510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k170524

B. Purpose for Submission:

New device

C. Measurand:

AMH (Anti-Müllerian [Mullerian] Hormone)

D. Type of Test:

Quantitative chemiluminescent immunoassay

E. Applicant:

Beckman Coulter, Inc.

F. Proprietary and Established Names:

Access AMH

G. Regulatory Information:

Test system	Regulation	Classification	Product Code	Panel
Anti-Mullerian Hormone Test System	21 CFR 862.1092	II	PQO	Toxicology (91)

H. Intended Use:

1. Intended use(s):

   See indication(s) for use below.

2. Indication(s) for use:

   The Access AMH assay is a paramagnetic particle chemiluminescent immunoassay for the quantitative determination of anti-Müllerian hormone (AMH) levels in human serum

and lithium heparin plasma using the Access Immunoassay Systems as an aid in the assessment of ovarian reserve in women presenting to fertility clinics. This system is intended to distinguish between women presenting with AFC (antral follicle count) values > 15 (high ovarian reserve) and women with AFC values ≤ 15 (normal or diminished ovarian reserve). The Access AMH is intended to be used in conjunction with other clinical and laboratory findings such as antral follicle count, before starting fertility therapy. The Access AMH is not intended to be used for monitoring of women undergoing controlled ovarian stimulation in an Assisted Reproduction Technology program.

3. Special conditions for use statement(s):

   For prescription use only.

   Samples for AMH levels should be drawn on days 2- 4 of the menstrual cycle

   The Access AMH assay is intended to be used for assessing the ovarian reserve in conjunction with other clinical and laboratory findings before starting any fertility therapy (including pre-treatment such GnRH agonist down-regulation therapy) and should be used in conjunction with AFC. The Access AMH assay is not intended to be used for monitoring of women undergoing controlled ovarian stimulation in an Assisted Reproduction Technology program.

4. Special instrument requirements:

   Performance data shown below was collected on the Access 2 Immunoassay System.

I. Device Description:

The Access AMH assay reagent pack (50 tests/pack) consists of the following components:

- R1a: Paragmagnetic particles coated with mouse monoclonal anti-AMH in TRIS buffer with surfactant, protein (bovine), <0.1% sodium azide, 0.1% ProClin 300
- R1b: Mouse anti-AMH alkaline phosphatase conjugate in MES buffer, surfactant, protein (bovine, recombinant), <0.1% sodium azide, 0.1% ProClin 300
- R1c: TRIS buffer with surfactant, protein (murine, bovine), <0.1% sodium azide, 0.1% ProClin 300

The Access AMH Calibrator kit is available separately and contains calibrators at six levels (S0 – S5): 0, 0.16, 0.6, 4, 10, and 24 ng/mL.

The Access AMH QC Quality Control kit is available separately and contains QC materials at three levels (QC1 – QC3): 1, 5, and 15 ng/mL.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Roche Elecsys AMH System

2. Predicate 510(k) number(s):

   DEN150057

3. Comparison with predicate:

Similarities and Differences		
Item	**Candidate Device: Access AMH**	**Predicate Device: Roche Elecsys AMH System (DEN150057)**
Intended Use	For the quantitative determination of anti-Müllerian hormone (AMH) levels in human serum and plasma as an aid in the assessment of ovarian reserve in women presenting to fertility clinics.	Same
Indications for Use	This system is intended to distinguish between women with AFC (antral follicle count) values >15 (high ovarian reserve) and women with AFC values <15 (normal or diminished ovarian reserve). This system is intended to be used for assessing the ovarian reserve in conjunction with other clinical and laboratory findings before starting any fertility therapy. The Elecsys AMH system is not intended to be used for monitoring of women	Same

Similarities and Differences		
Item	**Candidate Device: Access AMH**	**Predicate Device: Roche Elecsys AMH System (DEN150057)**
	under- going controlled ovarian stimulation in an Assisted Reproduction Technology program.	
Analyte	Anti-mullerian hormone	Same
Test Method	Chemiluminescence	Electrochemiluminescence
Sample Type	Serum and Lithium Heparin plasma	Same
Measuring Range	0.08 – 24 ng/mL	0.01 – 23 ng/mL
Antibody Source	Mouse monoclonal	Same

K. Standard/Guidance Document Referenced (if applicable):

CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures: Approved Guideline, Second Edition

CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures: Approved Guideline, Third Edition

CLSI EP06-A, Evaluation of Precision of Quantitative Measurement Procedures: Approved Guideline

CLSI EP07-A2, Interference Testing in Clinical Chemistry: Approved Guideline, Second Edition

CLSI EP09-A3, Measurement Procedure Comparison and Bias Estimation Using Patient Samples: Approved Guideline, Third Edition

CLSI EP28-A3c, Defining Establishing and Verifying Reference Intervals in the Clinical Laboratory: Approved Guideline, Third Edition

CLSI EP25-A, Evaluation of Stability of In Vitro Diagnostic Reagents: Approved Guideline

L. Test Principle:

The Access AMH assay is a simultaneous one-step immunoenzymatic ("sandwich") assay. A sample is added to a reaction vessel, along with a mouse monoclonal anti-AMH antibody conjugated to alkaline phosphatase in MES buffer, Tris buffered saline with proteins, and paramagnetic particles coated with a mouse monoclonal anti-AMH antibody in Tris buffer.

After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos 530 is added to the vessel and light generated by this reaction is measured with a luminometer. The light production is directly proportional to the concentration of AMH in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

An internal precision study was performed based on CLSI EP05-A3. Four serum samples spanning the claimed measuring range were tested in duplicate with two runs per day for 20 days using three reagent pack lots and three Access 2 instruments (N = 80). The results are as follows:

Lot 1:

Sample	Mean (ng/mL)	Within Run		Between Run		Total Imprecision	
		SD (ng/mL)	% CV	SD (ng/mL)	% CV	SD (ng/mL)	% CV
Sample 1	0.08	0.002	1.8	0.002	1.8	0.002	2.6
Sample 2	2.59	0.049	1.9	0.038	1.5	0.062	2.4
Sample 3	8.49	0.127	1.5	0.188	2.2	0.227	2.7
Sample 4	17.1	0.275	1.6	0.400	2.3	0.486	2.8

Lot 2:

Sample	Mean (ng/mL)	Within Run		Between Run		Total Imprecision	
		SD (ng/mL)	%CV	SD (ng/mL)	%CV	SD (ng/mL)	%CV
Sample 1	0.08	0.002	2.8	0.003	3.1	0.003	3.6
Sample 2	2.60	0.033	1.3	0.050	1.9	0.599	2.3
Sample 3	8.46	0.994	1.2	0.194	2.3	0.218	2.6
Sample 4	17.0	0.238	1.4	0.392	2.3	0.458	2.7

Lot 3:

Sample	Mean (ng/mL)	Within Run		Between Run		Total Imprecision	
		SD (ng/mL)	%CV	SD (ng/mL)	%CV	SD (ng/mL)	%CV
Sample 1	0.09	0.001	1.6	0.003	3.2	0.003	3.6
Sample 2	2.65	0.033	1.3	0.069	2.6	0.077	2.9
Sample 3	8.74	0.130	1.5	0.223	2.7	0.267	3.1
Sample 4	17.3	0.259	1.5	0.423	2.4	0.496	2.9

Combined lots:

	AMH (ng/mL) n=240	Within-run		Between-run		Intra-study (WL)		Inter-study		Total Imprecision	
Sample	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 1	0.09	0.002	2.8	0.001	1.7	0.003	3.3	0.004	4.4	0.005	5.5
Sample 2	2.61	0.052	2.0	0.041	1.6	0.066	2.5	0.035	1.3	0.075	2.9
Sample 3	8.56	0.161	1.9	0.175	2.0	0.238	2.8	0.152	1.8	0.282	3.3
Sample 4	17.16	0.380	2.2	0.289	1.7	0.478	2.8	0.155	0.9	0.502	2.9

Reproducibility

Reproducibility of the Access AMH assay was evaluated at three external sites and one internal site based on CLSI EP05-A3. The sample set included six serum panel samples (each panel was a mixture of six postmenopausal patient samples spiked with AMH) and three quality control samples. Samples were tested in quadruplicate with two runs per day for 10 days on four Access 2 instruments. The results are as follows:

	Result Summary		Within-Run (Repeatability)		Within Day		Between Day		Between Site		Reproducibility (Total Imprecision)	
Sample	N	Mean	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
P1	320	0.48	0.010	2.1%	0.003	0.6%	0*	NA	0.010	2.1%	0.015	3.1%
P2	320	0.94	0.017	1.8%	0.001	0.1%	0.005	0.5%	0.024	2.5%	0.030	3.2%
P3	320	2.58	0.049	1.9%	0.010	0.4%	0.018	0.7%	0.055	2.1%	0.076	2.9%
P4	320	5.14	0.100	1.9%	0.050	1.0%	0*	NA	0.094	1.8%	0.146	2.8%
P5	320	10.21	0.182	1.8%	0.060	0.6%	0.051	0.5%	0.158	1.5%	0.253	2.5%
P6	320	16.16	0.292	1.8%	0.107	0.7%	0*	NA	0.181	1.1%	0.360	2.2%
QC1	320	1.00	0.023	2.3%	0.002	0.2%	0.004	0.4%	0.021	2.1%	0.032	3.2%

QC2	320	5.04	0.106	2.1%	0.015	0.3%	0.035	0.7%	0.086	1.7%	0.141	2.8%
QC3	320	15.22	0.345	2.3%	0.054	0.4%	0.051	0.3%	0.006	0.0%	0.353	2.3%
*Default value when estimated variance was negative.												

b. Linearity/assay reportable range:

Linearity of the Access AMH assay was evaluated according to CLSI EP06-A. A high patient serum sample (containing ~24 ng/mL AMH) was diluted with a low patient serum sample (containing ~ 0 ng/mL AMH) for a total of nine concentration levels. The low sample was assayed in replicates of eight and the high samples as well as all intermediate dilution levels were assayed in replicates of four using three reagent pack lots on four Access 2 instruments. The observed values were compared to the expected values and a linear regression was performed. Results for a representative linear data fit are shown below:

$y = 0.96x + 0.0002$

The results support the claimed measuring range of 0.08 to 24 ng/mL.

Dilution study

A dilution study was performed by manually diluting three serum and three Lithium Heparin samples with AMH concentrations above the measuring range with Sample Diluent A in 1:2, 1:4, 1:8, and 1:16 ratios. The data support the following instruction for use:

"If a sample contains more than the stated value of the highest Access AMH Calibrator (S5), report the result as greater than that value (i.e., > 24 ng/mL [> 171 pmol/L]). Alternatively, dilute one volume of sample with 15 volumes (1/16) of Access Sample Diluent A or dilute one volume of sample with 9 volumes (1/10) of Access Sample Diluent A. Refer to the appropriate system manuals and/or Help system for instructions on entering a sample dilution in a test request. The system reports the results adjusted for the dilution."

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Traceability

The Access AMH assay is traceable to internal reference standards (primary reference calibrators), which consist of recombinant AMH in a BSA matrix.The applicant submitted a detailed traceability assurance plan which was reviewed and found to be acceptable.

Sample Stability

Sample stability studies were performed and demonstrated that both serum (gel and no gel) and Lithium Heparin plasma are stable for 48 hours at room temperature, 7 days at 2-8°C, and at least 1 month at ≤ -20°C. Samples should not be freeze/thawed more than two times.

d. Detection limit:

The Limit of Blank, Limit of Detection, and Limit of Quantitation were determined in accordance with CLSI EP17-A2.

The sponsor defined the Limit of Blank (LoB) as the non-parametric 95th percentile value from 120 measurements of the S0 calibrator obtained on three Access 2 instruments with three reagent pack lots. The sponsor claims an LoB of 0.0040 ng/mL.

For the Limit of Detection (LoD), the sponsor measured five low level serum samples using three reagent pack lots and one calibrator lot on three Access 2 Systems. Testing was performed over five days, with one run per day, nine replicates per run for a total of 45 replicates per sample per reagent pack lot and instrument. The sponsor calculated the LoD using the parametric approach, based on the LoB and the standard deviation of the five low level samples. The sponsor claims an LoD of 0.0098 ng/mL.

For the Limit of Quantitation (LoQ), the sponsor measured seven serum samples with low level AMH concentrations using three reagent pack lots and one calibrator lot on three Access 2 Systems. Testing was performed over five days, with one run per day, nine replicates per run for a total of 45 replicates per sample per reagent pack lot. The sponsor defined the LoQ as the lowest concentration of analyte that can be quantified with a total precision of ≤ 20% CV. The sponsor claims an LoQ of 0.0130 ng/mL.

e. Analytical specificity:

Endogenous and exogenous interference

Endogenous and exogenous interference was evaluated according to CLSI EP07-A2 by spiking two serum samples (~ 1-2 ng/mL and ~6-10 ng/mL AMH) with low and high concentrations of possible interferents. Each sample was tested in duplicate on one Access 2 instrument using three reagent pack lots and compared to control samples without interferent. The sponsor considered a percent difference between test samples and control samples > 10% to be significant interference.

The highest concentrations of endogenous substances tested that show non-significant interference are summarized in the table below:

Substance	Highest concentration tested with no significant interference
Acetaminophen	20 mg/dL
Acetylsalicylic acid	65 mg/dL
N-Acetyl-L-cysteine	150 mg/L
Ampicillin	1000 mg/L
Cefoxitin	2500 mg/L
Cyclosporine A	5 mg/dL
Doxycycline	50 mg/L
Folic acid	0.4 mg/L
Levodopa	20 mg/L
Levothyroxine	0.2 mg/L
Metformin	2000 mg/L
Methyldopa	20 mg/L
Metronidazole	200 mg/L
Phenylbutazone	400 mg/L
Rifampicin	60 mg/L
Theophylline	100 mg/L
Gonapeptyl	0.1 mg/L
Ibuprofen	50 mg/dL
Biotin	179 ng/mL
Heparin (low molecular weight)	3000 U/L
Autogloss	1% (v/v)
Ascorbic acid	170 µmol/L
Rheumatoid Factor	1000 IU/mL
IgM	0.5 g/dL
IgA	1.8 g/dL
IgG	2.5 g/dL
Unconjugated bilirubin	40 mg/dL
Conjugated bilirubin	43 mg/dL
Uric acid	1.4 mmol/L
Hemoglobin	1000 mg/dL
Total protein	12 g/dL
Gamma globulin	60 mg/mL

Substance	Highest concentration tested with no significant interference
Intralipid	37 mmol/L

The sponsor included the following limitation in the labeling:

"The following drugs may interfere with this test: Cetrotide, Ovitrelle, Endometrin and Follistatin: do not use this test to analyze samples from patients who have received one or more of these products within one to two weeks of testing."

HAMA/Heterophile interference

HAMA/heterophile interference has not been evaluated for this device.

The labeling contains the following limitation:

"For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g., HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples. Such interfering antibodies may cause erroneous results. In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies can occur. Carefully evaluate the applicability of this assay in patients suspected of having HAMA/heterophile antibodies."

Cross-reactivity

The cross reactivity of the Access AMH assay was evaluated using samples containing high (~ 5-10 ng/mL) or low (~ 1-2 ng/mL) concentrations of AMH spiked with potential cross-reacting compounds compared to unspiked control samples. All samples were tested in triplicate using three reagent pack lots and one calibrator lot on the Access 2 instrument. ≤ 5% Cross-reactivity was observed for the following cross reactants:

Cross-reactant	Concentration tested
Inhibin A	100 ng/mL
Activin A	16.32 µg/mL
LH	100 mIU/mL
FSH	115 mIU/mL
TGFbeta-1	65 ng/mL

High Dose Hook Effect

Two human serum samples spiked with a high concentration of recombinant AMH above the measuring range were tested using the Access AMH assay. No hook effect was observed up to 1000 ng/mL AMH.

f. Assay cut-off:

To predict an antral follicle count of > 15, the corresponding AMH cutoff is 1.77 ng/mL.

2. Comparison studies:

a. Method comparison with predicate device:

121 native serum samples with AMH concentrations ranging from 0.08 to 23 ng/mL were tested on both the Access AMH assay and the predicate device (Roche Elecsys AMH). Samples were run in singlicate with one reagent pack lot on one analyzer. Results analyzed using Passing-Bablok linear regression are shown below:

Slope (95% CI)	Intercept	Correlation Coefficient (r)
1.03 (1.01 – 1.06)	0.04	0.99

b. Matrix comparison:

A matrix comparison study was conducted to compare samples drawn into Lithium Heparin plasma, serum, and serum without gel tubes. 57 matched native samples were run in triplicate on two instruments using a single reagent pack lot. Results for the first measurement analyzed by Passing-Bablok regression are shown below:

Sample Type Comparison	Sample Range (ng/mL)	N	Slope	Intercept
Serum Gel vs. Serum No Gel	0.20 to 13.75	57	0.99	0.03
Lithium Heparin vs. Serum No Gel	0.20 to 13.75	57	1.03	0.06
Lithium Heparin vs. Serum Gel	0.19 to 13.71	57	1.05	0.01

3. Clinical studies:

a. Clinical Sensitivity:

The sponsor performed a prospective clinical study at 13 fertility clinics enrolling 164 women about to undergo their first cycle of controlled ovarian stimulation as part of an IVF or IVF/ICSI protocol. Venous blood samples were collected on days 2-4 of a spontaneous menstrual cycle and each patient underwent a transvaginal ultrasound scan (TVUS) at the same time to determine the antral follicle count. Serum AMH

values were correlated to the antral follicle count (AFC) of the women.

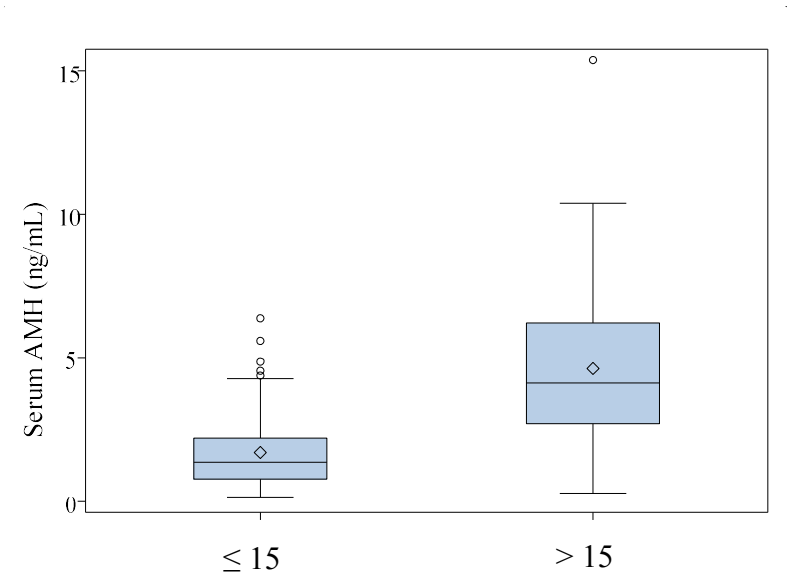
Patient BMI included in the study averaged 25.1 ± 5.3 and was distributed as shown below:

BMI	N
<18.50	5
18.50-24.99	88
25.00-29.99	44
>30.00-39.99	25
≥ 40.00	2

Based on the AFC, two groups are defined: AFC ≤ 15 and AFC > 15. Correlation of AMH (using the 1.77 ng/mL cutoff) in serum and AFC is presented in the table below (relationship is shown in both absolute numbers and percentages per AMH group):

	AFC ≤ 15	AFC > 15	N
AMH ≤ 1.77 ng/mL	65 (91.5%)	6 (8.5%)	71
AMH > 1.77 ng/mL	45 (48.4%)	48 (51.6%)	93
N	110	54	164

The following figure illustrates the clinical study AMH results, presented by AFC group/class (AFC ≤ 15 and AFC > 15).



Positive predictive value (PPV), negative predictive value (NPV), sensitivity, and specificity for predicting AFC > 15 (with 95% confidence intervals) observed in the study using the pre-specified 1.77 ng/mL cutoff are summarized in the following table:

	Result	95% CI
PPV	51.6%	41.6% - 61.5%
NPV	91.5%	82.8% - 96.1%
Sensitivity	88.9%	77.8% - 94.8%
Specificity	59.1%	49.7% - 67.8%

b. Clinical specificity:

See Clinical sensitivity above.

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off:

The clinical cutoff validated in the Access AMH clinical study was 1.77 ng/mL to distinguish women with an AFC >15 or ≤ 15. This cutoff is the same as for the predicate device.

5. Expected values/Reference range:

A reference range study was conducted based on CLSI C28-A3c to establish age-dependent reference ranges for AMH in 666 apparently healthy females ages 18 to 45. Native serum and Lithium Heparin samples were collected and testing was conducted at 5 geographically diverse sites. The reference values for different age groups are summarized below:

Serum

Age Range (years)	N	Median ng/mL (pmol/L)	95% Reference Interval ng/mL (pmol/L)
18-25	120	3.6 (25.70)	1.02 – 14.63 (7.28 - 104.46)
26-30	131	3.82 (27.27)	0.69 – 13.39 (4.93 - 95.60)
31-35	120	2.47 (17.64)	0.36 - 10.07 (2.57 - 71.90)

Age Range (years)	N	Median ng/mL (pmol/L)	95% Reference Interval ng/mL (pmol/L)
36-40	123	1.71 (12.21)	0.18 - 5.68 (1.29 - 40.56)
41-45	126	0.54 (3.86)	0.01 – 2.99 (0.07 - 21.35)

Plasma

Age Range (years)	N	Median ng/mL (pmol/L)	95% Reference Interval ng/mL (pmol/L)
18-25	120	3.75 (26.78)	1.04 – 15.25 (7.43 - 108.89)
26-30	131	4.06 (28.99)	0.74 – 13.81 (5.28 - 98.60)
31-35	120	2.56 (18.28)	0.38 - 10.50 (2.71 - 74.97)
36-40	123	1.78 (12.71)	0.19 – 6.06 (1.36 - 43.27)
41-45	126	0.56 (4.00)	0.01 – 3.17 (0.07 - 22.63)

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable and the special controls for this device type under 21 CFR 862.1092.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.