

# Quality Notification - IMM 2017-06

## *Actie – 14 maart 2017*

### Elecsys AMH plus

Lancering van de Elecsys AMH plus assay  
ter vervanging van de Elecsys AMH assay.

#### Overzicht

<b>Systemen</b>	cobas e 411 cobas e 601 cobas e 602 cobas e 801 MODULAR ANALYTICS <E>	
<b>Reagentia</b>	Elecsys AMH – e411/e601/e602	ref. 06 331 076 190
<b>Calibratoren</b>	Elecsys AMH – e801	ref. 07 026 714 190
<b>Controles</b>	Elecsys AMH CalSet	ref. 06 331 084 190
	PreciControl AMH	ref. 06 709 966 190
	Elecsys AMH Plus – e411/e601/e602	ref. 07 957 190 190
	Elecsys AMH Plus – e801	ref. 07 957 246 190
	Elecsys AMH Plus CalSet	ref. 07 957 203 190
	PreciControl AMH Plus	ref. 07 957 211 190

Roche introduceert een belangrijke uitbreiding van de intended use van haar Elecsys AMH assay: de Companion Diagnostics (CDx) claim in het kader van IVF-behandeling voor de 'bepaling van de individuele dagelijkse dosis follitropin delta van Ferring' (Andersen et al., Fertil Steril. 2017 Feb;107(2):387-396.e4).

Deze CDx claim en samenwerking met Ferring vereiste een bijkomende registratie door de autoriteiten zodat de Elecsys AMH plus als nieuwe assay geregistreerd en gelanceerd werd.

**De huidige AMH assay zal uiterlijk juni 2017 volledig vervangen worden door de Elecsys AMH plus assay.**

## 1) Overzicht van de wijzigingen

Inzake formulering, productie en standaardisatie, referentiewaarden, alsook prijs zijn de Elecsys AMH en Elecsys AMH plus assays identieke producten.

Het verschil tussen beide assays omvat:

- 1) de bijkomende indicatie zoals vermeld in de bijsluiter van de Elecsys AMH plus assay: 'bepaling van de individuele dagelijkse dosis follitropin delta van Ferring'.
- 2) De Elecsys AMH plus bijsluiter vermeldt dat het AMH-resultaat bij gebruik voor de dosering van follitropin delta van Ferring, vergezeld moet worden van volgend statement: 'Deze AMH waarde in pmol/L werd bekomen met de Elecsys AMH plus assay en is geschikt voor de geïndividualiseerde dosering van follitropin delta van Ferring'.
- 3) Bijkomende aandacht is vereist om ervoor te zorgen dat de accuraatheid en precisie van de AMH plus test binnen de QC-limieten blijft
  - Target ranges van de AMH Plus controles
  - Interrun variatie = < 8% CV
  - Bias < +/- 12%
  - Total error < +/- 25%

## 2) Praktische informatie

Onderstaand vindt u een vergelijking van de Elecsys AMH en AMH plus assays:

Elecsys AMH	
06 331 076 190 07 026 714 490	Elecsys AMH e411/e601/e602 e801
06 331 084 190	Elecsys AMH CalSet
06 709 966 190	PreciControl AMH

Elecsys AMH plus	
07 957 190 190 07 957 246 190	Elecsys AMH Plus e411/e601/e602 e801
07 957 203 190	Elecsys AMH Plus CalSet
07 957 211 190	PreciControl AMH Plus
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.'	

<p><u>Intended Use</u></p> <p>The determination of AMH is used for the assessment of ovarian reserve and the prediction of response to controlled ovarian stimulation (COS) in conjunction with other clinical and laboratory findings.</p>	<p><u>Intended Use</u></p> <p>The determination of AMH is used for the assessment of ovarian reserve and the prediction of response to controlled ovarian stimulation (COS) in conjunction with other clinical and laboratory findings.</p> <p>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 COS for the development of multiple follicles in women undergoing an assisted reproductive technology program.</p>
<p><u>Test principle</u></p> <p>Identical</p>	<p><u>Test principle</u></p> <p>Identical</p>
<p><u>Reagents - working solutions</u></p> <p>Identical</p> <p>The reagent RackPack is labelled AMH</p>	<p><u>Reagents - working solutions</u></p> <p>Identical</p> <p>The reagent RackPack is labelled AMHP</p>
<p><u>Calibration</u></p> <p>Identical</p>	<p><u>Calibration</u></p> <p>Identical</p>
<p><u>Quality Control</u></p> <p>For quality control, use PreciControl AMH. In addition, other suitable quality control material may be used.</p> <p>Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.</p> <p>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.</p> <p>Follow the applicable government regulations and local guidelines for quality control.</p>	<p><u>Quality Control</u></p> <p>For quality control, use PreciControl AMH Plus. Additional suitable quality control material may also be used in combination with PreciControl AMH Plus.</p> <p>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 values is within +/- 12%, the intermediate precision CV is ≤8% and the maximal total error is within +/- 25% (<math>TE = \text{bias} + [1.65 \times CV]</math>). It is recommended to use quality control rule software.</p> <p>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.</p> <p>Follow the applicable government regulations and local guidelines for quality control.</p>



**3) Bijlagen:**

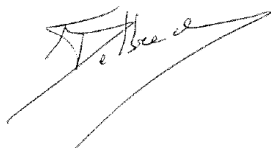
- Bijsluiter
- Fact Sheet

Wij danken u voor het vertrouwen dat u in ons stelt en blijven te uwer beschikking

Mocht u nog vragen hebben kunt u steeds terecht bij onze Regional Support

- op het nummer 02 247 45 16;
- via het e-mailadres: [vilvoorde.support\\_nl@roche.com](mailto:vilvoorde.support_nl@roche.com).

Alle informatie vindt u eveneens op onze website [www.e-labdoc.be](http://www.e-labdoc.be).



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Professional Diagnostics

# Elecsys® AMH Plus

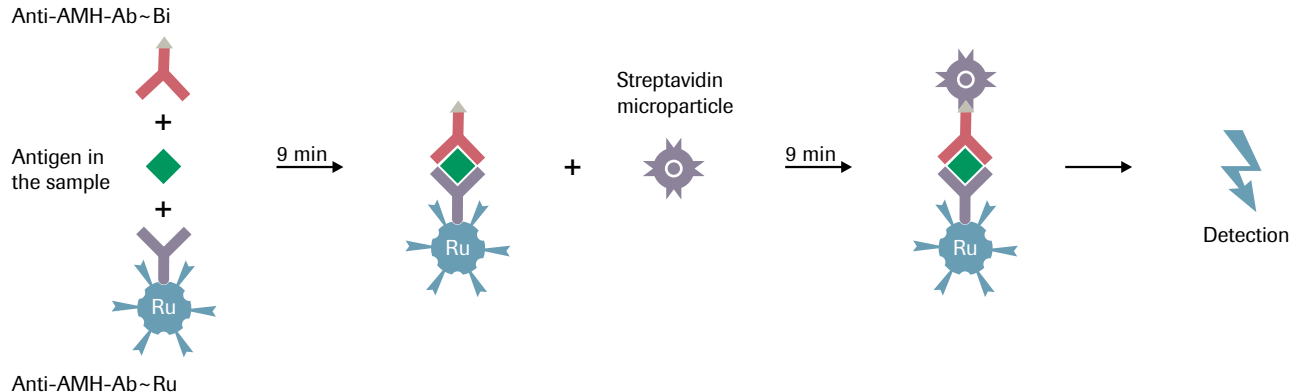
## *Electrochemiluminescence immunoassay (ECLIA) for the in vitro quantitative determination of anti-Müllerian Hormone in human serum and plasma*

### Indication

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. The Elecsys AMH Plus assay may also be used, in combination with body weight, to establish the individualised daily dose of the human recombinant follicle stimulating hormone (rFSH) follitropin delta of Ferring in women undergoing an assisted reproductive technology program.<sup>1</sup>

AMH plays a fundamental role in the regression of Müllerian ducts in male embryo and in its absence, Müllerian ducts develop into female inner reproductive organs.<sup>2</sup> In females, it is secreted by the granulosa cells of pre-antral and small antral ovarian follicles. AMH regulates follicle recruitment and growth of small ovarian follicles while preventing exhaustion of follicular pool.<sup>3,4</sup>

### Test principle: Sandwich principle



#### 1<sup>st</sup> Incubation (9 minutes):

50 µL of sample, a biotinylated monoclonal mammalian AMH-specific antibody and a monoclonal mammalian AMH-specific antibody labeled with a ruthenium complex react to form a sandwich complex.

#### 2<sup>nd</sup> Incubation (9 minutes):

After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

#### Measurement:

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. 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 reagent barcode.



Life needs answers

Elecsys® technology

ECL (ElectroChemiLuminescence) is a highly sensitive detection technology used with Roche immunoassays. Based on this technology and combined with well-designed, specific and sensitive immunoassays, Elecsys delivers reliable results. The development of ECL immunoassays is based on the use of a ruthenium-complex and tripropylamine (TPA). The chemiluminescence reaction for the detection of the reaction complex is initiated by applying a voltage to the sample solution resulting in a precisely controlled reaction. ECL technology can accommodate many immunoassay principles while providing superior performance.

Expected values

	N	2.5 <sup>th</sup> perc.		5 <sup>th</sup> perc.		Median		95 <sup>th</sup> perc.		97.5 <sup>th</sup> perc.	
		pmol/L	ng/mL	pmol/L	ng/mL	pmol/L	ng/mL	pmol/L	ng/mL	pmol/L	ng/mL
Healthy men											
	148	5.50	0.77	10.2	1.43	34.2	4.79	82.8	11.6	103	14.5
Healthy women (years)											
20 – 24	150	8.71	1.22	10.9	1.52	28.6	4.00	71.0	9.95	83.6	11.7
25 – 29	150	6.35	0.890	8.57	1.20	23.6	3.31	64.6	9.05	70.3	9.85
30 – 34	138	4.11	0.576	5.08	0.711	20.0	2.81	54.2	7.59	58.0	8.13
35 – 39	138	1.05	0.147	2.89	0.405	14.2	2.00	49.7	6.96	53.5	7.49
40 – 44	142	0.193	0.027	0.421	0.059	6.29	0.882	31.7	4.44	39.1	5.47
45 – 50	169	0.071	0.010	0.071	0.010	1.39	0.194	12.8	1.79	19.3	2.71
PCOS women**											
	149	13.3	1.86	17.2	2.41	48.6	6.81	122	17.1	135	18.9

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

\* LoB = Limit of Blank; LoD = Limit of Detection; LoQ = Limit of Quantitation (20 % total error)  
\*\* 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<sup>5</sup>

Order information

Elecsys AMH Plus	100 tests	07957190 190
Elecsys AMH Plus CalSet	4 x 1.0 mL	07957203 190
PreciControl AMH Plus	4 x 2.0 mL	07957211 190
Universal Diluent 2	2 x 36 mL	05192943 190

References

1 Anti-Müllerian Hormone. Elecsys and cobas e analyzers package insert 2016.  
2 La Marca, A., Broekmans, F.J. (2009). *Hum Reprod*; 24(9):2264-75.  
3 Visser, J.A., Themmen, A.P. (2005). *Mol Cell Endocrinol*; 234(1-2):81-6.  
4 La Marca, A., Volpe, A. (2007). *Hum Reprod Update*; 13(3):265-73.  
5 Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group (2004). Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod*; 19(1): 41-47.

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# Elecsys AMH Plus

cobas®

REF		SYSTEM
07957190 190	100	MODULAR ANALYTICS E170 cobas e 411 cobas e 601 cobas e 602

## English

### System information

For **cobas e 411** analyzer: test number 1590  
For MODULAR ANALYTICS E170, **cobas e 601** and **cobas e 602** analyzers: Application Code Number 472

### 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 electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

### Summary

The anti-Müllerian hormone is a homodimeric glycoprotein belonging to the transforming growth factor  $\beta$  (TGF  $\beta$ ) 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  $\beta$  family members, AMH is thought to require the N-terminal domain to potentiate activity of the C-terminal domain to attain full bioactivity.<sup>1,2</sup>

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.<sup>2</sup>

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.<sup>3</sup>

In females AMH plays an important role in the ovarian folliculogenesis.<sup>4</sup> 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 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.<sup>5,6</sup>

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.<sup>7,8</sup> Serum AMH levels have been shown to be relatively stable during the menstrual cycle with substantial fluctuations being observed in younger women.<sup>9,10,11</sup> AMH levels further demonstrate lower intra- and inter-cyclic variation than baseline FSH.<sup>10</sup> Serum AMH levels decrease significantly during the use of combined contraceptives.<sup>12</sup> Clinical applications of AMH measurements have been proposed for a variety of indications.<sup>13,14,15</sup> 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.<sup>13,15,16</sup> Further clinical applications of AMH are diagnosis of disorders of sex development in children<sup>17,18</sup> and monitoring of granulosa cell tumors to detect residual or recurrent disease.<sup>19,20</sup> AMH has been suggested as a surrogate biomarker for AFC in the diagnosis of polycystic ovary syndrome (PCOS)<sup>21,22</sup> and for the prediction of time to menopause.<sup>23,24</sup>

### Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 50  $\mu$ L of sample, a biotinylated monoclonal AMH-specific antibody, and a monoclonal AMH-specific antibody labeled with a ruthenium complex<sup>a)</sup> 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/ProCell 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 reagent barcode or e-barcode.

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

### Reagents - working solutions

The reagent rackpack is labeled as AMHP.

- M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:  
Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1 Anti-AMH-Ab-biotin (gray cap), 1 bottle, 8 mL:  
Biotinylated monoclonal anti-AMH antibody (mouse) 1.0 mg/L, phosphate buffer 50 mmol/L, pH 7.5; preservative.
- R2 Anti-AMH-Ab- $\text{Ru}(\text{bpy})_3^{2+}$  (black cap), 1 bottle, 8 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.

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



# Elecsys AMH Plus



## 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 read in from the respective reagent barcodes.

## Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **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
after opening at 2-8 °C	12 weeks
on the analyzers	8 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  $\pm 30\%$  of serum value  $\geq 3.57$  pmol/L ( $\geq 0.5$  ng/mL); recovery within  $\pm 1.43$  pmol/L ( $\pm 0.2$  ng/mL) for serum value  $< 3.57$  pmol/L ( $< 0.5$  ng/mL) and slope of 0.9-1.1 + intercept within  $\pm 0.7$  pmol/L ( $\pm 0.1$  ng/mL) + coefficient of correlation  $\geq 0.95$ .

Stable for 3 days at 20-25 °C, 5 days at 2-8 °C, 6 months at -20 °C ( $\pm 5$  °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, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls 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 mL
- [REF] 07957211190, PreciControl AMH Plus, for 2 x 2 mL each of PreciControl AMH Plus 1 and 2
- [REF] 05192943190, Diluent Universal 2, 2 x 36 mL sample diluent or [REF] 11732277122, Diluent Universal, 2 x 16 mL sample diluent or [REF] 03183971122, Diluent Universal, 2 x 36 mL sample diluent

- General laboratory equipment
- MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for **cobas e** 411 analyzer:

- [REF] 11662988122, ProCell, 6 x 380 mL system buffer
- [REF] 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- [REF] 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- [REF] 11933159001, Adapter for SysClean
- [REF] 11706802001, AssayCup, 60 x 60 reaction cups
- [REF] 11706799001, AssayTip, 30 x 120 pipette tips
- [REF] 11800507001, Clean-Liner

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- [REF] 04880340190, ProCell M, 2 x 2 L system buffer
- [REF] 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- [REF] 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- [REF] 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- [REF] 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- [REF] 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- [REF] 03023150001, WasteLiner, waste bags
- [REF] 03027651001, SysClean Adapter M

Accessories for all analyzers:

- [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. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers (except for the **cobas e** 602 analyzer).

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

## Calibration

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

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. 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 reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit 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 reagent kit, 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  $\pm 12\%$ , the intermediate precision CV is  $\leq 8\%$  and the maximal total error is within  $\pm 25\%$  ( $TE = |\text{bias}| + 1.65 \cdot CV$ ). 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.

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

### 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 assay is unaffected by icterus (bilirubin  $\leq 1129 \mu\text{mol/L}$  or  $\leq 66 \text{ mg/dL}$ ), hemolysis (Hb  $\leq 0.621 \text{ mmol/L}$  or  $\leq 1.0 \text{ g/dL}$ ), lipemia (Intralipid  $\leq 1000 \text{ mg/dL}$ ), biotin ( $\leq 143 \text{ nmol/L}$  or  $\leq 30 \text{ ng/mL}$ ), IgG  $\leq 2.5 \text{ g/dL}$ , IgA  $\leq 1.8 \text{ g/dL}$  and IgM  $\leq 0.5 \text{ g/dL}$ .

Criterion: Recovery within  $\pm 10\%$  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).

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	150
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

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 = 0.21 pmol/L (0.030 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 95<sup>th</sup> percentile value from  $n \geq 60$  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  $\leq 20\%$ .

## Dilution

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

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

After dilution by the analyzers, 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	2.5 <sup>th</sup> perc. ng/mL (95 % CI <sup>b</sup> )	5 <sup>th</sup> perc. ng/mL (95 % CI)	Median ng/mL (95 % CI)	95 <sup>th</sup> perc. ng/mL (95 % CI)	97.5 <sup>th</sup> 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)
• 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

# Elecsys AMH Plus

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	N	2.5 <sup>th</sup> perc. pmol/L (95 % CI)	5 <sup>th</sup> perc. pmol/L (95 % CI)	Median pmol/L (95 % CI)	95 <sup>th</sup> perc. pmol/L (95 % CI)	97.5 <sup>th</sup> 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.<sup>25</sup>

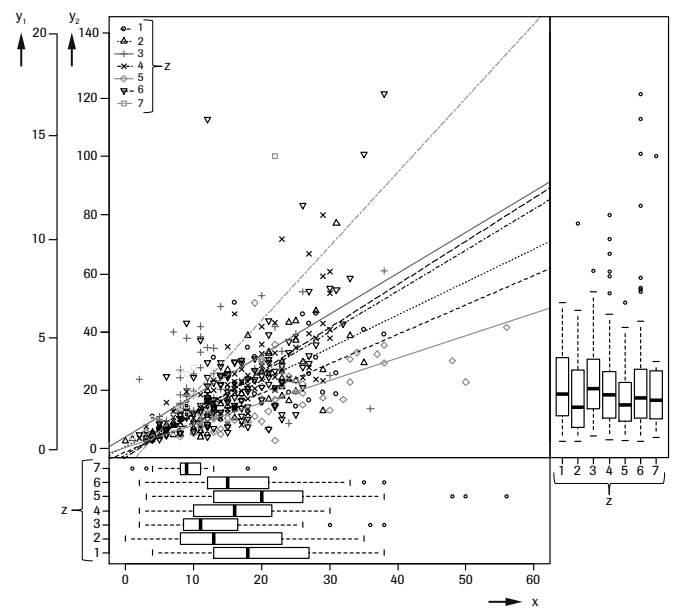
Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

## 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<sub>1</sub>: AMH (ng/mL)

y<sub>2</sub>: AMH (pmol/L)

z: Site

## Agreement table on absolute AFC numbers of 7 and 15

Three AFC groups were defined<sup>26,27</sup> 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

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

# Elecsys AMH Plus



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.<sup>28,29</sup> 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.<sup>29,30,31</sup> 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 in 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.<sup>32</sup> 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.<sup>33</sup>

## Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

## Precision

Precision was determined using Elecsys reagents, samples and controls in a protocol (EP5-A2) 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:

cobas e 411 analyzer					
		Repeatability		Intermediate precision	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	0.232	0.004	1.8	0.010	4.4
Human serum 2	0.705	0.008	1.1	0.028	4.0

cobas e 411 analyzer					
		Repeatability		Intermediate precision	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 3	2.44	0.030	1.2	0.082	3.3
Human serum 4	12.3	0.132	1.1	0.449	3.7
Human serum 5	18.8	0.287	1.5	0.711	3.8
PreciControl AMH 1	1.15	0.011	1.0	0.033	2.9
PreciControl AMH 2	5.68	0.057	1.0	0.214	3.8

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers					
		Repeatability		Intermediate precision	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	0.248	0.004	1.7	0.008	3.2
Human serum 2	0.745	0.009	1.2	0.020	2.7
Human serum 3	2.55	0.028	1.1	0.070	2.7
Human serum 4	13.0	0.165	1.3	0.397	3.0
Human serum 5	19.6	0.188	1.0	0.650	3.3
PreciControl AMH 1	1.19	0.014	1.2	0.042	3.5
PreciControl AMH 2	5.89	0.055	0.9	0.200	3.4

cobas e 411 analyzer					
		Repeatability		Intermediate precision	
Sample	Mean pmol/L	SD pmol/L	CV %	SD pmol/L	CV %
Human serum 1	1.66	0.030	1.8	0.072	4.4
Human serum 2	5.03	0.054	1.1	0.200	4.0
Human serum 3	17.4	0.213	1.2	0.584	3.3
Human serum 4	87.5	0.941	1.1	3.20	3.7
Human serum 5	134	2.05	1.5	5.08	3.8
PreciControl AMH 1	8.19	0.082	1.0	0.23	2.9
PreciControl AMH 2	40.5	0.405	1.0	1.53	3.8

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers					
		Repeatability		Intermediate precision	
Sample	Mean pmol/L	SD pmol/L	CV %	SD pmol/L	CV %
Human serum 1	1.77	0.030	1.7	0.057	3.2
Human serum 2	5.32	0.065	1.2	0.142	2.7
Human serum 3	18.2	0.197	1.1	0.497	2.7
Human serum 4	93.1	1.18	1.3	2.84	3.0
Human serum 5	140	1.34	1.0	4.64	3.3
PreciControl AMH 1	8.49	0.099	1.2	0.300	3.5
PreciControl AMH 2	42.0	0.392	0.9	1.43	3.4

## Method comparison

a) A comparison of the Elecsys AMH Plus assay (y) with a commercially available AMH assay (x) using clinical samples gave the following correlations (ng/mL):

Number of samples measured: 548

# Elecsys AMH Plus



Passing/Bablok<sup>34</sup>

$$y = 0.639x + 0.247$$

$$r = 0.919$$

The sample concentrations were between 0.046 and 16.5 ng/mL.

b) A comparison of the Elecsys AMH Plus assay (y) with a commercially available AMH assay (x) using clinical samples in the normal range up to 4 ng/mL gave the following correlations (ng/mL):

Number of samples measured: 340

Linear regression

$$y = 0.592x + 0.541$$

$$r = 0.976$$

Passing/Bablok<sup>34</sup>

$$y = 0.788x + 0.095$$

$$r = 0.884$$

The sample concentrations were between 0.046 and 3.99 ng/mL.

Linear regression

$$y = 0.720x + 0.206$$

$$r = 0.966$$

## 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. <sup>c)</sup>
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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- Applicable prescribing information of follitropin delta; Ferring.



REF			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 electrochemiluminescence immunoassay "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  $\beta$  (TGF  $\beta$ ) 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  $\beta$  family members, AMH is thought to require the N-terminal domain to potentiate activity of the C-terminal domain to attain full bioactivity.<sup>1,2</sup>

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.<sup>2</sup>

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.<sup>3</sup>

In females AMH plays an important role in the ovarian folliculogenesis.<sup>4</sup> 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 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.<sup>5,6</sup>

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.<sup>7,8</sup> Serum AMH levels have been shown to be relatively stable during the menstrual cycle with substantial fluctuations being observed in younger women.<sup>9,10,11</sup> AMH levels further demonstrate lower intra- and inter-cyclic variation than baseline FSH.<sup>10</sup> Serum AMH levels decrease significantly during the use of combined contraceptives.<sup>12</sup> Clinical applications of AMH measurements have been proposed for a variety of indications.<sup>13,14,15</sup> 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.<sup>13,15,16</sup> Further clinical applications of AMH are diagnosis of disorders of sex development in children<sup>17,18</sup> and monitoring of granulosa cell tumors to detect residual or recurrent disease.<sup>19,20</sup> AMH has been suggested as a surrogate biomarker for AFC in the diagnosis of polycystic ovary syndrome (PCOS)<sup>21,22</sup> and for the prediction of time to menopause.<sup>23,24</sup>

### Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 30  $\mu$ L of sample, a biotinylated monoclonal AMH-specific antibody, and a monoclonal AMH-specific antibody labeled with a ruthenium complex<sup>a)</sup> 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 ( $\text{Ru}(\text{bpy})_3^{2+}$ )

### 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~ $\text{Ru}(\text{bpy})_3^{2+}$ , 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.

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.

# Elecsys AMH Plus



Stability:	
unopened at 2-8 °C	up to the stated expiration date
on the <b>cobas e</b> 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  $\pm 30\%$  of serum value  $\geq 3.57$  pmol/L ( $\geq 0.5$  ng/mL); recovery within  $\pm 1.43$  pmol/L ( $\pm 0.2$  ng/mL) for serum value  $< 3.57$  pmol/L ( $< 0.5$  ng/mL) and slope of 0.9-1.1 + intercept within  $\pm 0.714$  pmol/L ( $\pm 0.1$  ng/mL) + coefficient of correlation  $\geq 0.95$ .

Stable for 3 hours at 20-25 °C, 5 days at 2-8 °C, 6 months at -20 °C ( $\pm 5$  °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, 45.2 mL sample diluent
- General laboratory equipment
- **cobas e** 801 analyzer

Accessories 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 Cups, 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). 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  $\pm 12\%$ , the intermediate precision CV is  $\leq 8\%$  and the maximal total error is within  $\pm 25\%$  ( $TE = |bias| + 1.65 \cdot CV$ ). 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.

## Calculation

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

Conversion factors:

$$\text{pmol/L} \times 0.14 = \text{ng/mL}$$

$$\text{ng/mL} \times 7.14 = \text{pmol/L}$$

## 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	$\leq 1129 \mu\text{mol/L}$ or $\leq 66 \text{ mg/dL}$
Hemoglobin	$\leq 0.62 \text{ mmol/L}$ or $\leq 1000 \text{ mg/dL}$
Intralipid	$\leq 1000 \text{ mg/dL}$
Biotin	$\leq 143 \text{ nmol/L}$ or $\leq 30 \text{ ng/mL}$
IgG	$\leq 2.5 \text{ g/dL}$
IgA	$\leq 1.8 \text{ g/dL}$
IgM	$\leq 0.5 \text{ g/dL}$

Criterion: Recovery within  $\pm 10\%$  of initial value.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e.  $> 5 \text{ mg/day}$ ) 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).



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## 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 95<sup>th</sup> percentile value from  $n \geq 60$  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  $\leq 20$  %.

## 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 analyzers, 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	2.5 <sup>th</sup> perc. ng/mL (95 % CI <sup>b)</sup> )	5 <sup>th</sup> perc. ng/mL (95 % CI)	Median ng/mL (95 % CI)	95 <sup>th</sup> perc. ng/mL (95 % CI)	97.5 <sup>th</sup> 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)
• 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	2.5 <sup>th</sup> perc. pmol/L (95 % CI)	5 <sup>th</sup> perc. pmol/L (95 % CI)	Median pmol/L (95 % CI)	95 <sup>th</sup> perc. pmol/L (95 % CI)	97.5 <sup>th</sup> 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)

	N	2.5 <sup>th</sup> perc. pmol/L (95 % CI)	5 <sup>th</sup> perc. pmol/L (95 % CI)	Median pmol/L (95 % CI)	95 <sup>th</sup> perc. pmol/L (95 % CI)	97.5 <sup>th</sup> perc. pmol/L (95 % CI)
• 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.<sup>25</sup>

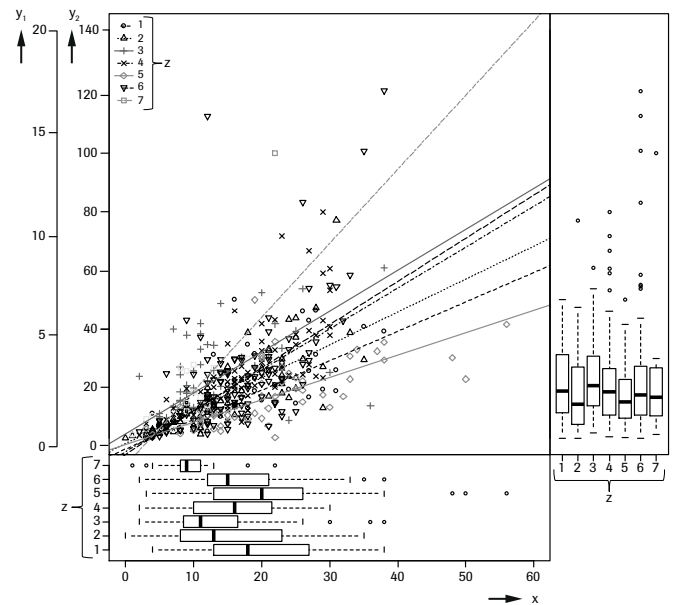
Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

## 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<sub>1</sub>: AMH (ng/mL)

y<sub>2</sub>: AMH (pmol/L)

z: Site

## Agreement table on absolute AFC numbers of 7 and 15

Three AFC groups were defined<sup>26,27</sup> 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

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

# Elecsys AMH Plus

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.<sup>28,29</sup> 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.<sup>29,30,31</sup> 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.<sup>32</sup> 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.<sup>33</sup>

## 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:

cobas e 801 analyzer					
		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

cobas e 801 analyzer					
		Repeatability		Intermediate precision	
Sample	Mean pmol/L	SD pmol/L	CV %	SD pmol/L	CV %
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

cobas e 801 analyzer					
		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 <sup>34</sup>	Linear regression
$y = 0.980x - 0.001$	$y = 0.951x + 0.100$
$r = 0.979$	$r = 0.999$

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. <sup>c)</sup>
Activin A	100 ng/mL	n. d.
LH	500 mIU/mL	n. d.
FSH	500 mIU/mL	n. d.

c) n. d. = not detectable

## References

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