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.

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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].
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 using Passing-Bablok regression, Bland-Altman plot and Spearman correlation.

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 SO 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-A2 [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 reagent 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
employees, MN, USA) were collected, aliquoted and stored at different temperatures for different time points. Room temperature stored samples were analyzed in triplicate on one Access 2 instrument at five different time points; immediately (day 0), after 8 h, 16 h, 24 h and 48 h. 2–8 °C stored samples were analyzed after 7 days and the frozen samples (−20 °C) after 30 days and 60 days. The average percent difference from the baseline was calculated.

Four serum gel tubes from five anonymized blood donors (Beckman Coulter employees, MN, USA) ranging from 5.89 to 22.19 ng/mL were collected but not centrifuged and stored up to 6 days at room temperature. Centrifugation was performed on one tube of each donor prior to testing after 0, 1, 2 and 6 days. The percent difference from the baseline was calculated for each donor at each time point.

For assessment of the long term sample stability; samples from 10 anonymized blood donors (Beckman Coulter employees, MN, USA) were collected with serum gel, serum no gel and lithium heparin plasma tubes. Samples ranging from 0.61 to 12.15 ng/mL were aliquoted and five were stored at −20 °C, the other five were stored at −70 °C. Aliquots were tested in triplicate after 60, 120, 240, 360, 390 and 452 days on one Access 2 instrument using one reagent and calibrator lot. The percent difference from the baseline over time was calculated for each sample and the mean percent difference of the 5 patient samples per storage condition (−20 °C and −70 °C) was plotted using linear regression with 95% confidence intervals (CI).

Comparison plots were used to evaluate between the three sample types. The slopes and 95% CI were calculated.

2.9. Reference interval

AMH reference intervals in healthy individuals were obtained from 864 serum samples (Cerba Specimen Services, Cergy Pontoise, France; Northwest Biological Inc., Bellevue, USA; BioreclamationIVT, New York, USA and Etablissement Francais du Sang Rhone Alpes, Lyon, France) which were run in duplicate on one Access 2 instrument with two reagent pack lots and one calibrator lot. Subjects with known endocrine or metabolic disorders, infertility, autoimmune and cancer diseases, infectious disease, diagnosed endometriosis, gonadal dysfunction were excluded. Results on 83 adult males, 483 adult females, 55 male newborns and 44 female newborns were analyzed using robust method applied to Box–Cox transformed data, according to recommendation in: Horn and Pesce (2005) [26]. One hundred ninety nine healthy pediatric males, aged between 8 and 19, were included to analyze the pediatric male reference range classified by Tanner stages. Healthy pediatric male donors were included in the protocol and classified using the Tanner scale of physical development based on external primary and secondary sex characteristics.

2.10. Lot-to-lot comparison

Four lots of two levels of assay controls and two lots of three levels of patient samples were run in duplicate on nine reagent pack lots using 12 calibrator lots over a year. Percent differences of doses obtained on each replicate were calculated versus their assigned values (target). The target for the controls level 1 and level 2 are respectively 4.6 and 14.1 ng/mL (lot 1); 4.6 and 14.1 ng/mL (lot 2); 5.07 and 15.03 ng/mL (lot 3); 4.92 and 14.9 ng/mL (lot 4). The target for the patient samples level 1, level 2, and level 3 are 2.81, 6.03, and 13.1 (lot 1) and 4.15, 5.71, and 13.9 (lot 2), respectively. The average of the percent differences from the target was also calculated per reagent pack lot.

2.11. Statistical analysis

The analysis of regression (Bland–Altman and Passing–Bablok methods) was performed using Analys-it software Version 3.5. All other statistical analyses were performed using JMP software Version 10.

3. Results

3.1. Imprecision

Repeatability, intermediate and total imprecision of Access AMH assay are similar between DxI 800 and Access 2 instruments. The total imprecision for AMH plasma pools between concentrations of 0.10–16.9 ng/mL ranged from 2.4 to 5.2% CV. Results of imprecision study are summarized in Table 1.

3.2. Sensitivity

The LoB and LoD ranged from 0.0024 ng/mL to 0.0077 ng/mL and from 0.0049 ng/mL to 0.0098 ng/mL, respectively for Access 2 and DxI 800 instruments. The LoQ was 0.01 ng/mL for Access 2 and DxI 800 instruments.

3.3. Linearity

Fig. 1 shows the results of linearity for the Access 2 instrument. The linearity study was conducted based on CLSI EP06-A using polynomial regression method. The Access AMH assay presented excellent linearity in the measuring range (0.15–22.5 ng/mL) with a maximum deviation of 2.45% for samples >0.16 ng/mL and <0.0037 ng/mL for samples ≤0.16 ng/mL (Fig. 1).

3.4. Method comparison

The Access AMH assay and the manual AMH Gen II assay displayed a strong correlation (Spearman rank correlation coefficient at 0.99 for Access 2 and DxI 800 instruments). The Passing-Bablok regression analysis resulted in slope values ranged from 0.89 to 0.92 on both instruments using two lots of reagent packs. The Access AMH measurements showed up to 11% lower sample concentrations as compared to the AMH Gen II assay over the entire measuring range (0.15–22.5 ng/mL). Correlation data for Access 2 system on one reagent lot is shown in Fig. 2. A mean bias of 1.3% was observed for the Access AMH on Access 2 and a mean bias of 0.8% was observed for the Access AMH on DxI 800 based on the Bland-Altman difference plots (see Supplementary data Fig. A2). These biases indicated good agreement across the measuring range between the AMH Gen II assay and the Access AMH assay on both instruments.

3.5. Sample stability

The AMH sample stability showed a variation <.5.5% between day 0 and 48 h at room temperature, 7 days at 2–8 °C or 60 days frozen stored at −20 °C for the three different tube types (serum gel/no gel and lithium heparin plasma) (data not shown, see Supplementary data Table A1). For the AMH whole blood stability study, the variation between day 0 and day 6 days at room temperature on the serum gel tubes was <.5% (see Supplementary data Fig. A2). For long term stability at −20 °C or −70 °C, no significant trend (p > 0.061) was observed over 15 months and the mean % difference between day 0 and 452 days was below 10% for the three different tube types (serum gel/no gel and lithium heparin plasma) (Table 2).

3.6. Reference interval

The median AMH values for the newborn (<60 days of age) were 46.94 ng/mL and 0.16 ng/mL, respectively for males and females. For the normal healthy males (>18 years old), the AMH median value is 4.87 ng/mL. The median AMH values for females decreased with age from 3.71 ng/mL to 0.29 ng/mL for 18 years old to 41–45 years old, respectively. After 46 years of age, the median AMH value for females was 0.01 ng/mL. For the different Tanner stages for males, which
represented a scale of physical pubertal development in children, adolescents and young adults, the median AMH values decreased most significantly from Tanner stage 1 to 3 with a median value decrease of 56.58 to 8.64 ng/mL. The reference interval results are displayed in the Table 3.

3.7. Lot-to-lot comparison

Lot-to-lot comparability on four different Access AMH quality control material lots showed a percent difference from the target value ranging from −4.1% to 4.3% per replicate of each control level and −6.2% to 5.6% for the two patient sample lots see Fig. 3. In average, per pack lot, the percent differences ranged from 0.13% to 2.25% for the four assay control lots with the two different concentration levels and from −3.7% to 2.5% for the two patient sample lots with the three different concentration levels using multiple calibrator lots over a year of testing.

### Table 1

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Sample</th>
<th>Grand mean (n = 80) (ng/mL)</th>
<th>Repeatability</th>
<th>Intermediate imprecision</th>
<th>Total imprecision</th>
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<td>Access 2 (1)</td>
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<td>0.002</td>
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<td>2.49</td>
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<td>1.5</td>
<td>0.413</td>
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<td>0.381</td>
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<td>15.9</td>
<td>0.327</td>
<td>2.1</td>
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Fig. 1. Linearity experiment for the Access AMH assay according to CLSI EP06-A guidelines. A high serum sample (124 ng/mL) and a low serum sample (<0.02 ng/mL) were analyzed in addition to 7 evenly distributed dilutions which were created by mixing the high and low sample. All diluted samples were measured in replicates of four. Measured AMH concentrations were plotted against the expected AMH concentrations. All results are calculated in ng/mL. Polynomial Fit Degree = 2 equation: AMH Observed value = 0.0002 + 0.956 * AMH Target value + 0.0019 * (AMH Target value − 0.0037)². Polynomial Fit Degree = 3 equation: AMH Observed value = 0.0001 + 0.9598 * AMH Target value + 0.0011 * (AMH Target value − 0.0037)² + 2.7476e-5 * (AMH Target value − 0.0037)³. The graph shows an example of the result obtained on one Access 2 using one reagent lot.

Fig. 2. Method comparison between the Access AMH assay and the AMH Gen II assay analyzed via Passing-Bablok regression. AMH values were measured for 104 serum samples across the range of the assay (0.15–22.5 ng/mL). The graph shows an example of the result obtained on one Access 2 instrument using one reagent lot. The line of identity is given as the dashed line, the Passing-Bablok regression line y = 0.12 + 0.91x as the solid line, the Spearman’s correlation coefficient r being 0.99.
4. Discussion

An analytical performance evaluation was conducted on a novel, automated AMH assay for the Access family of immunoassay systems. The results clearly demonstrate that the Access AMH assay is superior to current manual AMH assays [12]. As such, its remarkable analytical performance makes it suitable for implementation in routine practice. An LoQ of 0.01 ng/mL showed significantly higher sensitivity compared to manual AMH ELISA assays and also to the automated Elecsys AMH assay (Roche Diagnostics GmbH) [27,28]. All evaluated sensitivity parameters confirmed the excellent analytical sensitivity of the Access AMH assay and aligned with a recent publication of Pearson et al. reporting the LoB and LoD values to be lower than those designated in the Instruction for Use from Beckman Coulter [18].

Three previous studies on the Access AMH assay have shown good repeatability and intermediate imprecision assessed on a 10-day maximum experiment without calculation of total imprecision [17–19]. In accordance with these reports, we confirmed that the Access AMH assay exhibited low CVs and high reproductibility on both Access 2 and DxI 800 systems (CV ≤ 3.7% for repeatability, CV ≤ 4.2% for intermediate and CV ≤ 5.2% for total imprecision) based on a 20-day experiment. Furthermore, the Access AMH assay showed excellent linearity in the assay measuring range (0.15–22.5 ng/mL) using the polynomial regression method in accordance with CLSI EP06-A. Therefore, the Access AMH assay exhibited good analytical performance fulfilling the accuracy requirements for a fully automated assay.

Some recent studies revealed discrepancies of reported subject- and between-method variability suggesting that AMH may be prone to pre-analytical instability [29]. We showed highly stable AMH values under room temperature, refrigerated and frozen conditions on the three different sample tube types. Furthermore, long-term storage of samples at −20 °C and −70 °C for up to 15 months had no significant impact on measured AMH. In addition, this is the first paper to report the AMH whole blood stability study showing that for up to a 6-day period, unseparated serum gel tubes can easily be stored at room temperature or couriered to a remote site assay service without the need for centrifugation and refrigeration.

In method comparison studies of Access AMH assay and AMH Gen II assay there was a high correlation with r > 0.99 for samples covering the relevant concentration range of ovarian reserve assessment. This correlation study demonstrated a significant agreement of AMH values observed between the manual AMH Gen II assay and the Access AMH assay on both Access 2 and Dxl 800 systems with maximum difference of 11%. In support of our results, Pearson et al. and van Helden and Welskirchen showed high correlations between the automated Access AMH assay and AMH Gen II assay [17,18]. Nelson et al. also reported good linear relationship between the two AMH assays, but larger discrepancy (approximately 22%) between the average AMH results [19]. Furthermore, a similar type of discordance has been demonstrated between the AMH Gen II ELISA and the automated Elecsys AMH assay values [19,28]. A large between-laboratory variation has already been shown for the manual AMH Gen II assay despite good within-laboratory performance [30]. The Access AMH assay was harmonized with the AMH Gen II ELISA (Beckman Coulter Inc.) revised version ensuring that any complement interference issue is prevented. The Elecsys AMH assay was standardized against the AMH Gen II ELISA and the automated Elecsys AMH assay [27]. Interestingly, a high degree of accordance and correlation between the automated Elecsys AMH assay and the Access AMH assay has been demonstrated by both authors Nelson et al. and van Helden and Welskirchen [17,19]. These reports presumed that the conflicting results on the discordance between the values obtained by AMH Gen II ELISA and the automated AMH assays would more likely be due to a high degree of between-laboratory variability of the manual assay than to residual complement activity interference as discussed by Gassner and Jung [19,27,28]. The lack of internationally accepted standardization of AMH has been documented as a contributing factor to discrepancies between AMH assays [15,19]. Currently, there is no universal AMH calibration standard. There is an urgent need for development of a universal AMH standard to collectively

### Table 2

<table>
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<tr>
<th>Storage condition</th>
<th>Sample type</th>
<th>Slope (% Difference per month)</th>
<th>Std error of slope</th>
<th>p-Value</th>
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<td></td>
<td>Serum no gel</td>
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<td>0.13</td>
<td>0.171</td>
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<td>− 70 °C</td>
<td>Plasma gel tube</td>
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### Table 3

<table>
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<tr>
<th>Reference group</th>
<th>Age range (years)</th>
<th>N</th>
<th>Median ng/mL (pmol/L)</th>
<th>95% reference interval ng/mL (95% CI)</th>
<th>95% reference interval pmol/L (95% CI)</th>
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<tr>
<td>Adult males</td>
<td>&gt; 18</td>
<td>83</td>
<td>4.87 (34.77)</td>
<td>0.73–16.05 (0.36–1.43 and 11.99–19.92)</td>
<td>5.20–11.64 (2.54–10.21 and 8.56–11.99)</td>
</tr>
<tr>
<td></td>
<td>18–25</td>
<td>80</td>
<td>3.71 (26.49)</td>
<td>0.96–13.34 (0.72–1.22 and 9.49–16.97)</td>
<td>6.82–95.25 (5.13–9.39 and 67.73–121.19)</td>
</tr>
<tr>
<td></td>
<td>26–30</td>
<td>82</td>
<td>2.27 (16.21)</td>
<td>0.17–7.73 (0.08–0.34 and 5.96–8.72)</td>
<td>1.22–52.66 (0.59–24.44 and 42.55–62.24)</td>
</tr>
<tr>
<td></td>
<td>31–35</td>
<td>80</td>
<td>1.88 (13.43)</td>
<td>0.07–7.35 (0.02–0.27 and 5.65–6.93)</td>
<td>0.53–52.48 (0.11–1.96 and 40.36–63.78)</td>
</tr>
<tr>
<td></td>
<td>36–40</td>
<td>80</td>
<td>1.62 (11.60)</td>
<td>0.03–7.15 (0.00–0.11 and 5.45–8.77)</td>
<td>0.20–51.03 (0.03–0.82 and 38.88–62.63)</td>
</tr>
<tr>
<td></td>
<td>41–45</td>
<td>79</td>
<td>0.29 (2.05)</td>
<td>0.00–3.27 (0.00–0.00 and 2.11–4.31)</td>
<td>0.00–23.35 (0.00–0.00 and 15.08–30.76)</td>
</tr>
<tr>
<td></td>
<td>≥ 46</td>
<td>82</td>
<td>0.01 (0.06)</td>
<td>0.00–1.15 (0.00–0.00 and 0.72–1.53)</td>
<td>0.00–8.19 (0.00–0.00 and 5.11–10.91)</td>
</tr>
<tr>
<td>Male Tanner stage 1</td>
<td>8–13</td>
<td>39</td>
<td>56.58 (403.97)</td>
<td>4.95–144.48 (NA and 113.35–171.19)</td>
<td>35.37–1013.59 (NA and 809.31–1222.31)</td>
</tr>
<tr>
<td>Male Tanner stage 2</td>
<td>8–17</td>
<td>40</td>
<td>26.55 (189.58)</td>
<td>5.02–140.06 (NA and 93.20–183.17)</td>
<td>35.87–1000.03 (NA and 665.47–1307.83)</td>
</tr>
<tr>
<td>Male Tanner stage 3</td>
<td>10–19</td>
<td>40</td>
<td>8.64 (61.70)</td>
<td>2.61–75.90 (2.18–3.22 and 29.74–115.28)</td>
<td>18.03–541.92 (15.58–23.68 and 212.38–823.08)</td>
</tr>
<tr>
<td>Male Tanner stage 4</td>
<td>12–18</td>
<td>40</td>
<td>7.15 (51.02)</td>
<td>0.43–20.14 (0.014–2.53 and 14.64–26.04)</td>
<td>3.04–143.82 (0.10–18.05 and 104.51–185.96)</td>
</tr>
<tr>
<td>Newborn males</td>
<td>0 (&lt;60 days)</td>
<td>35</td>
<td>46.94 (335.17)</td>
<td>15.11–266.59 (11.42–20.71 and 189.28–331.06)</td>
<td>107.92–1905.49 (81.52–147.85 and 1351.43–2363.77)</td>
</tr>
<tr>
<td>Newborn females</td>
<td>0 (&lt;60 days)</td>
<td>44</td>
<td>1.16 (1.17)</td>
<td>0.01–3.35 (0.00–0.02 and 1.73–4.95)</td>
<td>0.04–24.19 (0.01–0.14 and 12.34–35.31)</td>
</tr>
</tbody>
</table>

* Lower limit with robust method was out of the range of observed data.
calibrate AMH assays and avoid misinterpretation of clinical values when different assays are used [19,31].

Reference intervals for Access AMH were determined to address the need for assay-specific ranges. For the first time, this study provides male Tanner stage AMH reference intervals established for the automated Access AMH assay. Grispon et al. determined AMH reference levels in normal males, from newborns to adults [4]; however, the evaluation was conducted with the manual AMH/MIS ELISA from Beckman-Coulter-Immunotech [32] by using a pair of antibodies different from those used in the Access AMH assay. We determined male Tanner stage AMH reference intervals for the Access AMH assay to address the need for evaluation of gonadal function and cryptorchidism, and for guidance of etiological diagnosis of pediatric male hypogonadism [4,10].

Notably, the Access AMH assay had high lot-to-lot precision based on the lot-to-lot comparison experiments on controls and patient samples showing a bias of <3.7% in the AMH range between 2.81 and 15.3 ng/mL for the nine assay lots using twelve calibrator lots over a year of testing. The reduced lot-to-lot variability with the Access AMH assay arises from the rigid lot standardization process, which involves comparison with a panel of native samples and calibrators with target values traceable to the AMH Gen II ELISA. Lack of universal standardization of current AMH assays to a certified reference material is one of the principal unmet needs in the quantification of AMH [17,19]. A recent guideline – published by the National Institute for Health and Clinical Excellence in 2013 (NICE, 2013) – stated specific AMH cut-off values to predict ovarian response to stimulation and guide treatment decisions [33]. Universal cut-off concentrations should only be recommend¬ed, however, when the results from AMH assays between lot, between laboratory, and over time are highly reproducible. The Access AMH assay, with an excellent lot-to-lot precision, fulfills all these criteria.

The Access AMH assay demonstrated excellent performance characteristics and exhibited good concordance with the AMH Gen II ELISA, recognized as the current clinical standard assay. In addition to the design of the assay, the excellent performance can also be attributed to the fully automated procedure on the Access system. The automated Access AMH assay is helpful in the investigation of women's fertility problems and management of reproductive health, and offers perspectives for wider acceptance of AMH testing into routine clinical practice. Additionally, this new AMH assay provided a high lot-to-lot comparability. In conclusion, the Access AMH assay demonstrates that results can be replicated to a high degree across laboratories and manufactured reagent lots. This represents a fundamental prerequisite for the establishment of a robust cut-off value for the future and for use as a new clinical reference assay pending availability of an international AMH standard.

Conflict of interest

GD, SB, CL, RM, DR, LN, PD and PYM are employed by Beckman Coulter, Inc., which is the manufacturer of the current Access AMH assay. There are no other conflicts of interest.

Funding

None declared.

Acknowledgments

The authors thank the following people for their contribution in the development and evaluation of the Access AMH assay: the assay development teams at Beckman Coulter Marseille France and MN, USA, Eric Rouvier and Jeff Todtleben. The authors’ appreciation also goes to Marian Navratil, Susan Retka and Lindsay Sun for reviewing this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.clinbiochem.2016.08.005.

References


[23] E. Han, M. McShane, R. Saherian, C. White, W. Ledger, Pre-mixing serum samples with assay buffer is a prerequisite for reproducible anti-Müllerian hormone measurement using the Beckman Coulter Gen II assay, Hum. Reprod. 29 (5) (May 2014) 1042–1048.

