HIGH SENSITIVITY CAPABILITY OF AN ANTI-MÜLLERIAN HORMONE ASSAY ON THE BECKMAN COULTER DxI 9000 IMMUNOASSAY ANALYZER*, A NEXT STEP TOWARD ENABLING OVARIAN FAILURE STAGING?

S. Kuklok, J. Thomforde, L.M. Pearson, K. Pritchard, T. Pfingsten, C. Bunkelman, L.V. Pearson, T. Her, B. Bolstad, B. Bilyeu, S. Santiago, R. Boettcher, S. Thornburgh, M. Posnansky, N. Malikowski-Hoffarth, S. Yancey, K. Walt, D. Lovett, M. Holland, M. Szabo, C. Carlson.
Beckman Coulter, Inc., Brea, CA USA

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BACKGROUND

Measurement of anti-Müllerian hormone (AMH) has proven value in the evaluation of ovarian reserve (Figures 1 & 2) using assays such as the Beckman Coulter Access AMH assay. If improved sensitivity of AMH measurement can be achieved, further clinical utilities are possible during assessment of reproductive aging to enable menopause diagnosis and ovarian response to enable staging of ovarian failure (Practice Committee of the American Society of Reproductive Medicine 2020[1] and Cappola et al. 2023[2]). The Beckman Coulter DxI 9000 analyzer has been designed with new technology that can be leveraged to improve assay sensitivity capabilities. Such technological advancements include the new Lumi-Phos PRO chemiluminescent substrate which yields markedly increased signal-to-noise, a new luminometer with increased dynamic range, and improved low-volume pipetting capabilities. Data herein summarize results from analytical verification studies of the Access AMH assay on the DxI 9000 analyzer, which demonstrate an example of these technologies enabling sensitivity claims that are up to 20-fold improved.

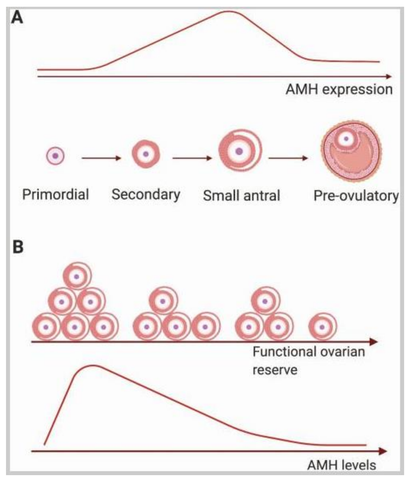

Figure 1. Anti-Müllerian hormone (AMH) expression and concentration in relation to folliculogenesis and ovarian reserve. (A) AMH expression increases from the secondary stage onward until the small antral follicle stage. In preovulatory follicles, AMH is only expressed in cumulus granulosa cells surrounding the oocyte (dark pink layer). (B) With increasing age, the functional ovarian reserve decreases as a result of exhaustion of the primordial follicle pool. This leads to a decrease in the number of small antral follicles and consequently to a decrease in serum AMH levels, reaching undetectable levels at menopause. (Moolhuijsen and Visser[3])

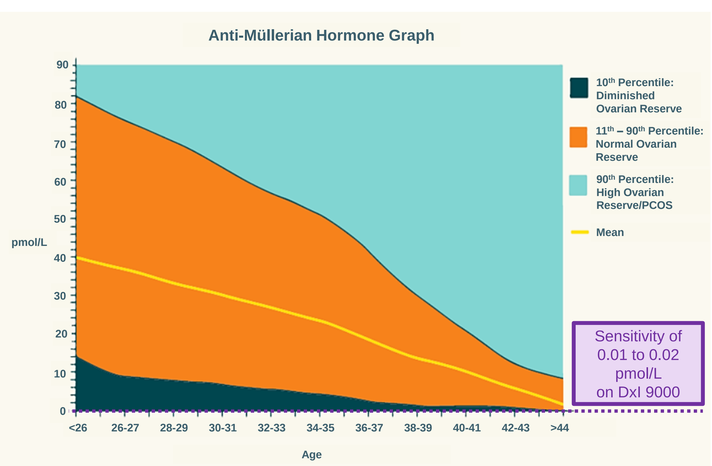

Figure 2. AMH levels by age for Diminished, Normal, and High Ovarian Reserves (Monash IVF Group[4]). The dashed purple line represents the observed LoQ estimates for the Access AMH assay on the DxI 9000 analyzer (0.001 to 0.003 ng/mL or 0.01 to 0.02 pmol/L). These LoQ estimates fall within the Diminished Ovarian Reserve population.

METHODS

A method comparison study was performed based on CLSI guideline EP09c, 3rd ed.[5] to compare results for the Access AMH assay on the DxI 9000 analyzer to the predicate device, the Access AMH assay on the Access 2 Immunoassay System. Within-laboratory precision was also evaluated following CLSI EP05-A3.[6] Finally, performance at low analyte levels was evaluated following CLSI EP17-A2,[7] and linearity was evaluated following CLSI EP06-Ed2.[8] Studies used serum samples, multiple reagent pack lots, one or more calibrator lots, and multiple DxI 9000 analyzers and Access 2 instruments. Quality controls were run in replicates of two on each day to verify the systems were in control.

RESULTS

Method comparison of the Access AMH assay on the DxI 9000 analyzer compared to the Access AMH assay on the Access 2 yielded excellent agreement with a Passing-Bablok slope of 1.02 for N=126 samples (Figure 3). Further, bias estimates for a number of key medical decision points suggest minimal bias (≤ 2%) across the assay range (Figure 3), and minimal non-linearity was observed (Figure 4). 20 -day imprecision studies yielded CVs between 2 and 5% across a broad range of concentrations evaluated (Figure 6). Sensitivity studies yielded estimates of limit of quantitation (LoQ) between 0.001 and 0.003 ng/mL (Figure 5). This represents an approximately 10- to 20-fold improvement in sensitivity claims compared to the AMH assay on the Access 2 and other commercially-available automated immunoassay analyzers

METHODS COMPARISON

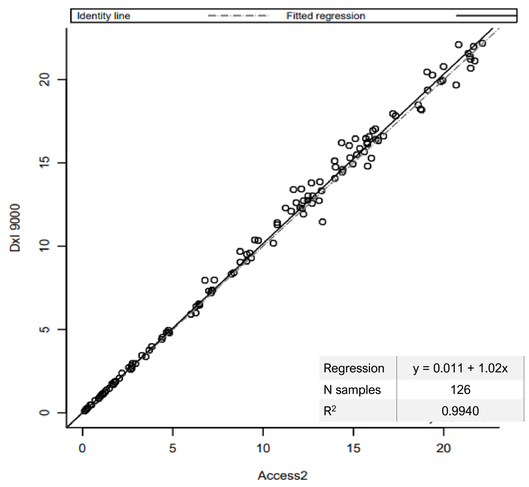

Figure 3. Method comparison regression and statistical descriptives showing strong agreement between the predicate Access 2 (x-axis) and DxI 9000 analyzer (y-axis) for the Access AMH assay (ng/mL). The results met all acceptance criteria. Bias estimates were derived for key medical decision points. Medical decision points assessed were 2, 5, 10, 11, and 20 ng/mL. Relative differences ranged from 1.6% to 2.1% on the DxI 9000 analyzer as compared to the Access 2.

LINEARITY

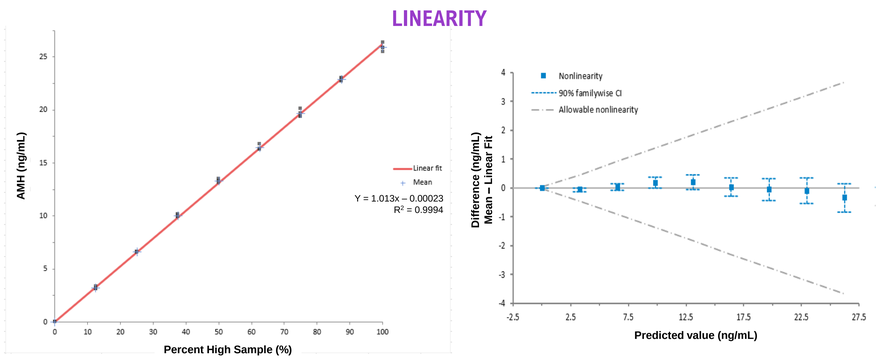

Figure 4a (left). Linear regression graph for a representative linearity study. The range of deviation from linearity was -3 to 9% across three studies. All studies met specifications.
Figure 4b (right). Absolute bias plot for a representative linearity study.

LIMIT OF QUANTITATION

Reagent Pack Lot	Instrument	N	Within-Lab %CV Target	LoQ (ng/mL)	LoQ (pmol/L)
1	1	495	20%	0.003	0.02
2	2	495	20%	0.002	0.01
3	3	493	20%	0.001	0.01

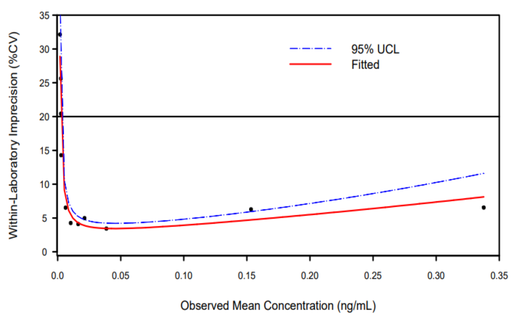

Figure 5a (above). Summary table for observed limit of quantitation (LoQ) results for Access AMH assay on the DxI 9000 analyzer. All studies met acceptance criteria.
Figure 5b (left). Precision profile of 20% CV Within-Laboratory (Total) Imprecision for a representative LoQ study. The resultant LoQ estimate is 0.003 ng/mL (0.02 pmol/L).

IMPRECISION

Concentration (ng/mL)			Repeatability (Within-run)		Between-run		Between-day		Within-Laboratory (Total)	
Sample	N	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 1	88	0.013	0.000	2.9	0.000	0.0	0.000	1.5	0.000	3.3
Sample 2	88	0.30	0.007	2.3	0.002	0.8	0.005	1.7	0.009	3.0
Sample 3	88	0.83	0.018	2.2	0.014	1.7	0.007	0.8	0.024	2.9
Sample 4	88	2.4	0.04	1.8	0.02	0.6	0.03	1.3	0.06	2.3
Sample 5	88	7.2	0.12	1.7	0.05	0.7	0.14	1.9	0.19	2.7
Sample 6	88	13	0.3	2.3	0.2	1.2	0.2	1.4	0.4	3.0
Sample 7	88	19	0.5	2.5	0.3	1.4	0.1	0.6	0.6	2.9
Sample 8	88	1.0	0.02	2.1	0.01	1.3	0.04	3.8	0.05	4.5
Sample 9	88	5.2	0.13	2.6	0.00	0.0	0.14	2.7	0.19	3.7
Sample 10	88	16	0.3	1.9	0.1	0.8	0.4	2.8	0.5	3.5

Figure 6. Summary table for a representative imprecision study. The range of Within-Laboratory (Total) Imprecision CV% across samples was 2.2% to 5.4% across three studies. All studies met acceptance criteria.

CONCLUSIONS

The data herein present performance data for the Access AMH assay on the DxI 9000 analyzer. The assay demonstrates excellent accuracy relative to the predicate device, while showing good linearity and imprecision. Of particular note, the Access AMH assay exhibits exceptional low-end performance on the DxI 9000 analyzer as demonstrated by the ability to enable claims of sensitivity that are up to 20-fold improved in comparison to existing immunoassays for the measurement of anti-Müllerian hormone. The sensitivity capabilities of the Access AMH assay on the DxI 9000 analyzer suggest opportunity for the addition of further intended uses for scenarios where extremely low AMH measurements can provide additional value of clinical significance (Figures 1 & 2). Further studies are needed to fully understand additional clinical utilities of the Access AMH assay with high sensitivity capability on the DxI 9000 analyzer, including the assessment of reproductive aging for menopause diagnosis or ovarian response for fertility treatment.

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** The official name is DxI 9000 Access Immunoassay Analyzer.*