510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K203757

B Applicant

Roche Diagnostics

C Proprietary and Established Names

Elecsys AMH

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
PQO	Class II	21 CFR 862.1092 - Anti-Mullerian Hormone Test System	TX - Clinical Toxicology

II Submission/Device Overview:

A Purpose for Submission:

Modifications to the previously authorized Elecsys AMH (DEN150057) including changes to mitigate the risk of biotin interference.

B Measurand:

AMH (Anti-Müllerian [Mullerian] Hormone)

C Type of Test:

Quantitative Electrochemiluminescence immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

Elecsys AMH is intended for the in vitro quantitative determination of anti-Müllerian hormone (AMH) in human serum and lithium heparin plasma. The determination of AMH is used for the assessment of the ovarian reserve in women presenting to fertility clinics. This immunoassay 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). This immunoassay is intended to be used for assessing the ovarian reserve in conjunction with other clinical and laboratory findings before starting any fertility therapy. Elecsys AMH is not intended to be used for monitoring of women undergoing controlled ovarian stimulation in an Assisted Reproduction Technology program.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on cobas e immunoassay analyzers.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

Roche cobas e 601

IV Device/System Characteristics:

A Device Description:

The Elecsys AMH reagent working solutions include:

- Rack Pack (kit placed on the analyzer)
- M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1 Anti-AMH-Ab~biotin (gray cap), 1 bottle, 8 mL: Biotinylated monoclonal anti-AMH antibody (mouse) 1.0 mg/L, phosphate buffer 50 mmol/L, pH 7.5; preservative.
- R2 Anti-AMH-Ab~Ru(bpy) (black cap), 1 bottle, 8 mL: Monoclonal anti-AMH antibody (mouse) labeled with ruthenium complex 1.0 mg/L, biotin scavenger antibody 1 mg/mL, phosphate buffer 50 mmol/L, pH 7.5; preservative.

B Principle of Operation:

The Elecsys AMH makes use of a sandwich test principle using a biotinylated monoclonal AMH-specific antibody and a monoclonal AMH-specific antibody labeled with a ruthenium complex to capture AMH present in the sample. The Elecsys AMH was updated to significantly

increase the biotin tolerance of the assay. For neutralization of free biotin in serum and plasma, an antibody, which binds specifically to free biotin and does not bind to or interact with the biotin-linker conjugates, was developed.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Elecsys AMH system

B Predicate 510(k) Number(s):

DEN150057

C Comparison with Predicate(s):

Device & Predicate Device(s):	K203757	DEN150057
Device Trade Name	Elecsys AMH	Elecsys AMH system
General Device Characteristic Similarities		
Intended Use/Indications For Use	For the quantitative determination of anti-Mullerian hormone (AMH) levels for the assessment of ovarian reserve in women presenting to fertility clinics.	Same
Detection Method	Electrochemiluminescence	Same
Measuring Range	0.03-23 ng/mL	Same
Sample Type/ Matrix	Human serum, lithium heparin plasma	Same
General Device Characteristic Differences		
Buffer composition R2	Phosphate buffer; Anti-Biotin Antibody; specific for free, unconjugated biotin (“scavenger antibody”)	Phosphate buffer
Biotin Tolerance	Up to 1200 ng/mL	Up to 30 ng/mL

VI Standards/Guidance Documents Referenced:

Standards No.	Standards Organization	Standards Title
EP05-A3	CLSI	Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition
EP17-A2	CLSI	Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, Approved Guideline, Second Edition
EP07-A3	CLSI	Interference Testing in Clinical Chemistry

VII Performance Characteristics:

A Analytical Performance:

1. Precision/Reproducibility:

Precision

An internal precision study was performed in accordance with CLSI EP05-A3, in which 5 human female serum (FS) samples, and 2 controls (C) were tested in 2 replicates per run, with 2 runs per day, for 21 days (N=84) on the cobas e 601 analyzer using 1 reagent lot. The following results were obtained:

Sample	Mean (ng/mL)	Repeatability (within run)		Intermediate Precision	
		SD	%CV	SD	%CV
FS 1	0.049	0.0008	1.6	0.001	2.5
FS 2	0.703	0.008	1.1	0.013	1.8
FS 3	3.77	0.046	1.2	0.082	2.2
FS 4	11.7	0.142	1.2	0.189	1.6
FS 5	21.4	0.327	1.5	0.451	2.1
C 1	0.907	0.006	0.7	0.011	1.2
C 2	4.78	0.041	0.9	0.061	1.3

Reproducibility

Reproducibility of the Elecsys AMH was performed at 3 sites (1 internal and 2 external) using three cobas e 601 analyzers and three reagent lots (with two reagent lots used per site). Two (2) levels of PreciControl AMH (PC) and 5 native human serum pools (HSP) were tested for 5 days, 1 run per day with 5 replicates per run. Assay calibration was performed as specified in the package insert. The results are summarized below:

Sample	Precision Type	N	Mean (ng/mL)	SD (ng/mL) (95% UCL*)	% CV (95% UCL)
HSP 1	Total	150	0.0503	0.00402 (0.00737)	8.0 (14.7)
	Reproducibility			0.00124 (0.00139)	2.5 (2.8)
HSP 2	Total	150	0.877	0.0337 (0.0526)	3.8 (6.0)
	Reproducibility			0.0125 (0.0140)	1.4 (1.6)
HSP 3	Total	150	4.57	0.201 (0.308)	4.4 (6.7)
	Reproducibility			0.0949 (0.106)	2.1 (2.3)
HSP 4	Total	150	13.8	0.662 (1.02)	4.8 (7.4)
	Reproducibility			0.278 (0.312)	2.0 (2.3)
HSP 5	Total	150	22.0	0.938 (1.62)	4.3 (7.4)
	Reproducibility			0.272 (0.305)	1.2 (1.4)
PC 1	Total	150	0.858	0.0284 (0.0428)	3.3 (5.0)
	Reproducibility			0.0117 (0.0131)	1.45 (1.5)
PC 2	Total	150	4.70	0.159 (0.245)	3.4 (5.2)
	Reproducibility			0.0542 (0.0607)	1.2 (1.3)

* UCL = Upper Confidence Level

2. Linearity/Assay Reportable Range:

Linearity
Linearity of the Elecsys AMH was evaluated using one human serum sample with high analyte content above the measuring range that was diluted to the lower end of the measuring range with an AMH-free serum for a total of 15 concentration levels across the measuring range. Samples were assayed in triplicate within a single run on one cobas e 601 analyzer, using one reagent lot. The observed values were compared to the expected values and a linear regression was performed and the deviations from linearity were acceptable.

The results support the claimed measuring range of 0.03 – 23 ng/mL.

Dilution
A dilution study was performed by diluting high concentration samples in duplicate with two different lots of Diluent Universal in 1:2 ratio. The data support the instructions for use.

3. Analytical Specificity/Interference:

Endogenous interference
Endogenous interference was evaluated by testing 3 human female serum samples (containing low, mid, and high AMH levels) in duplicate with three reagent lots. One part of each sample was spiked with endogenous interferent (i.e., interferent pool), while the other part was spiked with the same volume of solvent (i.e., dilution pool). A series of at least 9 dilution steps was prepared by mixing the interferent pools and dilution pools. The sponsor considered a percent difference between test samples and control samples of > 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
Biotin	1200 ng/mL
HAMA	17.1 ng/mL
Hemoglobin	1000 mg/dL
Intralipid	1000 mg/dL
Bilirubin	66 mg/dL
Rheumatoid Factor	1000 IU/mL
IgG	2.5 g/dL
IgA	1.8 g/dL
IgM	0.5 g/dL

The labeling for this device states the following:

This assay has no biotin interference in serum concentrations up to 1200 ng/mL. Pharmacokinetic studies have shown that serum concentrations of biotin can reach up to 355 ng/mL within the first hour after biotin ingestion for subjects consuming supplements of 20 mg biotin per day and up to 1160 ng/mL for subjects after a single dose of 300 mg biotin.

Exogenous interference
Exogenous interference was evaluated by spiking pharmaceutical compounds into two human female serum sample pools (low-AMH concentration and high-AMH concentration). Samples were tested in duplicate. When concentrations were not available in the guideline, concentrations of at least three times the maximum recommended daily dose were tested. The sponsor considered a percent difference between test samples and control samples > 10% to be significant interference.

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

Substance	Highest concentration tested with no significant interference
Acetylcysteine	150 mg/L
Acetylsalicylic Acid	30 mg/L
Ampicillin-Na	75 mg/L
Ascorbic acid	52.5 mg/L
Cefoxitin	750 mg/L
Doxycycline	18 mg/L
Heparin	3300 IU/L
Levodopa	7.5 mg/L
Methyldopa + 1.5	22.5 mg/L
Metronidazole	123 mg/L
Rifampicin	48 mg/L
Acetaminophen	156 mg/L
Cyclosporine	1.8 mg/L
Ibuprofen	219 mg/L
Theophylline	60 mg/L
Phenylbutazone	321 mg/L
Gonapeptyl	0.1 mg/L
Metformin	2000 mg/L
Folic acid	0.4 mg/L
Levothyroxine	0.2 mg/L

The labeling for this device states the following limitation:

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 on to two weeks of testing.

Cross-reactivity
The cross-reactivity of the Elecsys AMH was evaluated using female serum samples with no AMH content. Samples were spiked with potential cross-reactants and measured with 1 reagent lot on 1 cobas e 601 analyzer. Samples were tested in duplicate and ≤ 0.1% cross-reactivity at 0 ng/mL AMH was observed for the following cross reactants:

Cross-reactant	Concentration
Activin A	100 ng/mL
Inhibin A	100 ng/mL
LH	500 mIU/mL
FSH	500 mIU/mL

Hook effect
Two high concentration AMH serum samples were serially diluted using postmenopausal female serum. No hook effect was observed up to 1400 ng/mL.

4. Assay Reportable Range:

The claimed measuring range is from 0.03 ng/mL to 23 ng/mL.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Traceability
The AMH CalSet are traceable to manufacturer's internal reference standards (Master Calibrators), which consist of a panel of well characterized serum reference materials covering the entire measuring range. The detailed traceability assurance plan was reviewed in DEN150057 and found to be acceptable. The target values for AMH CalSet are shown below:

Level	Target Value (ng/mL)	Target Range (ng/mL)
Calibrator 1	<0.1	<0.1
Calibrator 2	8.0	7.2–8.8

6. Detection Limit:

The limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) were determined in accordance with CLSI EP17-A2.

The LoB was defined as the 95th percentile value from N ≥ 60 measurements of analyte-free samples over several independent series and was determined to be 0.007 ng/mL.

The LoD was calculated based on the LoB and the standard deviation of low concentration samples and was determined to be 0.01 ng/mL.

The LoQ was defined as the lowest concentration of analyte that can be quantified with an intermediate precision of ≤ 20% CV and was determined to be 0.03 ng/mL.

7. Assay Cut-Off:

The AMH cutoff, intended to predict an antral follicle count (AFC) >15, is 1.77 ng/mL.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Two-hundred and sixty-eight (268) native human samples with AMH concentrations ranging from 0.03 – 23 ng/mL were tested using two reagent lots of the predicate assay on one cobas e 411 analyzer and three reagents lots of the updated assay on three different cobas e 601 analyzers. A small number of samples were spiked with serum containing a high level of AMH to cover the upper end of the measuring range (>16 ng/mL). Results analyzed using a Passing-Bablok linear regression are shown below:

Slope (95% CI)	Intercept	Correlation Coefficient (r)
0.959 (0.950 – 0.969)	0.0207	0.973

2. Matrix Comparison:

The studies used to demonstrate analytical performance of the candidate device were conducted with serum samples. To demonstrate that lithium heparin plasma samples perform equivalently to serum samples, 82 matched serum and lithium heparin plasma samples covering the measuring range of the assay were tested with one reagent lot on one cobas e 601 analyzer. The results were assessed by Passing/Bablok regression analysis.

Slope	Intercept	Correlation Coefficient (r)
1.039	0.011	0.998

C Clinical Studies:

1. Clinical Sensitivity:

Not applicable.

2. Clinical Specificity:

Not applicable.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

The clinical performance of the device was established in DEN150057. See section B Comparison Studies, above.

D Clinical Cut-Off:

The AMH cutoff, intended to predict an antral follicle count (AFC) >15, was established in DEN150057 and is 1.77 ng/mL.

E Expected Values/Reference Range:

The expected values/reference range was established in DEN150057.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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