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Performance characteristics of the Access AMH assay for the quantitative determination of anti-Müllerian hormone (AMH) levels on the Access* family of automated immunoassay systems

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ABSTRACT

Objectives: Anti-Müllerian hormone (AMH) measurement is useful as an aid in the evaluation of ovarian reserve. In the past, its conventional use was restricted by the low-throughput and variability of existing manual AMH assays. We developed the automated Access AMH assay for the quantitative determination of AMH levels on the Access family of immunoassay systems. The analytical performance of this new assay was evaluated.

Design and methods: Sensitivity, dilution linearity, assay imprecision, AMH sample stability, lot-to-lot comparison and correlation with AMH Gen II assay (Beckman Coulter, Inc.) were evaluated. Reference intervals for Access AMH were established in healthy females, males, newborns (≤60 days) and pediatric males classified by Tanner stages.

Results: The limit of blank and limit of detection were below 0.0077 and 0.0098 ng/mL, respectively. The limit of quantitation was 0.010 ng/mL. The total imprecision ranged from 2.4 to 5.2%. Linearity was observed up to 24 ng/mL. Sample storage at room temperature up to 48 h, at 2–8 °C up to 7 days and at −20 °C up to 15 months had no impact on measured AMH. The correlation study gave a coefficient between 0.99 and 1 and a regression slope between 0.89 and 0.92. Excellent lot-to-lot comparability was observed on controls and patient samples with a maximum bias of 3.7% between 2.81 and 15.03 ng/mL.

Conclusions: The fully automated Access AMH immunoassay demonstrates excellent analytical performance. As a consequence, the availability of this assay will represent a robust, fast and precise alternative to manual AMH assay testing.

1. Introduction

Anti-Müllerian hormone (AMH) is a member of the transforming growth factor-β family. AMH is a glycoprotein, which circulates as a dimer composed of two identical 72 kDa monomers that are linked by disulfide bridges [1,2].

In males, AMH is secreted by Sertoli cells of the testes. AMH concentrations are high until puberty, and then decline slowly to residual levels after puberty [3]. This decrease of AMH production during puberty is associated with the pubertal development phase. The most significant reduction in AMH concentrations occurs between Tanner stages II and III, and is concurrent with the increase of testosterone concentrations within the testes [4].

In females, AMH expression has been observed in the fetus at approximately 36 weeks in granulosa cells of preantral ovarian follicles and is produced by these cells until menopause [5,6].

The measurement of AMH can be used in fertility investigations to help predict a women's response to ovarian stimulation, estimate of time to menopause and also to diagnose and monitor women with polycystic ovary syndrome (PCOS) [7–9]. Serum AMH levels are two to three times higher in PCOS compared with levels in women with normal ovaries and the level of AMH also correlates with the severity of PCOS [9]. Circulating levels of AMH serve as a reliable indicator of testicular function and descent, and also helps for neonatal gender determination [10].

With the increasing clinical importance of AMH, rapid development of several AMH assays has occurred [11]. The AMH Gen II assay (Beckman Coulter, Inc.) is one of the first manual AMH enzyme-linked immunosorbent assays (ELISA) which are still commonly used in clinical laboratory practice. [12–14].

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However, manual ELISA techniques are time consuming and labor intensive and their results are highly influenced by handling practices. Therefore, an automated AMH assay, providing more reproducible and accurate results, is needed [15].

In order to meet these challenges, Beckman Coulter developed a fully automated assay for AMH on the Access family of immunoassay systems (Access AMH, Beckman Coulter, Inc.) using the same pair of antibodies used in the AMH Gen II assay [16].

There are three publications on the Access AMH assay that have reported an analytical performance assessment carried out using a 10-day protocol [17,18] or intra-assay precision only [19].

We report here on the technical performance assessment of the Access AMH assay including an evaluation of sensitivity, linearity, repeatability over a 20-day period, intermediate imprecision, and total imprecision on the Access 2 and UniCel DxI 800 systems. Furthermore, we evaluated short-term sample stability and long-term frozen storage stability of AMH samples at −20 °C and −70 °C for up to 15 months and lot-to-lot comparability using 9 lots of reagents.

Moreover, we determined AMH reference interval values for healthy adult females, adult males, newborns (≤60 days) and pediatric males classified by Tanner stages.

2. Material and methods

2.1. Assay principle

The Access AMH assay is a simultaneous one-step sandwich chemiluminescence immunoassay using two mouse monoclonal antibodies recognizing total AMH [16,20]. Twenty microliters of a sample are added to the mouse monoclonal antibody F2B/7A conjugated to alkaline phosphatase, and paramagnetic particles coated with the mouse monoclonal antibody F2B/12H. After incubation and final wash, the test tubes are developed by adding a chemiluminescent substrate to produce a visible signal, which indicates the concentration of AMH in the sample determined by means of a stored, six-point calibration curve. Total assay time is approximately 40 min. Calibrators are prepared with human recombinant total AMH (140 kDa) produced in Chinese hamster ovary (CHO) epithelial cells which were transfected with a Simian Virus 40 (SV40) immortalizing gene along with the gene coding for human AMH [21].

2.2. Standardization

No international standard recognized in agreement with the International Federation of Clinical Chemistry is currently available. The Access AMH assay was harmonized with the AMH Gen II assay revised version (Beckman Coulter, Inc.) using 239 frozen samples covering the range of the assay (0–24 ng/mL) stored at −80 °C ($n = 159$) and −20 °C ($n = 75$) (Golden West Biologicals, Temecula, CA, USA and Hospital Saint Joseph, Marseille, France). The AMH Gen II ELISA kit procedure was revised in July 2013 with the addition of a premix step to eliminate the complement interference in fresh samples [22]. Mean of AMH concentrations obtained using two microplate lots and two calibrator lots for the Gen II assay were assigned to each of the 239 samples. These AMH concentrations were then transferred to Access AMH mean signal counts to assign values to reference calibrators prepared with recombinant total AMH (Immunotech (IOT), Marseille, France) in HEPES buffer using three reagent pack lots.

2.3. Samples

Written informed consent was obtained from all participants, which was approved by an Institutional Review Board of each participating facility. The Access AMH performances were evaluated using routine serum or lithium heparin plasma samples. The method comparison experiment was carried out using unused routine serum samples which were aliquoted and stored at −80 °C. The origin of the samples is mentioned for each performance paragraph.

2.4. Imprecision

The imprecision study was performed according to the Clinical and Laboratory Standards Institute (CLSI) EP5-A2 guideline [23] using four pooled plasma samples (Trina Bioreactives, Zurich, Switzerland) at AMH concentrations ranging from 0.10 to 16.9 ng/mL. Samples were randomized and measured in duplicate with two runs per day for a total of 20 days on three reagent lots, three DxI 800 and three Access 2 instruments. The standard deviation (SD) and coefficient of variation (%CV) were calculated for repeatability (within-run precision), intermediate imprecision (between-run precision) and total imprecision (within-lab precision).

2.5. Sensitivity

The Access AMH assay sensitivity was determined according to the CLSI guideline EP17-A2 [24].

For the limit of blank (LoB) determination, four 0-level analyte samples (calibrator S0 of four calibrator lots) were run over three days with four runs per day and five replicates per run on two Access 2 and two DxI 800 instruments using two reagent pack lots. The 95th percentile of the upper reference limit was calculated from a total of 120 replicates per sample and per reagent lot for Access 2 and DxI 800 instruments. The LoB corresponds to the highest apparent amount of AMH expected when replicates of a sample containing no AMH are measured.

The limit of detection (LoD) was determined using five low-level serum samples (Trina Bioreactives, Zurich, Switzerland) above the LoB with nine replicates per day over five days on two Access 2 and two DxI 800 instruments using one reagent pack and calibrator lot. The LoD corresponds to the lowest AMH concentration whose distribution of results shows 95% of the results above the LoB. Ninety-five percent represents the probability of detecting the AMH when it is present.

The limit of quantitation (LoQ) was determined over five days using seven low-level AMH serum samples (Trina Bioreactives, Zurich, Switzerland), two Access 2 and two DxI 800 instruments, two reagent pack lots with nine replicates per sample. The LoQ corresponds to the lowest AMH amount that can be accurately quantified with a 20% CV.

2.6. Linearity

Linearity of the reportable range was evaluated according to CLSI guideline EP06-A [25]. The study was completed on two Access 2 instruments using two reagent lots. One high serum sample (>24 ng/mL) and one low serum sample (<0.02 ng/mL) were used as neat and mixed samples to make seven evenly distributed sample concentrations. Measured AMH values were plotted against the expected AMH concentrations and linearity was determined using the polynomial regression method.

2.7. Method comparison

A comparison of the Access AMH assay and the AMH Gen II ELISA assay (Beckman Coulter, Inc.) was performed on 104 serum samples (internal blood draw and Hospital Saint Joseph, Marseille, France) across the range of the assay (0.15–22.5 ng/mL) using two reagents lots on Access 2 and DxI 800 instruments. Result analysis was performed using Passing-Bablok regression, Bland-Altman plot and Spearman correlation.

2.8. Sample stability

Serum with gel, serum no gel (without gel) and lithium heparin plasma samples from 11 anonymized blood donors (Beckman Coulter