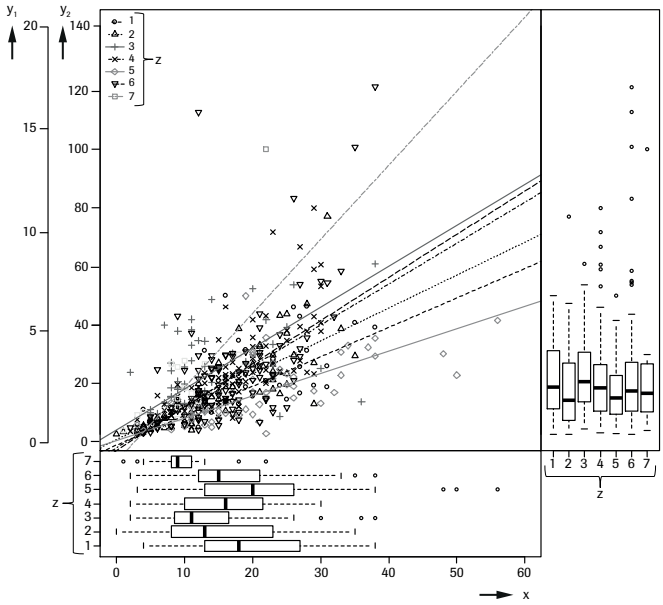
Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Use of AMH for the assessment of ovarian reserve

The use of AMH for the assessment of ovarian reserve was investigated in a prospective study with n = 451 women between 18-44 years old, where AMH values were correlated to the AFC of the women (Roche study No. RD001542). AFC was determined by transvaginal sonography measuring follicles of 2-10 mm diameter in size. Both AFC and AMH were determined on days 2-4 of the same menstrual cycle. Between 17 to 115 women were recruited per site at 6 different European sites and 1 Australian site.

No significant differences in mean AMH values were observed between the sites (pval = 0.301). The mean age values between the sites were significantly different, and also AMH and age showed a significant negative correlation (Spearman correlation coefficient -0.47). The age adjusted site effect of AMH showed no significance (pval = 0.193). The determined AFC values showed significant differences between the sites, with and without age adjustment. The overall correlation of AMH with AFC was 0.68 (Spearman's rank coefficient).

The figure below shows the scatterplot of AMH versus AFC, as well as the site-specific AMH and AFC distributions.



x: AFC (N)

y_1: AMH (ng/mL)

y_2: AMH (pmol/L)

z: Site

Agreement table on absolute AFC numbers of 7 and 15

Three AFC groups were defined[26,27] based on two cutoffs for AFC: 7 and 15 (0-7, 8-15, > 15). According to the prevalences within these groups (15 %, 37 %, 48 %), quantiles on AMH were computed (c1 = 4.86 pmol/L or 0.681 ng/mL, c2 = 16.2 pmol/L or 2.27 ng/mL) to define three groups. Agreement is presented in absolute numbers and percentages per AMH group.

Given the large variability of AFC results depending on site- and sonographer-specific variations, each site should review the agreement table for transferability to their own specific conditions.

	AFC 0-7	AFC 8-15	AFC > 15	N
AMH ≤ 4.86 pmol/L (0.681 ng/mL)	43 (63.2 %)	22 (32.4 %)	3 (4.4 %)	68
4.86 pmol/L (0.681 ng/mL) < AMH ≤ 16.2 pmol/L (2.27 ng/mL)	20 (12.0 %)	95 (56.9 %)	52 (31.1 %)	167
AMH > 16.2 pmol/L (2.27 ng/mL)	3 (1.4 %)	52 (24.1 %)	161 (74.5 %)	216
N	66	169	216	451

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For a patient with AMH ≤ 0.681 ng/mL the probability to have a low AFC (0-7) is 63 %, the probability to be in the middle AFC (8-15) group is about 32 % and only 4.4 % for having an AFC > 15.

The probability for patients with high AMH values (> 2.27 ng/mL) to have an AFC > 15 is 75 %, the probability for being in the middle AFC (8-15) group is 24 % and only 1.4 % to have an AFC < 8.

Use of AMH for the prediction of hyper-response to controlled ovarian stimulation

The following results were obtained in an external study "Clinical evaluation of the Elecsys AMH assay for the prediction of response to controlled ovarian stimulation" (Roche study No. CIM RD 001695).

AMH was determined in 149 women undergoing an antagonist treatment protocol in the course of their first cycle of controlled ovarian stimulation for in-vitro fertilization (IVF). Women included in the study were aged < 44 years, had a regular menstruation cycle and no major abnormalities at transvaginal sonography. Women with PCOS, endocrine or metabolic abnormalities and women undergoing IVF with oocyte donation were not included. All women received a standard FSH stimulation dose of 150 IU/day. Blood was drawn before start of FSH stimulation for post hoc analysis of AMH after completion of the treatment cycle. Hyper-response was observed in 16 women. Hyper-response was defined as > 15 oocytes retrieved or cancellation of stimulation cycle where more than 20 follicles > 12 mm were observed and estradiol values > 11700 pmol/L, or when more than 30 follicles > 12 mm were observed. The clinical performance of Elecsys AMH to predict hyper-response to controlled ovarian stimulation was evaluated by ROC (receiver operating characteristic) analysis and by applying a cutoff of 15 pmol/L (2.1 ng/mL) which has been previously published.[28,29] Prediction of hyper-response was significant with an AUC (area under the curve) of 82.1 % (CI 72.5-91.7 %). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for the AMH cutoff of 15.0 pmol/L (2.1 ng/mL) are shown in the table below.

Hyper-response		
AMH cutoff	15.0 pmol/L (2.10 ng/mL)	
	Estimate	95 % CI
Sensitivity	81.3 %	54.4-96.0 %
Specificity	64.7 %	55.9-72.8 %
PPV	21.7 %	12.1-34.2 %
NPV	96.6 %	90.5-99.3 %

Use of AMH for individual daily dose determination of follitropin delta of Ferring

Follitropin delta is produced in a human cell line (PER.C6®) by recombinant DNA technology.[29,30,31] Ferring is marketing authorisation holder of follitropin delta.

The AMH concentration (in pmol/L) determined by the Elecsys AMH Plus assay in combination with body weight was validated for the individual daily dose determination of follitropin delta in controlled ovarian stimulation for the development of multiple follicles in women undergoing an assisted reproductive technology program such as an IVF or intracytoplasmic sperm injection (ICSI) cycle. The Elecsys AMH Plus assay was assessed exclusively for the individual dose determination of follitropin delta (Ferring). The AMH-based individualised dosing regimen of follitropin delta was validated in the prospective phase III clinical study ESTHER-1, a randomised, controlled, assessor-blind, parallel groups, multicenter, multinational trial comparing the efficacy and safety of follitropin delta with follitropin alfa (randomized in 1:1 ratio). Women aged 18-40 years undergoing controlled ovarian stimulation for IVF or ICSI were enrolled and followed a GnRH antagonist protocol.[32] In this trial, 665 IVF/ICSI patients randomised to the follitropin delta group were treated with an individual dose of follitropin delta determined on the basis of their body weight and their AMH (in pmol/L) concentration measured with the Elecsys AMH Plus assay. The individual daily dose of follitropin delta was maintained throughout stimulation with no dose adjustments.

Important information: Clinicians who want to administer follitropin delta must read and understand the current prescribing information of follitropin delta (Ferring) applicable in their country prior to administration of the drug; this document provides the details on the actual dosing regimen, the clinical efficacy and safety of the drug.[33]

Specific performance data

Representative performance data on the analyzer is given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

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		Repeatability		**Intermediate precision**	
Sample	Mean pmol/L	SD pmol/L	CV %	SD pmol/L	CV %
Human serum 1	0.307	0.007	2.4	0.011	3.6
Human serum 2	6.50	0.098	1.5	0.151	2.3
Human serum 3	41.1	0.477	1.2	0.850	2.1
Human serum 4	112	1.66	1.5	3.33	3.0
Human serum 5	142	2.03	1.4	3.41	2.4
PreciControl AMH 1	6.44	0.070	1.1	0.109	1.7
PreciControl AMH 2	33.6	0.391	1.2	0.546	1.6

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		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	0.0430	0.001	2.4	0.002	3.6
Human serum 2	0.910	0.014	1.5	0.021	2.3
Human serum 3	5.76	0.067	1.2	0.119	2.1
Human serum 4	15.7	0.232	1.5	0.466	3.0
Human serum 5	19.9	0.284	1.4	0.478	2.4
PreciControl AMH 1	0.902	0.010	1.1	0.015	1.7
PreciControl AMH 2	4.71	0.055	1.2	0.077	1.6

Method comparison

A comparison of the Elecsys AMH Plus assay, REF 07957246190 (**cobas e** 801 analyzer; y) with the Elecsys AMH Plus assay, REF 07957190190 (**cobas e** 601 analyzer; x) gave the following correlations (ng/mL):

Number of samples measured: 126

Passing/Bablok[34]	Linear regression
y = 0.980x - 0.001	y = 0.951x + 0.100
τ = 0.979	r = 0.999

The sample concentrations were between 0.0117 and 22.1 ng/mL.

Analytical specificity

The monoclonal antibodies used are highly specific to human AMH. The following cross-reactivities were found:

Cross-reactant	**Concentration tested**	**Cross-reactivity %**
Inhibin A	100 ng/mL	n. d.[c)]
Activin A	100 ng/mL	n. d.
LH	500 mIU/mL	n. d.
FSH	500 mIU/mL	n. d.

c) n. d. = not detectable

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number

Additions, deletions or changes are indicated by a change bar in the margin.

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Elecsys AMH Plus

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