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Measurement of anti-Mullerian hormone: preliminary evaluation of an ABEI-based fully automated immunoassay

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ABSTRACT

Background

The added value of Anti-Müllerian hormone (AMH) measurement is recognized for several clinical applications such as assessment of the ovarian reserve, monitoring of *in vitro* fertilization protocol or in the field of oncofertility. Our study objective was to determine the performances of a novel fully automated chemiluminescent assay for AMH testing.

Methods

We evaluated the performances of the Maglumi® 800 AMH chemiluminescent immunoassay that applies N-(4-Aminobutyl)-N-ethylisoluminol (ABEI) labels. Assay imprecision was assessed with two levels of control materials. Method comparison was performed with an ultrasensitive AMH ELISA assay (Ansh Laboratories, Inc, Webster, TX, USA) with 88 patients' samples.

Results

The within-run and between-run coefficients of variation (CVs) were below 3% for both low and high internal quality controls. The automated and ELISA methods were significantly correlated. Bland-Altman plot evidenced a bias between the methods with a mean bias of 0.6 ng/mL.

Conclusions

Our preliminary evaluation showed overall good analytical performances for the Maglumi® AMH fully automated immunoassay and good concordance with a routinely used assay.

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INTRODUCTION

The Anti-Mullerian hormone (AMH) is a dimeric glycoprotein that belongs to the transforming growth factor-β (TGF-β) family and a key regulator of sexual differentiation and folliculogenesis (1,2). Measurement of AMH is helpful in several clinical situations (1–3). AMH is a widely used marker of functional ovarian reserve in the assessment and treatment of infertility (1,2). AMH testing also offers the advantage to detect ovarian reserve of those follicles that are not visible by ultrasound like small pre-antral follicles. Circulating levels of AMH help to establish patient profiles and predict ovarian response to stimulation in assisted reproduction techniques (1,2). AMH has recently been identified as an early predictor of ovarian follicle loss and menopause onset (1,2). AMH is also emerging in the field of oncofertility to understand the effects of different cytotoxic agents on ovarian function (4). Finally, AMH participates in the diagnosis of certain diseases such as granulosa cell tumors or Polycystic Ovary Syndrome (PCOS) (1–3,5).

Several AMH immunoassay methods are now commercially available ranging from manual Enzyme Linked Immunosorbent Assay (ELISA) methods to fully automated assays (2,6). According to the recognized clinical value of AMH testing, it is important to determine the performances of novel assays before their use in clinical practices.

The objective of our preliminary evaluation was to evaluate the performances of a novel chemiluminescent ABEI-based AMH automated immunoassay.

METHODS AND MATERIALS

We assessed the performances of the Maglumi® 800 (Snibe diagnostics, Shenzhen, China) AMH chemiluminescent immunoassay that applies ABEI labels. ABEI, *N*-(4-Aminobutyl)-*N*-ethylisoluminol, is a non-enzyme small molecule with a special molecular formula that enhances stability in acid and alkaline solutions. The chemical reaction process of ABEI using sodium hydroxide (NaOH) and hyperoxide (H_2O_2) finishes in three seconds (6). The lowest detection limit for this assay is 0.1 ng/mL.

The imprecision of the Maglumi® AMH was assessed by repeatedly measuring two different levels of Internal Quality Control (IQC). IQC at low concentration and IQC at high concentration were tested 3 times a day for 5 consecutive days according to the EP 15-A3:2014 protocol from CLSI guidelines. A method comparison was performed with ultrasensitive AMH quantitative three step ELISA method (Ansh Laboratories, Inc, Webster, TX, USA) by measuring 88 patients' serum samples (6). The limit of quantification for this assay is 0.06 ng/mL. Blood was taken by venipuncture from the antecubital vein and collected into dry serum tubes (S Monovette® 7.0 mL tubes, Sarstedt, Nümbrecht, Germany) and both methods were performed according to the manufacturer's specifications.

Data were analyzed with the Medcalc 7.2.1.0 package (Medcalc Software, Belgium). Passing and Bablock regression analysis was performed

for method comparison and Pearson's coefficient of correlation were calculated. Bland Altman plots were used to calculate the mean bias between methods.

RESULTS

Imprecision and accuracy

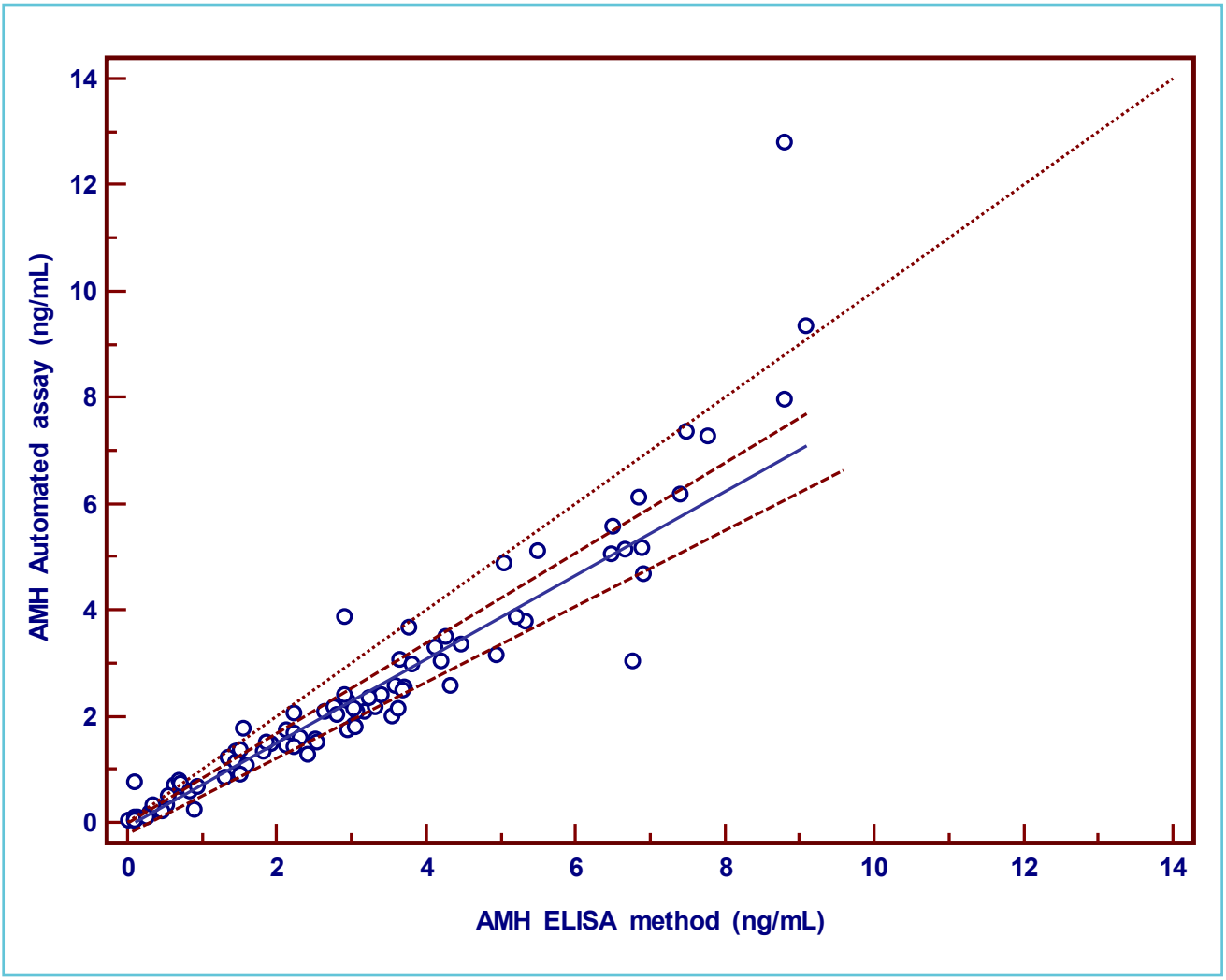
The within-run coefficients of variation (CVs) of the Maglumi® AMH assay were 2.2% and 1.4% for concentrations of 3.9 ng/mL and 15.9 ng/mL, respectively. For the same concentrations, the between-run CVs were 2.5% and 2.4%, respectively. Accuracy was also determined based on the targets IQC concentrations, and bias were 2.99% for the low IQC and 0.29% for the high IQC.

Comparison with the ELISA assay

The median AMH levels were 2.0 ng/mL (range: 0.1 – 12.8 ng/mL) with the Maglumi® assay and 2.9 ng/mL with the ELISA method (range: 0.1 – 9.1 ng/mL).

The correlation between both methods was good (r = 0.95, p<0.001). Passing-Bablok regression analysis showed a slope of 0.79 (95%

Figure 1 Passing and Bablok regression analysis between the automated and the ELISA AMH immunoassays



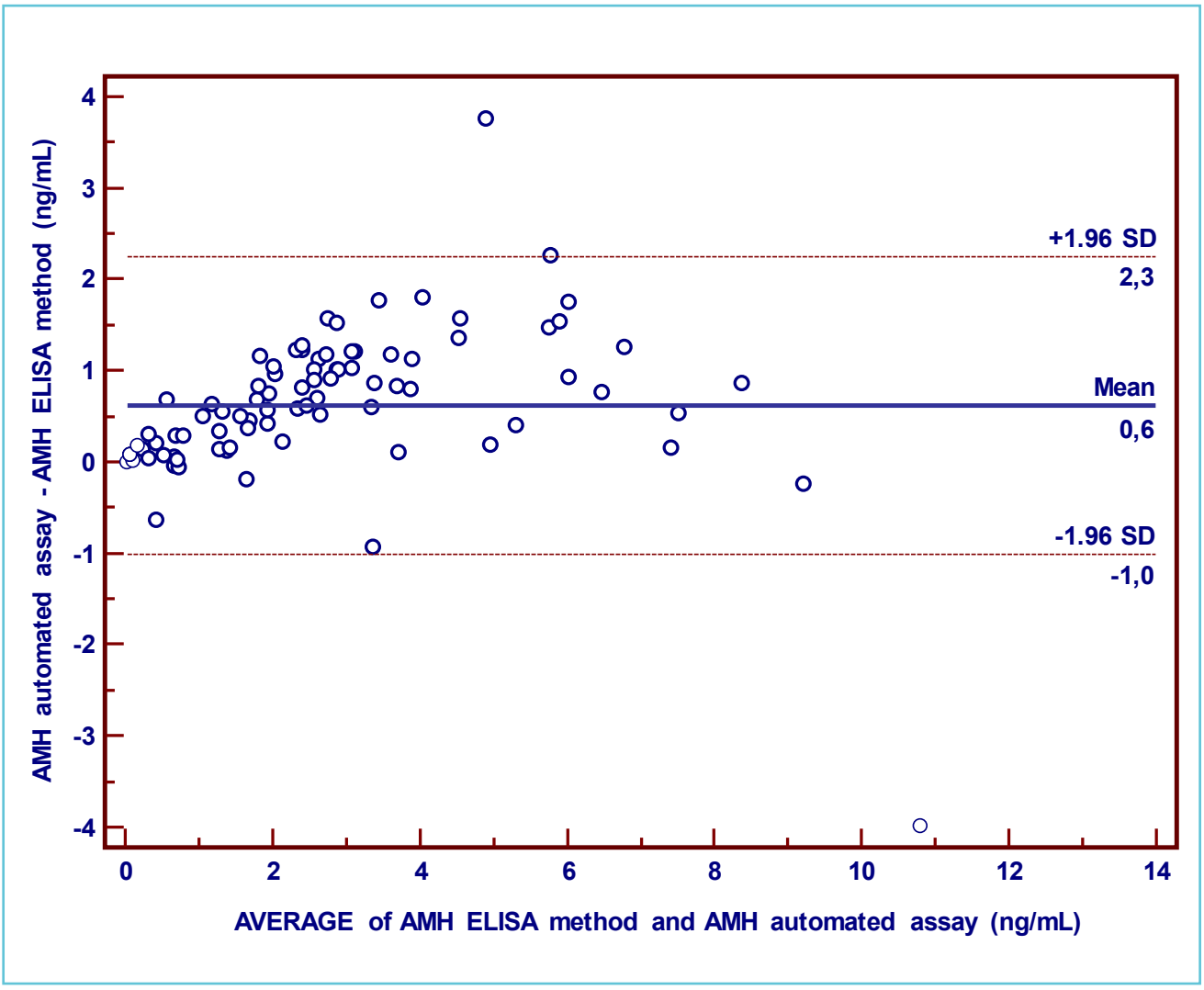
confidence interval (CI): 0.74 to 0.85) and an intercept of -0.07 (95% CI: -0.17 to -0.02) and no significant deviation from linearity (Figure 1).

The Bland Altman Plots revealed a mean difference of 0.6 ng/mL (95% CI: -1.0 to 2.3) between the two AMH immunoassays and a bias proportional to the concentration (figure 2). Looking at the lower AMH concentrations, 32 samples had concentrations below 2 ng/mL with the ELISA method. Eleven of these samples had AMH concentrations between 1 and 2 ng /mL with the ELISA. Two of these 11 had concentrations below 1 ng/mL (0.82 ng/mL and 0.88 ng/mL). Of the remaining 21 samples, 7 were with AMH concentrations below 0.1 ng/mL. Six of these samples were also below 0.1 ng/mL with the automated and the seventh one had a concentration of 0.74 ng/mL. We can conclude an overall good concordance between the two methods for lower AMH levels.

DISCUSSION

Our preliminary evaluation showed good analytical performances for the Maglumi® AMH ABEI-

Figure 2 **Bland Altman Plot between the automated and the ELISA AMH immunoassays**



based automated immunoassay, confirming that this method meets the expectations of clinical laboratories for use in routine practices.

AMH has emerged as value-added biomarker in clinical applications like the assessment of ovarian reserve, companion testing in in vitro fertilization, prediction of menopause, diagnosis of PCOS, serving as tumor marker for some cancers and monitoring the return of fertility in women with cancer treated with chemotherapy (1–5).

Because of this wide range of clinical indications, the measuring range of AMH assays needs to cover low concentrations in the case of low ovarian reserve but also high concentrations as in the case of PCOS. The imprecision of the assay should also be low to optimize monitoring of patients in the case of repeated measurements.

The advantage of automated immunoassay like the Maglumi® AMH method is the ability to integrate routine laboratory automated workflows and a faster delivery of results to the physicians for diagnosis purpose or monitoring of treatment efficiency. However, performance evaluation is necessary before routine diagnostic use. The analytical performance of the Maglumi® AMH ABEI based immunoassay was demonstrated by assessment of imprecision and by method comparison. Our results showed a very good precision of the ABEI based immunoassay with low CV for both low and high AMH concentrations. The coefficients of variation observed in our study agree with those reported in the literature for other automated immunoassays and was below 5% (8,9). Our study showed that Maglumi® AMH significantly correlated with a widely used ELISA assay with good overall agreement. A good concordance between the two methods was also observed for lower (below 2 ng/mL) AMH concentrations. However, a bias was observed with the Bland Altman analysis. This bias is not surprising, and is frequently reported in AMH methods comparison studies (6,8,10), and reflects the current lack of standardization that exists for AMH immunoassays (10). Differences are attributed to different assay formats, and also the different antibodies used by the manufacturers. The Ansh ELISA assay is based on a capture antibody specific to the pro region of AMH (Clone 39/6C) and a detection antibody specific for the mature region of AMH (Clone 39/30A), these are not used by other manufacturers. The antibodies used by Snibe for the Maglumi® AMH was not disclosed.

The commercially available AMH assays are, therefore, still not commutable and some important discrepancies have been also reported in the literature for low AMH concentrations (10). Clinical laboratories need therefore to establish specific reference limits for every individual assay to guide clinical decision-making. The recent development of a reference preparation by World Health Organization might help to standardize AMH immunoassays and to improve AMH measurement and interpretation (11).

CONCLUSION

Our preliminary evaluation showed overall good analytical performances of the Maglumi® AMH ABEI-based automated immunoassay. This assay offers an additional automated solution for AMH testing, a value based biomarker with increasing clinical applications in fertility medicine.

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Conflict of interest and funding

Reagents for the evaluation were kindly provided by Snibe diagnostics.

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