EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
Elecsys AMH system

DECISION SUMMARY

A. DEN Number:

DEN150057

B. Purpose for Submission:

De Novo request for evaluation of automatic class III designation of the Elecsys AMH system

C. Measurand:

AMH (Anti-Müllerian [Mullerian] Hormone)

D. Type of Test:

Electrochemiluminescence immunoassay

E. Applicant:

Roche Diagnostics

F. Proprietary and Established Names:

Elecsys AMH system including the following:
Elecsys AMH assay
AMH CalSet
PreciControl AMH
AMH CalCheck 5

G. Regulatory Information:

1. Regulation Section:

   21 CFR 862.1092. Anti-mullerian hormone test system

2. Classification:

   Class II (Special Controls)

3. Product Code(s):

   PQO
   JIT
   JJX

4. Panel:

   91 – Toxicology

H. Indications for use:

1. Indication(s) for use:

   Elecsys AMH system, consisting of the Elecsys AMH assay, AMH CalSet, PreciControl AMH, and AMH CalCheck 5, is intended for use in 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 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). 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 undergoing controlled ovarian stimulation in an Assisted Reproduction Technology program.

   The Elecsys AMH system is intended for use on cobas e 411 analyzer.

   AMH CalSet is used for calibrating the quantitative Elecsys AMH assay.

   PreciControl AMH is used for quality control of the Elecsys AMH assay.

   AMH CalCheck 5 is an assayed control for use in calibration verification and for use in the verification of the assay range established for the Elecsys AMH assay.

2. 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 Elecsys 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 Elecsys AMH assay is not intended to be used for monitoring of women undergoing controlled ovarian stimulation in an Assisted Reproduction Technology program.

3. Special instrument requirements:

For use on the cobas e 411 analyzer

I. Device Description:

The Elecsys AMH reagent working solutions are packed in Rack Pack (kit placed on analyzer), which include:

- Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- Reagent 1: 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.
- Reagent 2: Anti-AMH-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 8 mL: Monoclonal anti-AMH antibody (mouse) labeled with ruthenium complex 1.0 mg/L, phosphate buffer 50 mmol/L, pH 7.5; preservative.

The AMH CalSet is a lyophilized equine serum matrix with endogenous AMH (Cal 1) and a lyophilized equine serum matrix with added bovine AMH (Cal 2). The CalSet includes:

- AMH Cal 1: approximately 0.04 ng/mL endogenous AMH in an equine serum matrix, preservative.
- AMH Cal 2: approximately 8 ng/mL bovine AMH in an equine serum matrix, preservative.

PreciControl AMH is a lyophilized equine serum matrix with added bovine AMH (male fetal bovine serum) in two concentration ranges. The controls are used for monitoring the accuracy and precision of the Elecsys AMH assay. PreciControl AMH includes:

- PC AMH 1: approximately 1 ng/mL bovine AMH in an equine serum matrix, preservative.
- PC AMH 2: approximately 5 ng/mL bovine AMH in an equine serum matrix, preservative.

AMH CalCheck 5 is a (b) (4)

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J. Standard/Guidance Document Referenced:

CLSI EP05-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition

CLSI EP6-A: Evaluation of Linearity of Quantitative Measurement Procedures; A Statistical Approach; Approved Guideline

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

K. Test Principle:

The Elecsys AMH assay makes use of a sandwich test principle using monoclonal antibodies that are specifically directed against AMH. Endogenous AMH is bound by both a biotinylated monoclonal AMH-specific antibody and a monoclonal AMH-specific antibody labeled with a ruthenium complex to form a sandwich complex. Results are determined via a calibration curve which is instrument-specifically generated by a two-point calibration and a master curve provided via the reagent barcode.

L. Performance Characteristics:

1. Analytical performance:

a. Precision/Reproducibility:

Precision:
An internal precision study was performed in accordance with CLSI EP05-A2, in which 5 human serum samples and 2 controls were tested in 2 replicates per run x 2 runs per day for 21 days (N = 84) on the cobas e 411 analyzer. The following results were obtained:

Sample Material	Mean (ng/mL)	Repeatability (Within Run)		Intermediate Precision	
		SD	% CV	SD	% CV
Control 1	1.14	0.017	1.4	0.018	1.6
Control 2	5.61	0.088	1.6	0.101	1.8
Human Serum 1	0.055	0.001	1.6	0.001	2.3
Human Serum 2	1.05	0.014	1.3	0.019	1.8
Human Serum 3	3.56	0.055	1.5	0.058	1.6
Human Serum 4	11.7	0.113	1.0	0.144	1.2
Human Serum 5	19.0	0.265	1.4	0.281	1.5

Reproducibility:
At 3 external laboratory sites, 5 serum pools spiked with bovine AMH and 2 levels of PreciControl AMH were tested in triplicates per day for 5 days on the cobas e 411 analyzer. The results are summarized in the table below:

Sample	Precision Type	N	Mean	SD (95% CI)	%CV (95% CI)
Control 1	Total	90	0.93	0.049 (0.032, 0.099)	5.24 (3.46, 10.69)
	Repeatability			0.013 (0.011, 0.016)	1.42 (1.21, 1.73)
Control 2	Total	90	4.87	0.228 (0.155, 0.434)	4.70 (3.18, 8.91)
	Repeatability			0.092 (0.078, 0.112)	1.89 (1.60, 2.30)
Serum 1	Total	90	0.26	0.010 (0.007, 0.020)	3.99 (2.68, 7.77)
	Repeatability			0.004 (0.004, 0.005)	1.58 (1.34, 1.92)
Serum 2	Total	90	1.24	0.050 (0.034, 0.092)	4.05 (2.79, 7.42)
	Repeatability			0.022 (0.018, 0.026)	1.74 (1.48, 2.12)
Serum 3	Total	90	3.55	0.123 (0.083, 0.237)	3.45 (2.33, 6.66)
	Repeatability			0.050 (0.042, 0.061)	1.41 (1.19, 1.71)
Serum 4	Total	90	9.63	0.445 (0.290, 0.944)	4.62 (3.01, 9.80)
	Repeatability			0.163 (0.138, 0.198)	1.69 (1.44, 2.06)
Serum 5	Total	90	20.12	0.880 (0.592, 1.707)	4.38 (2.94, 8.48)
	Repeatability			0.340 (0.289, 0.414)	1.69 (1.43, 2.06)

b. *Linearity/assay reportable range:*

Linearity:
Linearity of the Elecsys AMH Assay were evaluated according to CLSI EP6-A. Three high level samples were diluted with postmenopausal female serum for a total of 15 concentration levels. Samples were assayed in triplicate on the cobas e 411 analyzer. The observed values were graphed against the calculated values and a linear regression was performed. Results are summarized in the table below:

Sample	Sample range (ng/mL)	Slope	Intercept	Correlation (r)
Set 1	0.004 – 27.5	1.0091	0.0105	0.999
Set 2	0.004 – 27.4	1.0054	0.0205	0.999
Set 3	0.004 – 25.8	1.0063	0.0013	0.999

The linear regression results support the Applicant's claimed measuring range (0.03

ng/mL – 23 ng/mL).

Dilution:
A dilution study was performed by diluting six human samples with Diluent Universal in 1:2 ratio both manually and by the analyzer. The data support the following instruction for use:

Samples with AMH concentrations above the measuring range can be diluted automatically with Diluent Universal 2. Manual dilution can be performed with Diluent Universal 2. The recommended dilution is 1:2 (either automatically by the cobas e analyzer or manually). The concentration of the diluted sample must be > 10 ng/mL. After manual dilution, multiply the result by the dilution factor. After dilution by the analyzer, the cobas e software automatically takes the dilution into account when calculating the sample concentration.

c. *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 Applicant submitted a detailed traceability assurance plan which was reviewed 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

Stability:
Shelf life stability studies were performed with assay reagents, calibrators and controls, and demonstrated that they are stable for at least 15 months when stored unopened at 2-8°C. Once opened, the AMH reagents are stable for 12 weeks at 2-8°C and for 8 weeks on board. The lyophilized AMH CalSet and lyophilized PreciControl AMH are for single use only on board. The reconstituted AMH CalCheck 5 is stable for 3 hours on board. The protocols for stability and acceptance criteria were reviewed and found to be adequate.

Sample Stability:
Sample stability studies were performed and demonstrated that both serum and Li-Heparin plasma are stable for 3 days at 15-25°C, 5 days at 2-8°C, 6 months at -25°C to -15°C, freeze only once.

Value assignment
Concentrations of the AMH CalSet, PreciControl AMH and AMH CalCheck 5 are assigned through internal procedures that were reviewed and found to be acceptable.

d. *Detection limit:*

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

The Limit of Blank (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 Limit of Detection (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 Limit of Quantitation (LoQ) was defined as the lowest concentration of analyte that can be quantified with within-laboratory precision of ≤ 20% and was determined to be 0.03 ng/mL.

e. *Analytical specificity:*

Endogenous interference:
Endogenous interference was evaluated by testing three serum samples (with low, mid, and high AMH levels) with various concentrations of possible endogenous interferents. Each sample was tested in duplicate. The Applicant states in their submission that interference is considered to be significant if analyte recovery is outside of ± 10% of the initial value.

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
Hemoglobin	1000 mg/dL
Biotin*	30 ng/mL
Intralipid	1000 mg/dL
Bilirubin	66 mg/dL
Rheumatoid Factor	1000 IU/mL
HAMA	805 µg/L
Human IgG	2.5 g/dL
Human IgM	0.5 g/dL
Human IgA	1.8 g/dL

* The labeling for this device states the following limitation:

Interference was observed at biotin concentrations above 30 ng/mL. Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day)

until at least 8 hours following the last biotin administration.

Exogenous interference

Exogenous interference was evaluated by spiking pharmaceutical compounds (at 2 concentrations with the higher concentration corresponding to at least 3 times the maximum recommended daily dose) into two human serum sample pools with low and high AMH levels. Each sample was tested in triplicate. The Applicant states in their submission that interference is considered to be significant if analyte recovery is outside of ± 10% of the initial value.

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 (mg/L)
Acetylcysteine	150
Ampicillin-Na	1000
Ascorbic acid	300
Cyclosporine	5
Cefoxitin	2500
Heparin	5000 U
Levodopa	20
Methyldopa +1.5	20
Metronidazole	200
Phenylbutazone	400
Doxycycline	50
Acetylsalicylic Acid	1000
Rifampicin	60
Acetaminophen	200
Ibuprofen	500
Theophylline	100
Gonapeptyl	0.1
Metformin	2000
Folic Acid	0.4
Levothyroxine	0.2

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 one to two weeks of testing.

Cross-reactivity:

The cross reactivity of the Elecsys AMH assay was evaluated using samples composed of Diluent Universal spiked with potential cross-reacting compounds. All

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 tested
Inhibin A	100 ng/mL
Activin A	100 ng/mL
LH	500 mIU/mL
FSH	500 mIU/mL

Hook effect:
Two serum samples spiked with high concentrations of AMH were serially diluted using Diluent Universal. No hook effect was observed up to 14000 ng/mL AMH.

f. Assay cut-off:

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

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable

b. Matrix comparaison:

The clinical study used to demonstrate clinical performance of the assay was performed in serum samples (see section L(3)(a) below). To demonstrate that Li-Heparin plasma samples perform equivalently to serum samples, 75 matched serum/Li-Heparin plasma samples with AMH concentrations covering the entire measuring range were tested on the cobas e 411 analyzer, and the results were assessed by Passing/Bablok regression analysis.

Slope	Intercept	Correlation coefficient (r)
1.017	-0.00186	0.999

3. Clinical studies:

a. Clinical sensitivity and specificity:

Clinical Studies:
The use of AMH for the assessment of ovarian reserve was investigated in a multicenter, prospective, non-interventional study with N = 856 women presenting at fertility clinics for evaluation. Patient BMI included in the study ranged from 14.76 to 39.99, as shown below:

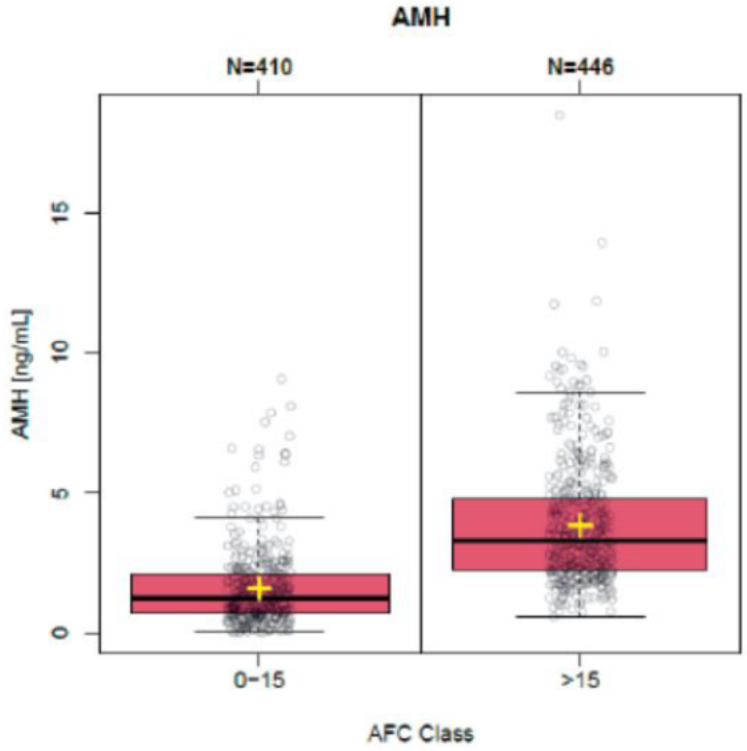
BMI	N
<18.50	29
18.50-24.99	469
25.00-29.99	200
>30.00-39.99	158

AMH values were correlated to the antral follicle count (AFC) of the women. AFC was determined by transvaginal ultrasonography (TVUS), which measures antral follicles (2-10 mm) of the ovary. Both AFC and AMH were determined on days 2-4 of the same menstrual cycle. Clinical serum samples were collected at 13 geographically diverse sites in the United States, and then tested at 3 laboratory sites in the United States on cobas e 411 analyzers.

Based on the AFC, two groups are defined: AFC ≤ 15 and AFC > 15. Correlation of AMH (using the 1.77 ng/mL cutoff) 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	280 (84.3 %)	52 (15.7 %)	332
AMH > 1.77 ng/mL	130 (24.8 %)	394 (75.2 %)	524
N	410	446	856

The following figure illustrates the Validation Arm 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 in parentheses) observed in the study using the 1.77 ng/mL cutoff are summarized in the following table:

	Result	95% CI
PPV	75.2%	(71.3-78.8%)
NPV	84.3%	(80.0-88.1%)
Sensitivity	88.3%	(85.0-91.2%)
Specificity	68.3%	(63.6-72.8%)

b. Other clinical supportive data (when a. is not applicable):

Not applicable.

4. Clinical cut-off:

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

5. Expected values/Reference range:

A reference range study was conducted to establish age-dependent reference ranges for AMH in 718 apparently healthy females of reproductive age between 20 and 44 years. Native serum samples were collected and testing was conducted at two sites. The reference range values for different age groups are summarized below:

Healthy Women (years)	N	2.5%-q ng/mL (95% CI)	5%-q ng/mL (95% CI)	Median ng/mL (95% CI)	95%-q ng/mL (95% CI)	97.5%-q ng/mL (95% CI)
20-24	150	1.22 (0.478-1.67)	1.52 (0.758-1.81)	4.00 (3.60-4.44)	9.95 (7.87-13.6)	11.7 (9.11-15.7)
25-29	150	0.890 (0.493-1.21)	1.20 (0.797-1.75)	3.31 (3.00-3.89)	9.05 (7.59-10.3)	9.85 (8.91-11.3)
30-34	138	0.576 (0.256-0.958)	0.711 (0.256-1.12)	2.81 (2.35-3.47)	7.59 (6.84-9.52)	8.13 (7.27-9.72)
35-39	138	0.147 (0.053-0.474)	0.405 (0.053-0.496)	2.00 (1.73-2.36)	6.96 (5.31-9.37)	7.49 (6.49-10.9)
40-44	142	0.030 (0.030-0.063)	0.059 (0.030-0.119)	0.882 (0.726-1.13)	4.44 (2.94-5.56)	5.47 (3.92-6.76)

M. Proposed Labeling:

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

N. Identified Risks and Identified Mitigations:

Identified Risks to Health	Identified Mitigations
Inaccurate test results that provide false positive results may lead to a modification, delay or cancellation before a controlled ovarian stimulation procedure is initiated.	General controls and special controls (1) and (2)
Inaccurate test results that provide false negative results may lead to the development of ovarian hyperstimulation syndrome in patients incorrectly thought to have normal and/or diminished ovarian reserve.	General controls and special controls (1) and (2)

O. Benefit/Risk Analysis:

Summary	
Summary of the Benefit(s)	The primary benefit of the Elecsys AMH system is its use in the prediction (in conjunction with other clinical and laboratory findings) of high ovarian reserve. The assessment of ovarian reserve prior to performing controlled ovarian stimulation (COS) can assist in the reduction of risk of patients developing ovarian hyperstimulation syndrome (OHSS) by identifying those patients with a higher OHSS risk (i.e., those with high ovarian reserve levels, which correspond to an antral follicle count (AFC) of >15). The AMH cut-off corresponding to an AFC > 15 was validated with a sensitivity and specificity of 88.3% and 68.3%, respectively.
Summary of the Risk(s)	The primary risks to patients through the use of the device are related to the consequences of clinical decisions based on false positive and the more clinically significant false negative results. False positive results may lead to a modification, delay or cancellation before a controlled ovarian stimulation procedure is initiated. Modifications to a stimulation regimen may lead to less oocytes for fertilization. False positive results may result in the need for a repeat procedure and cancelled or delayed stimulation. False negative results may lead to the development of ovarian hyperstimulation syndrome, a potentially life-threatening condition, in patients incorrectly thought to have normal and/or diminished ovarian reserve. However, this risk can be mitigated through appropriate labeling indicating that 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, and should be used in conjunction with Antral Follicle Count. Additional risks to patients and laboratory workers include those that are associated with routine phlebotomy performed as standard practice in a clinical setting.
Summary of Other Factors	There are no additional factors to consider.

Summary	
Conclusions Do the probable benefits outweigh the probable risks?	Given the performance characteristics, applicable general controls, including design controls, and proposed special controls, including labeling mitigations, the probable benefits outweigh the probable risks for this device.

Patient Perspectives:

This submission did not include specific information on patient perspectives for this device.

P. Conclusion:

Product Code: PQO, JIT, JJX
Device Type: Anti-mullerian hormone test system
Class: II (special controls)
Regulation: 21 CFR 862.1092

a) *Identification.* An anti-mullerian hormone test system is an in vitro diagnostic device intended to measure anti-mullerian hormone in human serum and plasma. An anti-mullerian hormone test system is intended to be used as an aid for assessing ovarian reserve in women.

b) *Classification*. Class II (special controls). An anti-mullerian hormone test system must comply with the following special controls:

1) Premarket notification submissions must include the following information:
   i. An adequate traceability plan to minimize the risk of drift in anti-mullerian hormone test system results over time.
   ii. Detailed documentation of a prospective clinical study to demonstrate clinical performance or, if appropriate, results from an equivalent sample set. This detailed documentation must include the following information:
      a. Results must demonstrate adequate clinical performance relative to a well-accepted comparator.
      b. Clinical sample results must demonstrate consistency of device output throughout the device measuring range that is appropriate for the intended use population.
      c. Clinical study documentation must include the original study protocol (including predefined statistical analysis plan), study report documenting support for the proposed indications for use(s), and results of all statistical analyses.
   iii. Reference intervals generated by testing an adequate number of samples from apparently healthy normal individuals in the intended use population.

2) Your 809.10(b) compliant labeling must include a warning statement that the device is intended to be used for assessing the ovarian reserve in conjunction with other clinical and laboratory findings before starting any fertility therapy, and that the device should be used in conjunction with the Antral Follicle Count.