Letter to the Editor

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A single-center performance evaluation of the fully automated iFlash anti-Müllerian hormone immunoassay

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To the Editor,

As a unique biomarker, the serum anti-Müllerian hormone (AMH) plays an important role in steroidogenesis and folliculogenesis within the ovary [1, 2]. The serum level of AMH has recently been well recognized as a biomarker of choice for evaluating ovarian reserve and forecasting the response of the ovary to stimulation during treatment of infertility in assisted reproductive technology [1, 3]. With recognition of its clinical utility, there has been continual development of manual enzyme-linked immunosorbent (ELISA) AMH immunoassays by several manufacturers [4, 5]. Conflicting results about the reliability of manual AMH assays have hampered their clinical application, most notably owing to issues with AMH sample stability, interference with AMH binding by complement by the commonly used Beckman Coulter AMH Gen II assay before the kit procedure has been modified and lack of reproducibility by substantial variability between laboratories [3, 5]. The release of two fully automated immunoassays, Elecsys AMH (Roche Diagnostics GmbH, Mannheim, Germany) and Access AMH (Beckman Coulter Inc., Brea, CA, USA) in 2014, has provided significantly more robust assays with reproducible results across numerous laboratories over a protracted time-scale [6], high sensitivity and no evidence for clinically relevant sample instability or variability [7–9].

The fully automated iFlash AMH immunoassay is a recently released one-step sandwich assay based on the acridinium direct chemiluminescence technology for use on iFlash 3000 and iFlash 1800 immunoanalyzers (manufactured by YHLO Biotech, Shenzhen, China). It uses two mouse monoclonal antibodies (anti-AMH coated to paramagnetic microparticles and anti-AMH acridinium-ester-labeled conjugate, respectively) directed against the AMH pro- and mature regions but different from the pair used in Access and Elecsys AMH assays [8, 9]. Consequently, all three assays seem to detect both the cleaved, non-covalent associated complex form of AMH and the uncleaved, full length proAMH, i.e. total AMH [8, 9]. The iFlash AMH assay detects AMH in the range of 0.01–25 ng/mL. The total assay time is 18 min and the sample volume is 50 µL of serum. Results are determined via a lot-specific calibration curve, which is instrument-specifically generated by a three-point recalibration and a master curve provided via the reagent QR code. iFlash AMH assay was calibrated to Elecsys AMH by the manufacturer.

This single-center study has evaluated the analytical performance of iFlash AMH assay with an assessment of repeatability and intermediate precision, linearity, functional sensitivity and short-term sample stability at 2–8 °C and 20–25 °C, respectively. In addition, the iFlash AMH was compared against Elecsys AMH and Access AMH in an extended method comparison study. Serum samples were donated by patients attending at the Reproductive Medical Center, Nanjing Jinling Hospital. Sample collection followed International Conference on Harmonization guideline for Good Clinical Practice E6. The study was approved by the Ethics Committees on Human Subject of Nanjing Jinling Hospital and conducted in accordance with the Declaration of Helsinki (as amended in Tokyo,

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Venice, Hong Kong, and Edinburgh). The informed consents were obtained from all participants.

Repeatability and intermediate precision of iFlash AMH assay were determined according to EP5-A2 guidelines of the Clinical and Laboratory Standards Institute (CLSI) during 20 days measuring each sample material in two runs per day in duplicate measurement (n = 80). Repeatability and intermediate precision were calculated

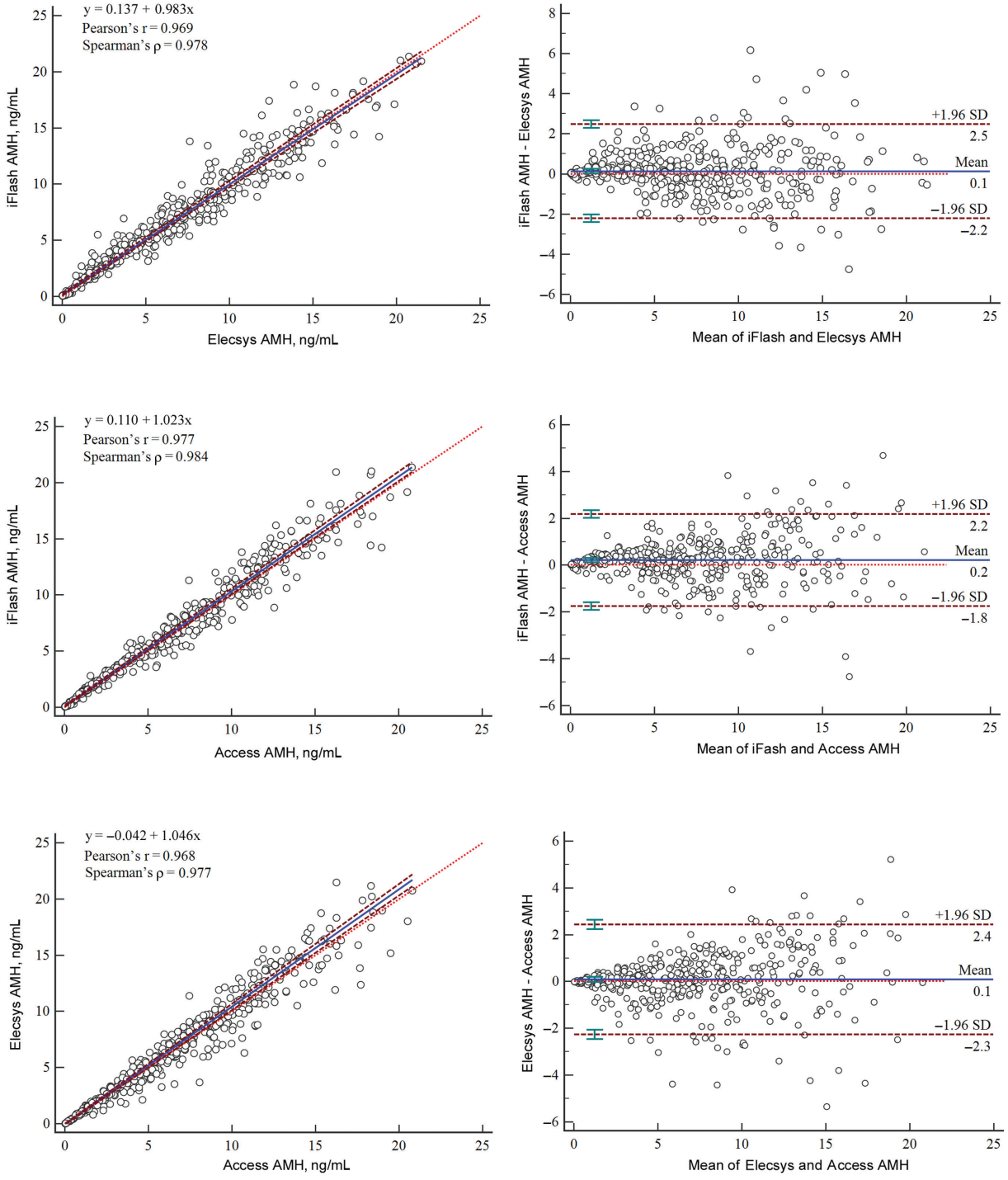

Figure 1: Passing-Bablok regression analysis (left panel) and Bland-Altman difference plots (right panel) between AMH values measured with iFlash AMH assay, Elecsys AMH assay or Access AMH assay.
n = 438; in the left panel, the blue line shows the regression curve; the dashed brown lines show the 95% CI around the regression curve; the dashed red line shows the perfect correlation.

in view of within-run, between-run and between-day variance components.

Precision results for four levels of human serum and two levels of AMH quality control samples are shown in Supplementary Table 1 for AMH concentrations ranging from 0.918 to 17.28 ng/mL. The repeatability coefficient of variation (CV) values were from 2.09% to 5.85%, and the intermediate precision CV values were from 4.54% to 8.82%.

Linearity was evaluated following CLSI EP6-A guidelines. One high serum sample and one low serum sample were used as neat and mixed samples to make eight sample concentrations in the range of 0.84–21.29 ng/mL AMH. Samples were subsequently assayed in triplicates in one run on the iFlash system. The mean results of observed values were plotted against the expected values and evaluated by linear regression analysis. Percentage deviations in the tested AMH concentration range were always below the predefined specification of 10% with $y = 0.989x - 0.21$ ($r = 0.998$, $n = 8$).

Limit of quantitation (LoQ) was determined according to CLSI EP17-A requirements. LoQ was established in terms of imprecision only corresponding essentially to the previous functional sensitivity defined as the lowest concentration that can be reproducibly measured with an interassay CV of <20%. Serum samples with low AMH concentrations were prepared to target four concentrations below 0.3 ng/mL. Two runs per day for each sample in single determination were performed on 5 testing days. Mean AMH values were plotted against corresponding CV values. The lowest measured AMH concentration was 0.022 ng/mL at 19.17% which is less than the LoQ of 0.03 ng/mL provided by the manufacturer.

To evaluate AMH sample stability with iFlash AMH assay five serum samples in the range of 1.66–10.01 ng/mL AMH were measured in duplicate determination freshly, i.e. within 2 h of blood draw, and after storage at 2–8 °C and 20–25 °C for 3 and 5 days. The stored samples showed no significant storage issues as shown in Supplementary Figure 1. Hence, the serum samples can conveniently be stored at room temperature or transported to a remote site measurement service without the need for refrigeration [8, 9].

In a method comparison study, the iFlash AMH assay was compared to the Elecsys AMH and Access AMH using fresh human serum samples from routine diagnostic testing from subjects ($n = 438$) attending at Reproductive Medical Center, Jinling Hospital, Nanjing University School of Medicine due to causes of infertility. The patients included both men and women.

Fresh human serum samples were measured gradually on the same day each within 8 h after blood draw with automated AMH assays. Data were analyzed using both Passing-Bablok regression analysis and Bland-Altman difference plots (Figure 1). The iFlash AMH assay strongly correlates with both the Elecsys AMH (Spearman's correlation coefficient of 0.978) and Access AMH (Spearman's correlation coefficient of 0.984). Likewise, the Elecsys AMH strongly correlates with the Access AMH (Spearman's correlation coefficient of 0.977). The values measured by the three assays were also in close agreement (iFlash AMH = Elecsys AMH*0.983; iFlash AMH = Access AMH*1.023; Elecsys AMH = Access AMH*1.046). A mean bias of 4.79% was observed for the iFlash AMH versus Elecsys AMH and a mean bias of 4.62% was observed for the iFlash AMH versus Access AMH based on the Bland-Altman difference plots (Figure 1). These biases indicated good agreement across the measuring range between the iFlash AMH assay and the Elecsys AMH and Access AMH, respectively. Yet, agreement between assays tended to become progressively weaker as mean AMH levels obtained with the three assays increased. With the increased availability of more AMH assays in the market there is still an urgent need for an international standard (IS) under the auspices of the National Institute of Biological Standards and Control [10].

The new fully automated iFlash AMH assay demonstrated good analytical performance in a single laboratory environment. The values measured by iFlash AMH, Elecsys AMH and Access AMH were well correlated. However, examination of the performance of the new assay across numerous different laboratories, and over a protracted time-scale, still requires rigorous assessment, to gain trust in use of the new test.

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References

1. Jamil Z, Fatima SS, Ahmed K, Malik R. Anti-Mullerian hormone: above and beyond conventional ovarian reserve markers. Dis Markers 2016;2016:5246217.
2. Dewailly D, Andersen CY, Balen A, Broekmans F, Dilaver N, Fanchin R, et al. The physiology and clinical utility of anti-Mullerian hormone in women. Hum Reprod Update 2014;20:370–85.
3. Broer SL, Broekmans FJ, Laven JS, Fauser BC. Anti-Mullerian hormone: ovarian reserve testing and its potential clinical implications. Hum Reprod Update 2014;20:688–701.
4. Kumar A, Kalra B, Patel A, McDavid L, Roudebush WE. Development of a second generation anti-Mullerian hormone (AMH) ELISA. J Immunol Methods 2010;362:51–9.
5. Iliodromiti S, Anderson RA, Nelson SM. Technical and performance characteristics of anti-Mullerian hormone and antral follicle count as biomarkers of ovarian response. Hum Reprod Update 2015;21:698–710.
6. Fleming R, Fairbairn C, Gaudoin M. Objective multicentre performance of the automated assays for AMH and estimation of established critical concentrations. Hum Fertil (Camb) 2017;8:1–5.
7. Li HW, Wong BP, Ip WK, Yeung WS, Ho PC, Ng EH. Comparative evaluation of three new commercial immunoassays for anti-Mullerian hormone measurement. Hum Reprod 2016;31:2796–802.
8. Demirdjian G, Bord S, Lejeune C, Masica R, Riviere D, Nicouleau L, et al. Performance characteristics of the Access AMH assay for the quantitative determination of anti-Mullerian hormone (AMH) levels on the Access* family of automated immunoassay systems. Clin Biochem 2016;49:1267–73.
9. Gassner D, Jung R. First fully automated immunoassay for anti-Mullerian hormone. Clin Chem Lab Med 2014;52:1143–52.
10. Whiting G, Ferguson J, Fang M, Pepin D, Donahoe P, Matejtschuk P, et al. Quantification of Mullerian inhibiting substance/anti-Mullerian hormone polypeptide by isotope dilution mass spectrometry. Anal Biochem 2018;6. pii: S0003-2697(18)30222-7. DOI: 10.1016/j.ab.2018.05.006. [Epub ahead of print].

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