



Analytical

Multicenter analytical performance evaluation of a fully automated anti-Müllerian hormone assay and reference interval determination



E. Anckaert^{a,*}, M. Öktem^b, A. Thies^c, M. Cohen-Bacrie^d, N.M.P. Daan^e, J. Schiettecatte^a, C. Müller^f, D. Topcu^b, A. Gröning^c, F. Ternaux^d, C. Engel^g, S. Engelmann^h, C. Milczynski^{f,1}

^a Laboratory of Hormonology and Tumour Markers, Universitair Ziekenhuis Brussel, Free University of Brussels (VUB), Brussels, Belgium

^b Duzen Laboratories Group, Tunus Cad. No. 95, Kavaklıdere, Ankara, Turkey

^c MVZ wagnerstibbe für Laboratoriumsmedizin and Pathologie GmbH, Georgstraße 50, 30159 Hannover, Germany

^d Laboratoire Eylau Saint Didier, 55, rue Saint Didier, 75116 Paris, France

^e Department of Reproductive Medicine and Gynecology, University Medical Center Utrecht, PO Box 85500, 3508 GA Utrecht, The Netherlands

^f Limbach Laboratory, Medical Care Center, Im Breitspiel 15, 69126 Heidelberg, Germany

^g Roche Diagnostics GmbH, Nonnenwald 2, 82377 Penzberg, Germany

^h Roche Diagnostics GmbH, Sandhofer Str. 116, 68305 Mannheim, Germany

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ABSTRACT

Objectives: Anti-Müllerian hormone (AMH) is an established biomarker for assessing ovarian reserve and predicting response to controlled ovarian stimulation. Its routine clinical use is hampered by the variability and low-throughput of available enzyme-linked immunosorbent assays (ELISA). The presented study examined if a fully automated AMH electrochemiluminescence assay (ECLIA; Elecsys® AMH assay, Roche Diagnostics) was suitable for measuring AMH levels in healthy women and in those diagnosed with polycystic ovary syndrome (PCOS).

Design and methods: Five European laboratories evaluated the Elecsys® AMH assay independently under routine conditions over eight months. Within-run imprecision, repeatability, intermediate precision, linearity and functional sensitivity were assessed. The Elecsys® AMH assay was compared to a manual ELISA microtiter plate format test (AMH Gen II ELISA, modified version; Beckman Coulter Inc.) using 1729 routine serum samples. AMH reference intervals were determined in 887 healthy women with regular menstrual cycle aged 20–50 years, and 149 women diagnosed with PCOS.

Results: The fully automated Elecsys® AMH assay showed excellent precision, linearity, and functional sensitivity. The coefficient of variation was 1.8% for repeatability and 4.4% for intermediate precision. Values measured with the Elecsys® AMH assay were highly correlated with the manual ELISA method (modified version) but 24–28% lower. Reference intervals showed the expected AMH decline with age in healthy women and increased AMH levels in women with PCOS.

Conclusions: The Elecsys® AMH assay demonstrated good precision under routine conditions, and is suitable for determining AMH levels in serum and lithium-heparin plasma.

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1. Introduction

Anti-Müllerian hormone (AMH) is a dimeric glycoprotein that belongs to the transforming growth factor β family [1]. In women, AMH is produced by ovarian granulosa cells of pre-antral and small antral follicles and is involved in the control of ovarian follicle growth

Abbreviations: AMH, anti-Müllerian hormone; COH, controlled ovarian hyperstimulation; CLSI-EP, Clinical and Laboratory Standards Institute - Evaluation Protocols; CV, coefficient of variation; ECLIA, electrochemiluminescence immunoassay; ELISA, enzyme-linked immunosorbent assay; FSH, follicle-stimulating hormone; PCOS, polycystic ovary syndrome; CI, confidence interval.

* Corresponding author.

E-mail address: ellen.anckaert@uzbrussel.be (E. Anckaert).

¹ Present address: ALAB Laboratories, 00-739 Warsaw, Poland.

through paracrine and autocrine effects [2–4]. Serum AMH is associated with ovarian response to controlled ovarian hyperstimulation (COH), and is also associated with gonadotropin dose adjustments [5]. AMH levels correlate with antral follicle count (AFC) and increasing evidence suggests that AMH is currently the best available test for ovarian reserve assessment [6,7]. Furthermore, AMH offers the convenience of simple blood sampling and AFC is prone to considerable operator-dependent variability [8]. A recent large prospective, non-randomized study suggested that an AMH-based treatment approach for COH may result in reduced treatment burden and reduced risk for hyper-response with maintained pregnancy rates [9]. Moreover, inter- and intra-cycle variations are low for AMH, reflecting the non-cyclic (follicle-stimulating hormone [FSH]-independent) growth of pre-antral and small antral follicles [10,11]. Therefore, serum AMH measurement is both a reliable

and a practical tool for ovarian reserve assessment. Accurate, straightforward and consistent serum AMH measurements are necessary for optimal assessment of the ovarian reserve.

The 2013 National Institute for Health and Care Excellence (NICE) clinical guideline for fertility and the European Society of Human Reproduction and Embryology (ESHRE) recommend AMH measurement as one method to predict ovarian response to gonadotropin stimulation [12,13]. The NICE guideline states that AMH concentration of ≤ 0.75 ng/ml (≤ 5.4 pmol/L) indicates a low ovarian response to stimulation, whereas AMH concentration ≥ 3.50 ng/ml (≥ 25.0 pmol/L) indicates a high response [12]. It follows that precise and accurate AMH measurement in these intervals is a prerequisite for reliable interpretation of AMH results in a clinical setting. However, limited assay reproducibility (precision) was observed with the AMH Gen II ELISA assay, unmodified version (Beckman Coulter) [14–16]. Beckman Coulter modified the assay to include a pre-dilution step to avoid interference from complement binding (AMH Gen II ELISA, modified version). Manual immunoassay techniques are labor-intensive, time-consuming and subject to the influence of handling techniques on results, making them less reproducible than fully automated assays. Therefore, there is a need for a precise and reliable fully automated AMH assay [6].

Recently, the first fully automated AMH electrochemiluminescent immunoassay ('ECLIA AMH', Elecsys® AMH assay, Roche Diagnostics) was developed and an initial analytical evaluation of the assay found no evidence for sample instability or variability [17].

Other studies on the Elecsys® AMH assay that included an analytical performance evaluation reported the intra-assay precision only [18] or linearity, within-run and intermediate precision based on a 10-day experiment [19] without specification of the immunoanalyzers used.

The aim of the present study was to perform a thorough analytical evaluation of the automated Elecsys® AMH assay with an assessment of within-run imprecision, 21-day repeatability and intermediate precision, linearity and functional sensitivity on **MODULAR ANALYTICS E 170**, **cobas e 411** and **cobas e 601** immunoanalyzers in a large multicenter study.

In addition, appropriate age-specific reference interval values were established for this assay in a large female population, as no international reference material for AMH is yet available. Women with regular menstrual cycles and women diagnosed with polycystic ovary syndrome (PCOS) were included in this study for reference interval determination. PCOS is the most common endocrinopathy among women of reproductive age and is characterized by a combination of menstrual cycle dysfunction, hyperandrogenism, and polycystic ovary morphology (≥ 12 antral follicles per whole ovary) [20,21]. An increased number of antral follicles is a prevailing element within PCOS, therefore these women were included in the current study to assess the Elecsys® AMH assay in this patient population.

2. Materials and methods

2.1. Investigational sites

The multicenter evaluation of the analytical performance of the Elecsys® AMH assay was conducted from May 2013 to December 2013 at five laboratories: UZ Brussels, Free University of Brussels (VUB), Belgium; Duzen Laboratories, Ankara, Turkey; Laboratoire Eylau, Paris, France; Limbach Laboratory, Heidelberg and MVZ wagnerstibbe für Laboratoriumsmedizin und Pathologie GmbH, Hannover, Germany. Each laboratory routinely used ELISA testing for quantification of AMH.

2.2. Study design

The study design of the multicenter evaluation comprised within-run precision and method comparison experiments conducted at five different laboratories on one **MODULAR ANALYTICS E 170**, three **cobas e 411** and two **cobas e 601** immunoanalyzers. In addition, at

two laboratories, repeatability and total imprecision according to CLSI EP5 protocol and testing of the functional sensitivity and linearity of the assay were performed on **MODULAR ANALYTICS E 170** ($n = 1$), **cobas e 411** ($n = 1$) and **cobas e 601** ($n = 1$) systems. Furthermore, reference intervals were determined on one **MODULAR ANALYTICS E 170**, **cobas e 411** and **cobas e 601** immunoanalyzers using two different AMH assay reagent lots.

Within the context of the performance evaluation of Elecsys® AMH assay a reference interval study was initiated in a population of apparently healthy women and in women diagnosed with PCOS. Reference intervals were determined in at least two independent runs using one **cobas e 411**, one **MODULAR ANALYTICS E 170** and one **cobas e 601** analyzer as the measurement devices and two different AMH reagent lots per instrument. Each run was calibrated with AMH CalSet and validated by a quality control measurement with PreciControl AMH 1 and 2, prior to each experimental run.

The Elecsys® AMH was calibrated using two calibration levels of AMH CalSet (Roche Diagnostics GmbH, Germany, catalog number 06331084 190). Each run was validated by measuring two levels of quality control material (Elecsys® PreciControl AMH 1 and 2 assay, Roche Diagnostics GmbH, Germany, catalog number 06709966 190) prior to starting the experiment. The assay was standardized to be traceable to AMH Gen II ELISA (Beckman Coulter Inc., USA, catalog number A79765/A79766, unmodified version).

Reference intervals were determined for PCOS subjects aging from 18 to 43 years. For reference interval determination of the apparently healthy female sample set including women from 20 to 50 years, six cohorts were established, each spanning an age range of five years. All sample sets were equally distributed among the three study instruments and measured in a randomized order.

2.3. Ethical approval

All investigation and sample collection sites followed International Conference on Harmonization guideline for Good Clinical Practice E6 and conducted the study in accordance with the Declaration of Helsinki (as amended in Tokyo, Venice, Hong Kong, and Edinburgh). Where required, Ethics Committee approval of the respective institutions was obtained. The reference interval study included samples only from subjects who signed an informed consent agreement.

2.4. Statistical methods

Measurements were captured using the WinCAEv software [23].

Reference intervals were determined in accordance with the International Federation of Clinical Chemistry recommendations [22]. For sample cohorts 2.5%, 5%, 95% and 97.5% non-parametric percentiles were calculated (with 95% CI according to the method of Hahn and Meeker [24]). No outlier detection method was used when analyzing the data. All statistical analyses were performed using R Version 3.0.1.

2.5. Assay procedure

The fully automated AMH assay is an electrochemiluminescence immunoassay for use on Elecsys® and **cobas e** immunoassay analyzers (manufactured by Roche Diagnostics GmbH, Germany, catalog number 06331076 190) [17].

The test design corresponds to a sandwich immunoassay, based on the streptavidin-biotin-technology. The capture antibody is biotinylated, the detection antibody is covalently linked with a Ruthenium complex. Successfully formed antigen-antibody complexes can be detected via electrochemiluminescence within a total assay time of 18 min.

The fully automated Elecsys® immunoassay detects AMH in the range of 0.01 to 23 ng/ml (0.07 to 164 pmol/L) and requires 50 μ l of serum or lithium-heparin plasma.

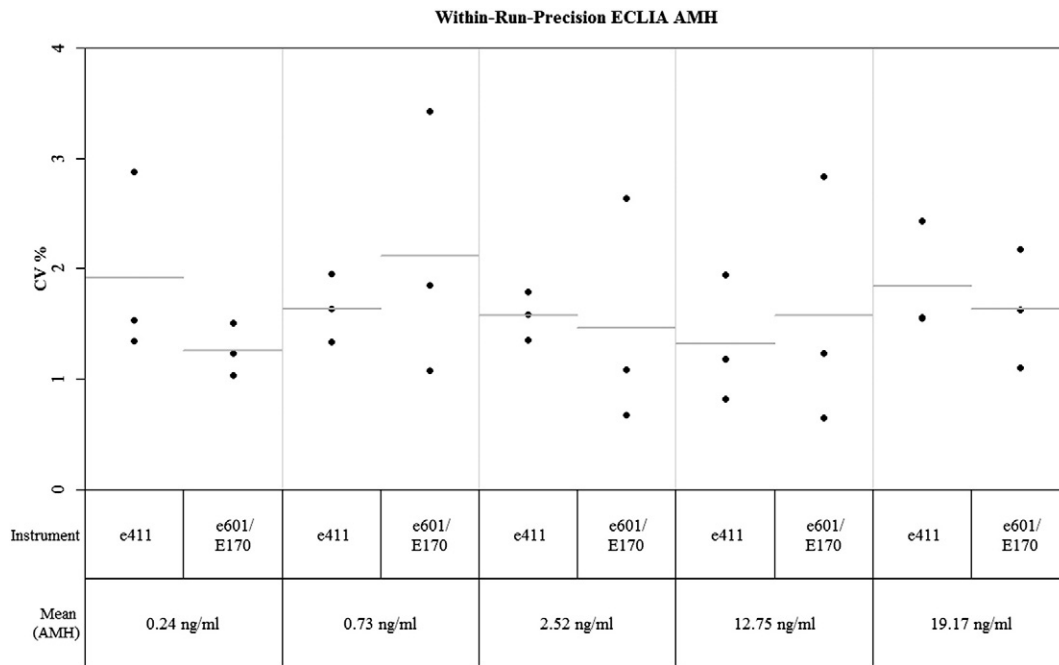


Fig. 1. Within-run precision CV for spiked serum sample material at five levels covering the entire measuring range. Each sample level was analyzed 21 times in a single analytical run on a MODULAR ANALYTICS E 170, cobas e 411 and cobas e 601 systems. The gray bar indicates the mean CV among all instruments.

2.6. Samples

All study sites evaluated the Elecsys® AMH performance independently using male fetal bovine spiked serum samples and routine serum samples. Left-over routine serum samples for the method comparison experiment were aliquoted and stored at -20 °C. Spiked serum sample material was stored at -20 °C until measurement. Samples were thawed shortly before measurement. For long term storage of reference interval

samples, aliquots of PCOS samples were kept frozen at -20 °C, healthy subject samples at -80 °C for less than two years.

2.7. Within-run imprecision

The study sponsor provided each laboratory with spiked serum sample material in five levels covering the entire measuring interval. Each sample level was analyzed 21 times in one analytical run. Mean,

Table 1

Repeatability and intermediate precision CV for five spiked serum samples and two quality control sample materials (PeciControl AMH 1 and 2) at levels covering the entire measuring range. Repeatability and intermediate precision were determined over 21 days measuring each sample in duplicate during two runs per day (n = 84) according to CLSI EP5-A2 protocol on the MODULAR ANALYTICS E 170 analyzer, cobas e 411 and cobas e 601 systems.

Instrument	Mean (ng/mL)	Mean (pmol/L)	Repeatability CV (%)	Intermediate precision CV (%)
cobas e 601			1.22	1.80
cobas e 411	0.24	1.72	1.79	4.37
MODULAR ANALYTICS E 170			1.68	3.24
cobas e 601			1.04	1.78
cobas e 411	0.72	5.14	1.07	3.97
MODULAR ANALYTICS E 170			1.23	2.67
cobas e 601			1.09	2.15
cobas e 411	1.14	8.14	1.00	2.87
MODULAR ANALYTICS E 170			1.17	3.53
cobas e 601			0.92	1.59
cobas e 411	2.47	17.63	1.22	3.35
MODULAR ANALYTICS E 170			1.08	2.73
cobas e 601			1.10	2.23
cobas e 411	5.70	40.70	1.00	3.77
MODULAR ANALYTICS E 170			0.93	3.40
cobas e 601			1.07	1.90
cobas e 411	12.58	89.82	1.07	3.66
MODULAR ANALYTICS E 170			1.26	3.05
cobas e 601			1.43	1.83
cobas e 411	19.04	136	1.53	3.79
MODULAR ANALYTICS E 170			0.96	3.32

Linearity for e601

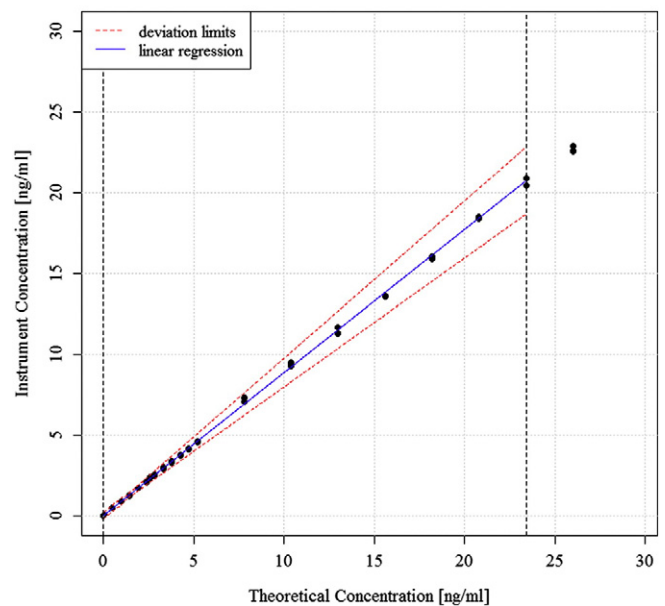


Fig. 2. Linearity experiment for the cobas e 601 system according to CLSI EP6 guidelines. Twenty-three dilution series were prepared by mixing a high concentrated sample pool with a low concentrated sample pool. Three replicates of each dilution step were subsequently measured in one analytical run on each system and are displayed for each concentration. Observed values were plotted against the expected values.

standard deviation and the coefficient of variation (CV) were calculated from these data.

2.8. Repeatability and intermediate imprecision according to CLSI EP5-A2

The precision experiment was performed on **MODULAR ANALYTICS** E 170, **cobas e 411** and **cobas e 601** systems, respectively. Repeatability and intermediate imprecision were determined during 21 days measuring each sample material in two runs per day in duplicate measurement ($n = 84$) [25]. Repeatability and intermediate precision was calculated for each analyzer and sample based on a variance-components model taking repeatability, run-to-run and day-to-day variance components into account. Repeatability and intermediate imprecision were investigated using two levels of quality control material (PeciControl AMH 1 and 2) and five levels of spiked serum pool material covering the lower and upper measuring interval. The spiked serum material was provided to the sites in frozen aliquots.

2.9. Functional sensitivity

Functional sensitivity was assessed on **MODULAR ANALYTICS** E 170, **cobas e 411** and **cobas e 601** systems, respectively. Each laboratory pooled low concentration native AMH serum samples targeting five concentration ranges below 0.1 ng/ml (0.7 pmol/L). For 10 days, one aliquot per sample pool was measured in one analytical run. The mean of each serum pool level was then plotted against the CV and the data fitted to an exponential curve. The lowest analyte concentration that can be reproducibly measured with an inter-assay CV of $\leq 20\%$ was derived from the fitted equation.

2.10. Linearity

Linearity was assessed according to CLSI EP6 guidelines. Two laboratories collected native serum sample material in order to generate a high and a very low concentration AMH sample pool. Twenty-three dilutions were prepared by mixing the high concentration sample pool (exceeding the upper measuring range) with the low concentration sample pool (below measuring range). Three replicates of each dilution step were subsequently analyzed in one analytical run on one **MODULAR ANALYTICS** E 170, **cobas e 411** and **cobas e 601** systems each. Observed values were plotted against the expected values and a regression model was selected according to CLSI EP6-A [26].

2.11. Method comparison

Anonymized left-over serum samples from routine diagnostic testing were used for comparing Elecsys® AMH assay against AMH Gen II ELISA, modified version (Beckman Coulter Inc., USA, catalog number A79765, lot numbers 327449 and 325128).

The presented method comparison combines data measured at four laboratories using material from at least 250 subjects per site, with 1729 serum samples in total. Data were analyzed using both Passing-Bablok and weighted Deming regressions.

2.12. Reference interval groups

Samples from 887 female subjects were collected from self-reported and apparently healthy donors between 20 and 50 years, with regular menstrual cycles (length 21–35 days). Subjects with a BMI exceeding 30 and/or receiving hormone replacement therapy or using combined hormonal contraceptives were excluded from the study. Furthermore, subjects with infertility, gonadal disorder/dysfunction, diagnosed endometriosis, known previous or current endocrine or metabolic disorders were excluded. Samples of 149 PCOS-subjects aged 18–43 years were collected. PCOS was diagnosed according to the revised diagnostic criteria of PCOS defined by the Rotterdam

ESHRE/ASRM-sponsored PCOS consensus workshop group [20]. Exclusion criteria were hyperprolactinaemia (prolactin > 1.23 IU/L), thyroid dysfunction (thyroid-stimulating hormone outside of 0.35–5.0 mIU/L), previous gynecological surgery and baseline elevated serum FSH (FSH > 12 IU/L).

3. Results

3.1. Within-run imprecision

Within-run CV for AMH concentrations ranging between 0.24 and 19.2 ng/ml (1.71 to 136 pmol/L) are shown in Fig. 1. CV values ranged between 0.8 and 2.9% for the **cobas e 411** analyzers and between 0.7 and 3.4% for **cobas e 601/MODULAR ANALYTICS** E 170 analyzers.

3.2. Repeatability and intermediate precision according to CLSI EP5-A2

Precision results for AMH concentrations between 0.24 and 19.0 ng/ml (1.71 and 136 pmol/L) are shown in Table 1. The repeatability CV values for **cobas e 601**, **cobas e 411** and **MODULAR ANALYTICS** E 170 immunoanalyzers were respectively 1.2%, 1.8%, and 1.7%. Intermediate precision CV ranges for **cobas e 601**, **cobas e 411** and **MODULAR ANALYTICS** E 170 systems were respectively 2.2%, 4.4%, and 3.5%.

3.3. Functional sensitivity

Functional sensitivity is defined as the lowest analyte concentration that can be measured with an inter-assay CV of $\leq 20\%$. The lowest concentration sample in this experiment had a mean concentration of 0.021 ng/ml (0.15 pmol/L) on **cobas e 601**, 0.025 ng/ml (0.18 pmol/L) on **MODULAR ANALYTICS** E 170, and 0.026 ng/ml (0.19 pmol/L) on **cobas e 411** and the respective inter-assay CVs were 4.6%, 5.0%, and 4.0%. Therefore, the functional sensitivity of the assay is well below the limit of quantification of 0.03 ng/ml (0.21 pmol/L) provided by the manufacturer.

3.4. Linearity

Linearity data for the **cobas e 601** system are depicted in Fig. 2. In addition linearity data for the **cobas e 411** and **MODULAR ANALYTICS** E 170 systems are provided in Supplementary Fig. 1A and B. According to the selected CLSI EP6-A regression model, the Elecsys® AMH assay showed linear results in the measuring range of 0.01–23 ng/ml (0.07–164 pmol/L), the lowest value based on the limit of detection (LOD), with percentage deviations below 10% for all analyzers.

3.5. Method comparison

The Elecsys® AMH assay was compared to the AMH Gen II ELISA, modified version using 1729 serum samples. Passing-Bablok and weighted Deming regression analyses are shown for all laboratories and instruments combined in Fig. 3. Although linearity according to CLSI EP6 regression models were demonstrated for the Elecsys® AMH assay, the method comparison did not meet the definition of linearity over the entire measuring range. Therefore an analysis of both the entire measuring range up to 23 ng/ml (164 pmol/L, top) and an analysis for AMH values within the normal physiological range, up to 4 ng/ml (28.6 pmol/L, bottom), is presented. The Elecsys® AMH assay strongly correlates with the AMH Gen II ELISA modified assay (Spearman's correlation coefficient 0.987, Pearson's 0.976), but has a sample recovery of up to 24% less than the AMH Gen II ELISA for samples with concentration ≤ 4 ng/ml (≤ 28.6 pmol/L) (applying Passing-Bablok as regression model). Over the entire measuring range, the Elecsys® AMH measurements showed up to 28% lower sample concentrations as compared to the AMH Gen II ELISA modified version.

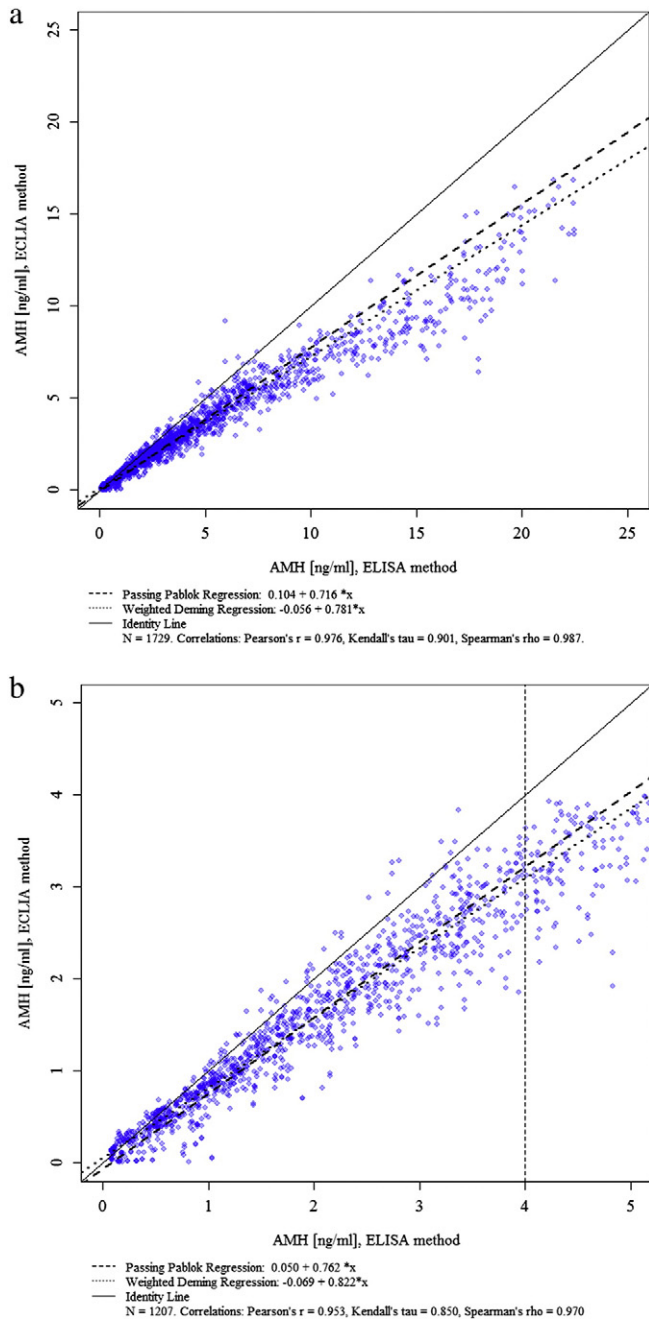


Fig. 3. Passing-Bablok and weighted Deming regression analysis between AMH values measured with the Elecsys® AMH assay and the Beckman AMH Gen II ELISA assay modified version for all laboratories and instruments combined: (A) for AMH values covering the whole measuring range (up to 23 ng/ml [164 pmol/L]) and (B) for AMH values within the normal physiological range, up to 4 ng/ml (28.6 pmol/L).

Due to its lower LOD of 0.01 ng/ml (0.07 pmol/L), the Elecsys® AMH assay could measure results in 86 samples (5.0% of all samples) for which AMH was undetectable using the AMH Gen II ELISA.

3.6. AMH values in healthy women and in polycystic ovary syndrome (PCOS) patients

AMH values measured in healthy women and in women diagnosed with PCOS are shown in Table 2 and Fig. 4. In healthy women, median AMH values declined with increasing age. In women aged 20–24 years, a median AMH concentration of 4.0 ng/ml (28.6 pmol/L) was observed and in women aged 35–39 years, the median AMH

concentration was 2.00 ng/ml (14.2 pmol/L). Median AMH values in women diagnosed with PCOS were higher compared to healthy women and did not markedly decline with increasing age. In women diagnosed with PCOS, a median of 7.19 ng/ml (51.3 pmol/L) was observed in subjects aged 20–24 years, and a median of 6.46 ng/ml (46.1 pmol/L) was observed in subjects aged 35–39 years.

Discussion

Several studies have shown that AMH is the best biomarker currently available for ovarian reserve assessment [6,27]. However, the reliability of commercially available AMH assays has been questioned, which is hampering the routine clinical use of AMH [14–16]. The results of the multicenter trial reported here demonstrate excellent analytical performance of the first fully automated Elecsys® AMH assay.

The AMH Gen II ELISA was reported to be influenced by pre-analytical instability [14], and in 2013 Beckman Coulter released an urgent Field Safety Notice (FSN-20434-3) stating that the AMH Gen II ELISA was susceptible to complement interference in freshly drawn and freshly frozen samples. This might have led to artificially lower AMH concentration results with a shift of up to 70%, depending on samples and sample storage conditions. Complement in freshly drawn samples may bind to the Fc portion of the assay capture antibody of the IgG2a subclass coating the assay wells. The magnitude of the interference by complement was reported to be dependent on sample storage conditions and pre-analytical dilution steps [28]. The assay was subsequently modified to include a pre-dilution step to eliminate the complement interference (AMH Gen II ELISA, modified version).

The Elecsys® AMH assay is based on the same monoclonal antibody pair as the AMH Gen II ELISA assay however with a different assay design and technology [17]. Capture and detection antibody bind preferentially to the mature and pro-region of AMH respectively [29], and detect AMH in human, bovine and other mammalian species. The capture antibody reacts with unbound AMH in solution, therefore the Elecsys® assay is not prone to interference by complement. In a performance evaluation study, the Elecsys® AMH assay was unaffected by sample instability or by different storage conditions for up to 7 days [17].

The analytical performance of the AMH Gen II ELISA was previously evaluated in a multicenter trial [30]. A functional sensitivity of 0.21 ng/ml (1.50 pmol/L) was observed, and within-batch and between-batch imprecision measurements assessed over the concentration range of 0.70–9.8 ng/ml (5.00–70.0 pmol/L) were respectively 5.3–11.4% and 3.8–17.3%. By comparison, in the present study the Elecsys® AMH assay has shown a functional sensitivity at least 7-fold more sensitive (≤ 0.03 ng/ml [≤ 0.21 pmol/L]), and CVs across a wider measuring range that are consistently lower ($CV \leq 1.8\%$ for repeatability, $\leq 4.4\%$ for intermediate precision). Furthermore, the Elecsys® AMH assay showed linearity across the entire measuring range applying CLSI EP6 regression models. Therefore, the Elecsys® AMH assay showed superior technical and inter-laboratory performance as compared to the AMH Gen II ELISA assay.

The increased sensitivity of the Elecsys® AMH assay allows the determination of AMH concentration in samples that are undetectable when using the AMH Gen II ELISA assay. Less sensitive AMH ELISA methods were previously shown to be of no value following orthotopic transplantation of ovarian tissue after gonadotoxic treatment [31]. The improved sensitivity of the Elecsys® AMH assay may thus represent an important step forward and open up new clinical applications.

Within the scope of this performance evaluation the Elecsys® AMH assay was compared to the AMH Gen II ELISA modified version using 1729 anonymized routine samples. AMH values measured by the Elecsys® AMH assay showed a strong correlation with the AMH Gen II ELISA modified assay, but had analyte recovery 24 to 28% lower than AMH Gen II ELISA modified version. This confirms method comparison results between Elecsys® AMH and AMH Gen II ELISA modified version (Passing-Bablok Regression $Y = 0.81x - 0.046$, with $n = 57$ and $r =$

Table 2
AMH values measured by the Elecsys® AMH assay in healthy women with regular menstrual cycles aged 20–50 years and in PCOS patients, aged 18–43 years. Median values and 2.5th, 5th, 95th and 97.5th percentiles (with 95% CIs) are displayed for each group.

Age (years)	N	2.5th percentile (95% CI)		5th percentile (95% CI)		Median (95% CI)		95th percentile (95% CI)		97.5th percentile (95% CI)	
		ng/ml	pmol/L	ng/ml	pmol/L	ng/ml	pmol/L	ng/ml	pmol/L	ng/ml	pmol/L
20–24	150	1.22 (0.478–1.67)	8.71 (3.41–11.9)	1.52 (0.758–1.810)	10.9 (5.41–12.9)	4.00 (3.60–4.44)	28.6 (25.7–31.7)	9.95 (7.87–13.60)	71.0 (56.2–97.1)	11.7 (9.11–15.70)	83.6 (65.0–112.0)
25–29	150	0.890 (0.493–1.21)	6.35 (3.52–8.64)	1.20 (0.797–1.750)	8.57 (5.69–12.5)	3.31 (3.00–3.89)	23.6 (21.4–27.8)	9.05 (7.59–10.30)	64.6 (54.2–73.5)	9.85 (8.91–11.30)	70.3 (63.6–81.0)
30–34	138	0.576 (0.256–0.958)	4.11 (1.83–6.84)	0.711 (0.256–1.120)	5.08 (1.83–8.00)	2.81 (2.35–3.47)	20.0 (16.8–24.8)	7.59 (6.84–9.52)	54.2 (48.8–68.0)	8.13 (7.27–9.72)	58.0 (51.9–69.4)
35–39	138	0.147 (0.053–0.474)	1.05 (0.378–3.38)	0.405 (0.053–0.496)	2.89 (0.378–3.54)	2.00 (1.73–2.36)	14.2 (12.4–16.9)	6.96 (5.31–9.37)	49.7 (37.9–66.9)	7.49 (6.49–10.90)	53.5 (46.3–77.9)
40–44	142	0.027 (0.010–0.063)	0.193 (0.071–0.450)	0.059 (0.017–0.119)	0.421 (0.121–0.850)	0.882 (0.726–1.130)	6.29 (5.18–8.07)	4.44 (2.94–5.56)	31.7 (21.0–39.7)	5.47 (3.92–6.76)	39.1 (28.0–48.3)
45–50	169	0.010 (0.010–0.010)	0.071 (0.071–0.071)	0.010 (0.010–0.010)	0.071 (0.071–0.071)	0.194 (0.144–0.269)	1.39 (1.03–1.92)	1.79 (1.43–2.99)	12.8 (10.2–21.3)	2.71 (1.79–4.16)	19.3 (12.8–29.7)
PCOS women	149	2.41 (1.67–3.01)	17.2 (11.9–21.5)	3.12 (2.29–3.77)	22.3 (16.4–26.9)	6.81 (6.30–7.42)	48.6 (45.0–53.0)	12.6 (11.5–17.1)	90.1 (82.3–122)	17.1 (13.3–20.3)	122 (95.0–145)

0.98) [32]. However, the observed differences are larger than those described by van Helden et al. (analyte recovery 12% lower than AMH Gen II modified version, with $n = 89$) [19], possibly due to the high between-laboratory variability previously described for the AMH Gen II ELISA assay [15].

The Elecsys® AMH assay was standardized to be traceable to AMH Gen II ELISA (Beckman Coulter Inc.), unmodified version. This was accomplished through sample value transfer from the AMH Gen II ELISA unmodified version, using frozen aged serum samples, presumed to be unaffected by complement interference, covering the entire measuring range [17]. However, this approach may be limited. Firstly, high between-laboratory variability has been previously described for the AMH Gen II ELISA assay [15]. Secondly, aged serum samples might still be affected by some degree of complement interference. It has been observed in several publications that the lack of an international AMH reference material makes comparison between assays difficult [6,9]. The variability between the two immunoassays reported here, despite attempts to harmonize the assays, further underscores the need for development of an international AMH standard and AMH assay standardization allowing the use of consensual AMH cut-offs.

Reference intervals were determined for Elecsys® AMH to address the need for an assay-specific AMH cut-off. In healthy, regularly cycling women, median AMH decreased from 4.00 ng/ml (28.6 pmol/L) in women aged 20–24 years to 0.194 ng/ml (1.39 pmol/L) in women aged 45–50 years. In women diagnosed with PCOS aged 18–43 years, median AMH values (6.81 ng/ml [48.6 pmol/L]) were elevated in each age cohort compared to the median AMH values in healthy women. Two- to four-fold increases in serum AMH have been described previously in PCOS patients compared to healthy patients [33–36] and we also observed an approximate two- to four-fold increase. Increasing evidence suggests that serum AMH measurement has a potential role in diagnosing PCOS, replacing polycystic ovary morphology assessment [37,38]. Moreover, AMH levels correlate with PCOS severity [34,39] and may predict therapeutic responses to clomiphene, or recombinant FSH.

In conclusion, this multicenter evaluation of the first fully automated Elecsys® AMH assay demonstrates excellent analytical performance. In addition, the AMH reference values determined in this study provide clinicians with age-dependent reference intervals in a population of apparently healthy women with regular menstrual cycles, and in women diagnosed with PCOS. In conjunction with other clinical and laboratory findings, the Elecsys® AMH assay is well-suited for the assessment of ovarian reserve.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.clinbiochem.2015.10.008>.

Authors' roles

The manuscript was drafted by EA. SE designed the study. NMPD contributed literature references and well characterized PCOS patient samples to the project. EA, MÖ, AG, MCB, CMi, CMü, SE and CE interpreted study data and critically discussed the study findings. JS, AT, DT, FT assayed the samples, performed the data analysis and interpretation of results. Statistical analysis was performed by CE. All authors revised the manuscript and approved the final version.

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Conflict of interest

MÖ and AG have received financial benefits for speaking from Roche. EA, AT, MCB, NMPD, JS, CMü, DT, FT and CMi have no conflict of interest in relation to this work. CE and SE are employed by Roche Diagnostics GmbH, which is a manufacturer of in vitro diagnostic products, including the current Elecsys® AMH assay.

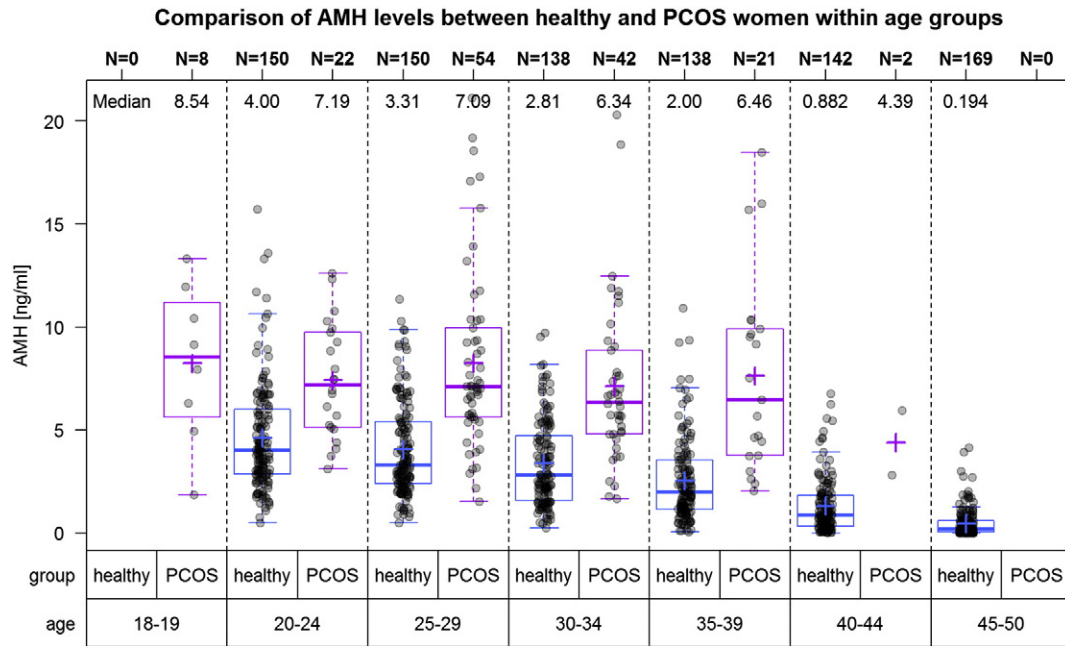


Fig. 4. AMH values measured by the Elecsys® AMH assay in apparently healthy women with regular menstrual cycle of different age groups and in PCOS patients, respectively.

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