Evaluation of a new automated immunoassay for the quantification of anti-Müllerian hormone

Manuela Lotierzo [a,b,*], Victor Urbain [a], Anne-Marie Dupuy [a], Jean-Paul Cristol [a,b]

[a] Département de Biochimie et Hormonologie, Centre de Ressources Biologiques, CHU de Montpellier, Montpellier, France
[b] PhyMedExp, Université de Montpellier, INSERM, CNRS, Département de Biochimie et Hormonologie, Centre de Ressources Biologiques, CHU de Montpellier, Montpellier, France

A B S T R A C T

Objectives: A newly developed fully automated Lumipulse G AMH method (Fujirebio Diagnostics) was recently introduced in clinical laboratories for quantitative determination of anti-Müllerian hormone (AMH) level in human serum or plasma. AMH has emerged as value-added biomarker in the assessment of ovarian reserve, in diagnosis of granulosa cells cancer and in the investigation of gonadal disorders. We compared Lumipulse G AMH assay performances with other methods largely applied for AMH measurements.

Design and Methods: The Lumipulse G AMH method based on two-step sandwich chemiluminescence enzyme immunoassay was assessed on Lumipulse G600II analyzer. The evaluation study included imprecisions, sensitivity and linearity whereas a comparison study was performed on a heterogeneous population of 114 patients by using the Elecsys AMH Plus assay on COBAS 8000 e602 module (Roche Diagnostics).

Results: Lumipulse G AMH system showed good repeatability (within-run imprecision) with CV values below 1% (0.5% and 0.9% for high and low serum pools). Similarly within-laboratory imprecision was assessed with CV values of 2.5% and 1.6% for high and low level controls respectively. A linearity regression formula of 1.0119x-0.067 with a coefficient of determination (r^2) equal to 0.999 was obtained in a range from 0.044 to 22.42 ng/ml. Passing-Bablok regression analysis was performed for assay comparability of AMH measurements. Results were closely correlated (correlation coefficient = 0.997) with a regression equation (y = 1.230x-0.025) showing a positive slope. Also, Bland-Altman analysis confirmed a good agreement between Lumipulse G AMH and Roche Elecsys AMH Plus assays with a bias of 17.76% in a large measurement range.

Conclusions: The performance of Lumipulse G AMH system was highly comparable with that of Roche Elecsys AMH Plus assay although approximately 10% higher values of AMH levels were observed for Lumipulse AMH system at all range of concentrations. Nevertheless the Lumipulse G system seems to be largely suitable for quantitative determination of AMH level in small-scale laboratory because of the reduced size and the use of single cartridge per test assuring flexibility and easy handling.

* Corresponding author. Département de Biochimie-Hormonologie, Hôpital Lapeyronie-CHRU Montpellier, 191, Avenue du Doyen Gaston Giraud, 34295, Montpellier, Cedex 5, France.
 *E-mail address:* m-lotierzo@chu-montpellier.fr (M. Lotierzo).

1. Introduction

Anti-Müllerian hormone (AMH) is a homodimeric glycoprotein belonging to the transforming growth factor β (TGF- β) family. During male embryonic development, AMH is secreted by Sertoli cells and it is responsible for regression of the Müllerian ducts. In female secretion is mainly secreted by granulosa cells and play an essential role in ovarian follicles regulation. Firstly, the pro-AMH dimer, composed of two identical 70 KDa monomers linked by disulfide bridges, is produced after glycosylation and dimerization of its precursor (pre-proAMH). To become active a proteolytic cleavage of both arms of the proAMH is necessary, producing an N-terminal pro-region (115 kDa AMHN dimer) and a C-terminal mature region (25 kDa AMHC dimer), associated in a non-covalent complex (AMHN,C). Both the proAMH and AMHN,C complexes circulate in approximately equal amount and are detected by the antibodies used in the AMH assays [1,2]. The most common clinical reason for AMH measurement is doubtless the assessment of ovarian reserve, notably in study design to improve ovarian stimulation strategy or to diagnose premature ovarian failure. Serum AMH levels have been shown to strongly correlate with the number of antral follicles (AFC) considered as the reference marker for the poor response predictor and the association of these two parameters may have some crucial advantages [3–5]. Moreover in women, AMH level is widely requested in the cancer of granulosa cells or to monitor reproductive potential undergoing gonadotoxic chemotherapy. In pre-pubertal boys, as a marker of Sertoli cell function, AMH level can be explored in association with genetics for suspicious of hypogonadism or Klinefelter's syndrome [6]. Robust and reliable methods of quantification are thus required. Commercial assays for the measurements of AMH have been available since the late 1990s, basically with the development of specific antibodies anti-AMH applied in the AMH enzymatic-linked immunoassays (ELISA). The societies Beckman Coulter and Diagnostic System Laboratories (DSL) developed two different ELISA kits which converged later into a second generation ELISA termed the Beckman Coulter Gen II AMH ELISA [7–9]. This second generation assay was modified with few optimizations in 2013 and simultaneously other ELISA kits were commercialized from
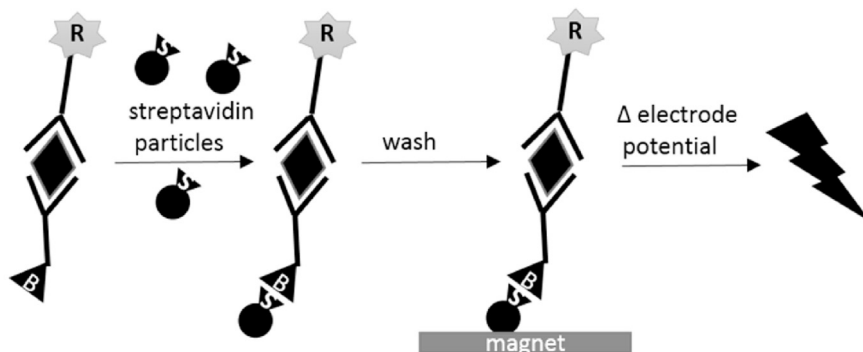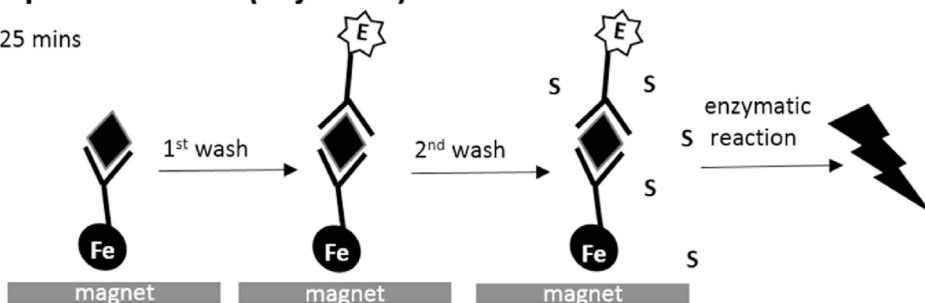

Fig. 1. Schematic representation of the two immunoassays.

AnshLabs, mainly increasing in sensitivity [10,11]. Since 2014, automated immunoassays appeared on the market, the most widely used of which are the Access AMH assay by Beckman Coulter and the Elecsys AMH Plus assay by Roche Diagnostics. Several studies have shown satisfactory analytical performances for both assays and good correlations with the modified AMH Gen II ELISA reference assay [12]. Nevertheless, the lack of international standard can still lead to differences in results. The aim of this work was to evaluate analytical performances of the newly developed Lumipulse G AMH assay by Fujirebio and to perform a comparative study between Lumipulse G AMH assay and Roche Elecsys AMH Plus assay, the first 3rd generation fully automated system, considered as reference method in our study.

2. Materials and methods

2.1. Principle of methods

Elecsys AMH Plus assay (Roche Diagnostics, Meylan France) is a one-step sandwich method based on electrochemiluminescence immunoassay (ECLIA) technology for the quantitative determination of AMH level in human serum or plasma. The Elecsys AMH Plus method is applied on COBAS e411 analyzer and on different COBAS modules (e601, e602 e801). As previously described, the immunoassay is based on a sandwich format between the analyte and the two antibodies: a biotinylated AMH-specific mouse monoclonal capture antibody (F2B/12H) and a second sulfo-ruthenium-labeled AMH mouse monoclonal antibody (F2B/7A). The antibodies are licenced from Beckman Coulter and are the same as the ones used in the AMH Gen II assay [12]. Results are determined via a lot-specific calibration curve which is generated specifically for the instrument by two-point recalibration with AMH CalSet and a master curve provided via the reagent barcode.

The Lumipulse G system consisted on Lumipulse G AMH assay on Lumipulse G600II analyzer (Fujirebio Diagnostics AB, Courteboeuf, France) It is a two-step sandwich immunoassay based on chemiluminescent enzyme immunoassay (CLEIA) technology for measuring of AMH concentrations in human serum and plasma. The Lumipulse G600II is a compact bench analyzer featuring a constant throughput of 60 tests per hour. The system uses a unique mono test cartridge concept. In this assay, the AMH antigen (calibrator or specimen) specifically binds to anti-AMH monoclonal antibody (mouse) coated on particles, and antigen-antibody immunocomplexes are formed. The particles are washed and rinsed to remove unbound materials. Alkaline phosphatase (ALP)-labeled anti-AMH monoclonal antibody (mouse) is added and specifically binds to the prior forms immunocomplexes on the particles, and additional immunocomplexes are formed. The two monoclonal antibodies are the same as those of the Roche assay (named A7 and A12) licensed by Beckman Coulter. After washing the particles, a substrate solution is added. Luminescence (at a maximum wavelengths of 477 nm) is generated by the cleavage reaction of dephosphorylated substrate. The luminescent signal reflects the amount of AMH present in the sample. No international standard existing, both methods use a recombinant AMH (standardized with AMH Gen II ELISA from Beckman Coulter).

A schematic representation of the two systems is presented in Fig. 1.

2.2. Precision verification study

Lumipulse G AMH assay on Lumipulse G600II analyzer was evaluated according to the CLSI (Clinical and Laboratory Standards Institute) EP15-A3 [13]. To determine repeatability (within-run imprecision), two levels of human serum pool were measured in 30 replicates for each level within the same run. Within-laboratory imprecision was determined using two levels of AMH controls (ref. 660–20, Fujirebio Diagnostics Inc.) five times a day during five following days.

2.3. Analytical performances

2.3.1. Sensitivity

The limit of blank (LoB) was calculated with a parametric option by using the mean and standard deviation (SD) of all blank results in the dataset according to the CLSI EP17-A2 standards [14]. Similarly calculation of the limit of detection (LoD) followed a parametric analysis by calculating the SD for each of the low level samples in the dataset and using the formula reported in the CLSI EP17-A2. The limit of quantification was the lowest concentration of analyte with a CV $\leq$ 20%.

2.3.2. Linearity

Linearity was determined by nine consecutive dilutions from a serum pool of 22.42 ng/ml to reach a final concentration of 0.044 ng/ml with adequate % of mean recovery. High samples pools were created using patients serum samples that contained naturally expressed AMH. Dilutions were performed by using low serum samples (post-menopausal women serum with non-detectable AMH). AMH measured values were plotted against expected AMH concentrations and linearity was determined using the polynomial regression method. The % of the mean recovery was also assessed [15].

2.4. Comparison study

The comparison study was evaluated by using serum samples from 114 patients (20 males and 94 females) admitted to the Endocrinology Departments of Lapeyronie University Hospital (Montpellier, France), covering the analytical range from 0.01 to 46 ng/ml according to routine results obtained from Roche Elecsys AMH Plus method on COBAS 8000 e602 analyzer. Samples with concentration superior to detection limit of 23 ng/ml were automatically diluted to ½ (universal diluent provided) as suggested by Roche.

Simultaneously, residual samples were analyzed with Lumipulse G AMH on Lumipulse G600II analyzer for method comparison with no need of supplementary sampling. The study was approved by local ethical committee and an informed consent was obtained for experimentation with human subjects. Passing-Bablok regression analysis was calculated to compare the results of Lumipulse G AMH assay versus those of Elecsys AMH Plus assay which is the method used in daily practice [16]. In addition, the Bland-Altman plots described agreement between the two quantitative measurements by using the mean and the percentage of differences [17,18]. For all comparisons, p-value < 0.05 was considered statistically significant. In the presence of discordant results between two methods, the samples were re-analyzed on the two instruments and the clinical record was checked. Statistical analyses were performed using XLSTAT® software, version 2016.06.35661 (NY, USA).

3. Results and discussion

3.1. Precision verification study

Repeatability was satisfactory with coefficient of variation (CV) values below 1% for both levels of the pools. Similarly for within-laboratory imprecision the CVs were 2.5% and 1.6% for high and low controls respectively, lower than that claimed by the manufacturer (Table 1).

3.2. Analytical performances

3.2.1. Sensitivity
The calculated LoB was = 0.005 ng/ml. LoD average value was = 0.01 ng/ml, almost the same as that reported by the two manufacturers (Table 1) as well as the calculated LoQ (0.02 ng/ml).

3.2.2. Linearity
Measurements from the Lumipulse system were correlated with expected concentrations with a linear regression formula: 1.0119x-0.067 and high coefficient of determination (r^2 = 0.999), showing highly acceptable linearity over the most clinically relevant range of AMH concentrations. A trend of gradual increase in recovery was obtained in the same range (0.044 ng/ml to 22.42 ng/ml) as presented in Table 2. The CUSUM test for both assays did not show significant deviation from linearity.

3.3. Comparison study

The AMH median of all patients (total n = 114) was comparable for Elecsys AMH Plus and Lumipulse G AMH (1.97 ng/ml and 2.29 ng/ml respectively) as well as the first (0.91 ng/ml versus 1.02 ng/ml) and third quartile (4.82 ng/ml versus 5.37 ng/ml), although there is a constant Lumipulse G AMH overestimation of the Elecsys AMH Plus results corresponding to approximately 10% for all range of concentrations. Passing-Bablok regression analysis was performed on both instruments for assay comparability of AMH measurements. As depicted in Fig. 2A, results were closely correlated (correlation coefficient = 0.997) with a regression equation (y = 1.227x-0.028) showing a positive slope. The Bland-Altman plot allowed us to evaluate an acceptable trend of differences with most of the points within the 95% of confidential interval (95% CI) for all range of concentrations. Precisely, good agreement between the two methods was assessed with a bias of 17.76% (Fig. 2B).

4. Conclusion

In 2014 third generation AMH assay started with automatization of two different kits: the Access AMH kit from Beckman Coulter and the Elecsys AMH kit from ROCHE. Both used the antibodies from the previous AMH Gen II kit and they developed a CLEIA and ECLIA

Table 1
Precision verification study and analytical performances of the Lumipulse G AMH.

		Our study			Fujirebio data (Lumipulse G AMH)			Roche data (Elecsys AMH Plus)		
		Mean, ng/ml	SD	% CV	Mean, ng/ml	SD	% CV	Mean, ng/ml	SD	% CV
Repeatability (Within-run imprecision)	High serum pool	14.75	0.08	0.5	9.06	0.08	0.9	13.0	0.16	1.3
	Low serum pool	1.08	0.01	0.9	1.89	0.02	1.1	0.75	0.01	1.2
Within-laboratory imprecision	High level control	17.08	0.42	2.5	22.43	0.30	1.3	5.89	0.20	3.4
	Low level control	1.22	0.02	1.6	1.90	–	0.2	1.19	0.04	3.5
LoB, ng/ml		0.005			0.005			0.007		
LoD, ng/ml		0.010			0.011			0.010		
LoQ, ng/ml		0.022			0.022			0.030		
Measuring interval, ng/ml		0.044–22.42			0.024–25			0.01–23		
Extended Measuring interval, ng/ml					0.024–60			0.01–46		

CV: Coefficient of Variation, SD: Standard Deviation, LoB: Limit of Blank; LoD: Limit of Detection, LoQ: Limit of Quantification.

Table 2
Linearity values and % of mean recovery of Lumipulse G AMH assay.

Theoretical values, ng/ml	Mean of observed values, ng/ml	% of mean recovery
22.42	22.64	99
11.21	11.28	99.4
5.61	5.9	100.2
2.80	2.62	107.2
1.40	1.32	106.4
0.70	0.64	110
0.35	0.32	111.2
0.17	0.15	116.8
0.09	0.07	120
0.04	0.04	121.6

Linear regression formula, r^2: 1.0119x-0.067, 0.999.
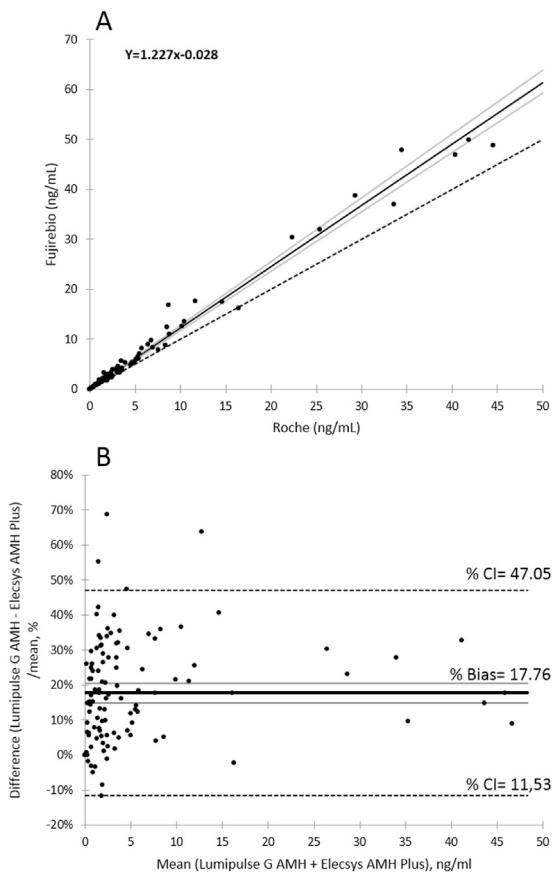
Fig. 2. (A) Passing-Bablok regression analysis against Lumipulse G AMH assay. (B) Bland-Altman analysis with bias of 17.76%.

technologies to adapt to the respective automated analyzers. Both systems allow large scale sample throughput, fast turnaround times, increased sensitivity and broader measuring ranges than previously commercial AMH assays. Now a new method is positioned basically in between the manual ELISA kits and the large scale automatization proposed by Roche Diagnostics and Beckman Coulter. In fact the Lumipulse G system is an easy-to-use benchtop analyzer suited for personalized workflow solutions within a time comparable to the other systems (25 min against 18 min of Roche system) and with highly comparable performances. The fact to have mono test cartridge allow more easily small series of analysis and with no risk of waste for expiry or contamination of the cartridge. This represents an advantage compared to the Access instrument (Beckman Coulter) which has got the classical system of test cartridge (50 tests/pack). At the moment Roche did not provide the AMH kit on small automated such as the COBAS 411 but only on larger COBAS clinical chemistry analyzers. Lumipulse G AMH assay on Lumipulse G600II analyzer exhibited equally good analytical performance compared to Roche Elecsys AMH Plus assay with lower analytical variability and close correlation with AMH Gen II ELISA, considered as the reference method [19]. Patient results were closely correlated between the two methods although Lumipulse G AMH assay globally overestimated the AMH results obtained with the Roche method at approximately 10%. This difference is probably due to the lack of an international

standard which could unify the calibration across different assay platforms. AMH is increasingly being used as a biochemical marker for the assessment of the growing ovarian pool and thus as a surrogate marker for the ovarian reserve in conjunction with other clinical and laboratory findings such as antral follicle count, before starting fertility therapy. Therefore references values have already been established with commonly used systems such as Access AMH and Elecsys AMH Plus, notably to distinguish between women with AFC values > 15 (high ovarian reserve) and women with AFC values ≤ 15 (normal or diminished ovarian reserve). In addition, important studies for paediatric reference intervals were recently carried on comparing data from the Roche Elecsys assay and the Beckman Coulter Access assay. Thus it is crucial to extend this kind of comparison studies with selected target populations also to the more recent automated Lumipulse G AMH method for AMH measurements.

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CRediT author contribution statement

Manuela Lotierzo: Writing - original draft preparation; conceptualization, methodology, software and editing; Victor Urbain: Data curation; Anne-Marie Dupuy: Writing manuscript, conceptualization, methodology and editing; Jean-Paul Cristol: Supervision.

Declaration of competing interest

None.

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