EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
picoAMH ELISA

DECISION SUMMARY

A. DEN Number:

DEN180004

B. Purpose for Submission:

New device

C. Measurand:

Anti-Müllerian Hormone (AMH)

D. Type of Test:

Quantitative enzyme-linked immunosorbent assay (ELISA)

E. Applicant:

Ansh Labs

F. Proprietary and Established Names:

picoAMH ELISA

G. Regulatory Information:

Regulation	Name	Product Code	Panel
21 CFR 862.1093	Menopause test system	QDH	Chemistry (75)

H. Indications for Use:

1. Indication(s) for use:

The picoAMH ELISA is an enzyme-linked immunosorbent assay (ELISA) for the in vitro quantitative measurement of anti-Müllerian hormone (AMH), also known as Müllerian Inhibiting Substance (MIS), concentrations in human serum. It is intended to be used as an aid in the determination of menopausal status in women between 42 and 62 years of age. This assay should only be used in conjunction with other clinical and laboratory findings and results from this test alone should not be used to make diagnostic or treatment decisions. It is intended for *in vitro* diagnostic use and for prescription use only.

2. Special conditions for use statement(s):

   For prescription use only.

   picoAMH results should always be assessed in conjunction with the patient's medical history, clinical examination, and other findings when being interpreted for diagnostic purposes.

   picoAMH test results < 10 pg/mL should be carefully evaluated in the context of a full clinical work up to ensure that the use of contraceptives is not discontinued in women who have not yet reached menopause.

   picoAMH test results > 10 pg/mL should be carefully evaluated in the context of a full clinical work up to ensure that uterine bleeding due to endometrial cancer is not dismissed as a potential diagnosis.

   This test should not be used to assess a woman's fertility status or for use in monitoring or predicting the ovarian response in women undergoing or planning to undergo fertility treatments.

3. Special instrument requirements:

   Microplate reader capable for absorbance measurement at 450 nm, 405 nm, and 630 nm.

I. Device Description:

The picoAMH ELISA device is supplied as a reagent kit containing the following components in buffer with preservatives:

- AMH/MIS (Müllerian Inhibiting Substance) Coated Microtitration strips: one strip-holder, containing 12 strips and 96 microtitration wells with mouse monoclonal AMH antibody immobilized to the inside wall of each well.
- AMH/MIS Assay Buffer: one 12 mL bottle containing protein-based buffer with preservative.
- picoAMH Biotin Conjugate Ready-To-Use: one 12 mL bottle containing biotinylated mouse anti-AMH antibody in protein-based buffer with preservative.
- picoAMH Streptavidin-Enzyme Conjugate Ready-To-Use: one 12 mL bottle containing streptavidin-HRP (horseradish peroxidase) in a protein-based buffer and preservative.
- TMB Chromogen Solution: one 12 mL bottle containing a solution of tetramethylbenzidine (TMB) in buffer with hydrogen peroxide.
- Stopping Solution: one 12 mL bottle containing 0.2 M sulfuric acid.
- Wash Concentrate A: one 60 mL bottle containing buffered saline with a nonionic detergent that requires a 25-fold dilution with deionized water prior to use.

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

CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Methods, 3rd Edition

CLSI EP06-A: Evaluation of the Linearity of Quantitative Measurement Procedures; A Statistical Approach, 2nd Edition

CLSI EP07-A2: Interference Testing in Clinical Chemistry, 2nd Edition

CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition

CLSI EP25-A: Evaluation of Stability of In Vitro Diagnostic Reagents, 1st Edition

CLSI CP28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory, Third Edition

K. Test Principle:

The picoAMH ELISA is a quantitative three-step sandwich type immunoassay that is designed to measure human AMH. In the first step, calibrators, controls, and unknown samples are added to AMH antibody-coated microtiter wells. After an incubation and washing, biotinylated AMH antibody solution is added. After a second incubation and washing, the wells are incubated with streptavidin horseradish peroxidase conjugate (SHRP) solution. Finally, after a third incubation and washing step, the wells are incubated with substrate solution (TMB) followed by an acidic stopping solution.

The AMH antibody-biotin conjugate binds to the solid phase antibody-antigen complex, which in turn binds to the streptavidin-enzyme conjugate. The antibody-antigen-biotin conjugate-SHRP (streptavidin horseradish peroxidase conjugate solution) complex bound to the well is detected by enzyme-substrate reaction. The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 nm as the primary test filter and 630 nm as the reference filter. The absorbance measured is directly proportional to the concentration of AMH in the calibrators, controls, and specimens tested.

L. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

   Precision was evaluated using four human serum specimens containing AMH concentrations ranging from about 15 pg/mL to about 930 pg/mL. Samples were tested at one site in replicates of four, in two runs per day, for 20 days with three reagent lots (n = 160 per lot).

Results for within-lab imprecision are as follows:

Sample	Lot	N	Mean pg/mL	Repeatability SD (pg/mL)	Repeatability % CV	Intermediate Precision SD (pg/mL)	Intermediate Precision %CV
Serum 1	1	160	14.6	0.8	5.5%	1.2	8.1%
	2	160	14.2	0.6	4.2%	0.8	5.9%
	3	160	15.5	0.7	4.5%	1.0	6.7%
Serum 2	1	160	80.1	2.2	2.8%	3.6	4.5%
	2	160	80.0	2.0	2.5%	3.4	4.2%
	3	160	80.8	2.8	3.5%	3.4	4.2%
Serum 3	1	160	620.3	17.4	2.8%	22.9	3.7%
	2	160	609.6	16.8	2.8%	24.2	4.0%
	3	160	643.2	19.2	3.0%	24.0	3.7%
Serum 5	1	160	942.8	28.9	3.1%	51.3	5.4%
	2	160	924.1	23.7	2.6%	48.5	5.3%
	3	160	935.2	36.8	3.9%	47.4	5.1%

b. *Linearity/assay reportable range:*

Linearity

Linearity was evaluated based on CLSI EP06-A. A high analyte (1200 pg/mL) concentration specimen ("High Pool") was prepared by adding recombinant human AMH to low-analyte pooled human serum. 14 intermediate levels were prepared by mixing low-analyte serum with the High Pool for a total of 16 level (b) (4) Each level was measured in triplicate with three lots and the allowable nonlinearity was calculated for each lot. Representative data for one reagent lot is shown below:

y = (b) (4)

The data supports the sponsor's claimed measuring range of 6.0 to 1150 pg/mL.

Dilution

A dilution study was performed using nine human serum samples spiked with recombinant AMH (ranging from (b) (4) pg/mL). Samples were manually diluted 20-fold with picoAMH Calibrator A/Sample diluent and tested in triplicate using four reagent lots. The data support the sponsor's instructions for use stating that samples containing AMH concentrations above the claimed measuring range (6 to 1150 pg/mL) and up to 23,000 pg/mL can be measured with the picoAMH ELISA assay when diluted up to 20-fold.

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

The picoAMH ELISA assay is traceable to an internal recombinant AMH standard

manufactured at Ansh Labs. The applicant submitted a detailed traceability assurance plan which was reviewed and found to be acceptable.

The picoAMH Calibrators consist of a picoAMH Calibrator A/Sample Diluent containing AMH in equine serum with preservative. The target values for the calibrators are shown below:

Calibrator	AMH Concentration (pg/mL)
CAL A	0
CAL B	8
CAL C	31
CAL D	105
CAL E	360
CAL F	1091

The picoAMH Controls consist of two vials, labeled Levels I and II, containing low and high AMH concentrations in serum with preservative. Controls are reconstituted with 1 mL of deionized water.

d. Detection limit:

Detection limits were evaluated based on CLSI EP17-A2.

The Limit of Blank (LoB) was evaluated using 18 independent human serum pools containing no detectable AMH. Samples were tested with four reagent lots for a total of 73 replicates per specimen. The overall LoB (n = 324) was calculated using a non-parametric analysis.

The Limit of Detection (LoD) and Limit of Quantitation (LoQ) were evaluated using 12 low-level serum pools (containing between 2.76 and 9.95 pg/mL AMH) measured over four reagent lots (n = 144 per lot). The LoD was calculated as the Mean LoB + 1.645*SDL (SDL = standard deviation ofpooled low level specimens). The sponsor defined the LoQ as the lowest amount of analyte in a sample that can be quantified reliably with an intra-assay coefficient of variance (CV) of 20% based on precision profiling.

The data supports the following claims:

LoB = 0.5 pg/mL

LoD = 1.3 pg/mL

LoQ = 3.2 pg/mL

e. *Analytical specificity:*

Endogenous and exogenous interference

Endogenous and exogenous interference was evaluated according to CLSI EP07-A2 using low (27 pg/mL) and high (300 pg/mL) serum samples spiked with possible interferents. High samples were tested in replicates of six and low samples were tested in replicates of four. Test samples were compared to control samples without interferent. The sponsor considered a percent difference between test samples and control samples of >10% to be significant interference.

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

Substance	Highest Concentration Tested Without Significant Interference
Acetaminophen	0.2 mg/mL
Acetylcysteine	0.15 mg/mL
Acetylsalicylic acid	1 mg/mL
Ampicillin-Na	1 mg/mL
Ascorbic acid	0.3 mg/mL
Bazedoxifene	1 µg/mL
Bilirubin	0.66 mg/mL
Biotin*	10,000 ng/mL
Bisphosphonate (Alendronate (Fosamax))	0.02 mg/mL
Cefoxitin Na	2.5 mg/mL
Cholesterol	5 mg/mL
Citalopram	0.01 mg/mL
Cyclosporine	5 µg/mL
Cyproterone acetate	0.3 mg/mL
Doxycycline Hyclate	50 µg/mL
Escitalopram Oxalate	0.1 mg/mL
Estradiol (beta)	1 ng/mL
Estrone sulfate	1 ng/mL
Estropipate	0.015 mg/mL
Fluoxetine HCl	3.5 µg/mL
Folic Acid	0.4 µg/mL
Gabapentin	0.09 mg/mL
Hemoglobin (Human)	10 mg/mL
Heparin	30 U/mg
Ibuprofen	0.5 mg/mL
Levodopa	30 µg/mL
Levonorgestrel	3 µg/mL
Levothyroxine	0.2 µg/mL

Substance	Highest Concentration Tested Without Significant Interference
Medroxyprogesterone acetate	1 µg/mL
Metformin	2 mg/mL
Methyldopa	0.02 mg/mL
Metronidazole	0.2 mg/mL
Norethindrone	0.03 mg/mL
Paroxetine HCl	1 µg/mL
Phenylbutazone	0.1 mg/mL
Pregabalin	0.01 mg/mL
Progesterone	0.4 mg/mL
Raloxifene HCl	0.12 mg/mL
Rifampicin	60 µg/mL
Theophylline	0.1 mg/mL
Intralipid 20%	20 mg/mL
Triptorelin acetate	15 µg/mL
Venlafaxine HCl	15 µg/mL

*Though this assay uses biotin-streptavidin binding technology, no interference is observed in specimens containing up to 10,000 ng/mL biotin.

Cross-reactivity

The cross reactivity of the picoAMH ELISA assay was evaluated using pooled serum samples containing undetectable concentrations of AMH spiked with potential cross-reacting compounds. Pro+Mature recombinant human AMH was included as a positive control. All samples were tested in duplicate with a single reagent lot. The AMH measured as pg/mL after addition was used to calculate a percent cross reactivity based on the mass of potential cross reactant tested as follows:

% cross reactivity = (2.5 pg/mL divided by pg/mL cross-reactant tested) x 100

For cross-reactants yielding detectable AMH concentrations, the formula for calculating percent cross reactivity was:

% cross-reactivity = ((measured value - true value)/concentration of cross-reactant tested)*100)

None of the potential cross-reactants showed > 0.5% cross-reactivity.

Cross-Reactant	Concentration Tested (pg/mL)	AMH Value (pg/mL)	% Cross-reactivity
Activin B	50,000	< 1.3	0.003%
Inhibin A	100,000	< 1.3	0.001%
Inhibin B	100,000	< 1.3	0.001%

Cross-Reactant	Concentration Tested (pg/mL)	AMH Value (pg/mL)	% Cross-reactivity
alpha-2	65,000	< 1.3	0.002%
Follistatin-288	50,000	< 1.3	0.003%
Follistatin-315	50,000	< 1.3	0.003%
hAMH, Pro+Mature	600	627.8	104.641%
hAMH, Mature	600	< 1.3	0.217%
Myostatin	50,000	< 1.3	0.003%
FSH	39,683	< 1.3	0.003%
TSH	869,565	< 1.3	0.000%
LH	9,312	< 1.3	0.014%
Prolactin	211,000	< 1.3	0.001%
Testosterone	100,000	< 1.3	0.001%
Estrone Sulphate	100,000	< 1.3	0.001%
DHEA	100,000	< 1.3	0.001%
Progesterone	100,000	< 1.3	0.001%
Estradiol	50,000	< 1.3	0.003%

Hook effect

In order to assess hook effect, recombinant human AMH was added to pooled human serum AMH to generate a specimen containing known AMH levels at least (b) (4) the upper limit of the measuring range. The specimen was tested neat and at a series of dilutions in each of three reagent lots. The sponsor concludes that there is no hook effect on the picoAMH ELISA by AMH concentrations in human serum up to 256,000 pg/mL.

f. Assay cut-off:

Please see section 4 (clinical cut-off) below.

2. Comparison studies:

a. Method comparison with predicate device:

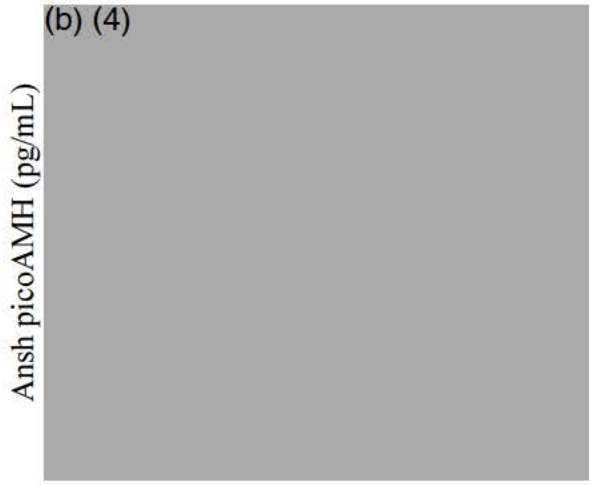
Not applicable.

b. Matrix comparison:

Not applicable. Serum is the only claimed sample matrix.

3. Clinical studies:

A clinical study was performed to evaluate the clinical performance of the device. 690 retrospective serum samples from 690 apparently healthy women ages 42.9 to 62.4 (no history of ovarian surgery, no diagnosis of PCOS, no oral contraceptive intake) participating in the Study of Women's Health Across the Nation (SWAN) study were randomly chosen and assigned to the clinical validation cohort. Each woman contributed one sample and one outcome to the study. The sponsor provided data supporting the stability of the frozen serum specimens used in the clinical validation study for the length and conditions of storage of SWAN study specimens. Menopausal status for each woman was determined based on time to final menstrual period (FMP). The sponsor defined three menopausal status categories: "> 5 years from FMP", "< 5 years from FMP", and "at FMP or later". The samples were evenly distributed across all three menopausal categories (n = 230) based on outcome and each category contained women with a range of ages.



Age distribution in each menopausal category:

Age	Menopausal category			Total
	> 5 years from FMP	< 5 years from FMP	At FMP or later	
42.9 – 45	50	12	2	64
45 – 49.9	164	108	47	319
50 – 54.9	16	103	138	257
55 – 62.4	0	7	43	50
Total	230	230	230	690

The following table describes the distribution of picoAMH ELISA test results across the three menopausal categories. Percentages indicate the agreement of the picoAMH ELISA test result with the corresponding menopausal category.

picoAMH	Menopausal category			Total
	> 5 years from FMP	< 5 years from FMP	At FMP or later	
< 10 pg/mL	19 (8.2%)	89 (38.7%)	198 (86.1%)	306
10 – 99.9 pg/mL	22 (9.6%)	80 (34.8%)	28 (12.2%)	130
≥ 100 pg/mL	189 (82.2%)	61 (26.5%)	4 (1.7%)	254
Total	230 (100%)	230 (100%)	230 (100%)	690

Clinical performance was estimated for each menopausal category. The results are summarized below:

picoAMH	Menopausal Category	Detection Rate (%) (95% CI)
≥100 pg/mL	>5 years from FMP	82.2 (76.6 – 86.9)
<10 pg/mL	at FMP or later	86.1 (80.4 – 89.9)
10-99.9 pg/mL	< 5 years from FMP	34.8 (28.6 – 41.3)

picoAMH	Classified Menopausal Category	True Menopausal Status	False Positive Rate (%) (95% CI)
≥100 pg/mL	>5 years from FMP	< 5 years from FMP	26.5 (20.9-32.7)
		at FMP or later	1.7 (0.4 – 4.4)
<10 pg/mL	at FMP or later	>5 years from FMP	8.2 (5.0-12.6)
		< 5 years from FMP	38.7 (32.4 – 45.3)
10-99.9 pg/mL	< 5 years from FMP	>5 years from FMP	9.6 (6.1-14.1)
		at FMP or later	12.2 (8.2, 17.1)

The labeling contains the following statement:

"This analysis shows that the picoAMH ELISA performs reasonably well in distinguishing women at FMP or later and women > 5 years from FMP. Specifically, 86.1% of women who were at FMP or later also had a picoAMH level <10 pg/mL and 82.2% of women who were > 5 years from FMP also had a picoAMH level > 100 pg/mL.

However, the picoAMH ELISA test result has limited deterministic value when AMH values fall between 10 and 99.9 pg/mL, and test results falling into this range should be interpreted with caution."

To aid in the interpretation of picoAMH ELISA test results obtained for women in the menopausal transition (i.e., < 5 years away from their FMP), the following table provides the likelihood ratios for the comparison of women < 5 years away from their FMP vs. adjacent menopausal categories given the AMH concentrations obtained in the clinical study.

Menopausal Category Comparison	Positive Likelihood Ratio (95% CI)	Negative Likelihood Ratio (95% CI)
< 5 years from FMP vs. at FMP or later	2.86 (1.94, 4.22)	0.74 (0.67, 0.83)
< 5 years from FMP vs. > 5 years from FMP	3.64 (2.35, 5.62)	0.72 (0.65, 0.80)

The results above show that, comparing to any woman with an unknown AMH level who has reached her FMP or is < 5 years from her FMP, a woman with an AMH test result between 10 and 99.9 pg/mL is 2.86 times more likely to be < 5 years away from her FMP than to be at FMP or later. Likewise, comparing to any woman with an unknown AMH level who is < 5 years away from her FMP or > 5 years away from her FMP, a woman with an AMH test result between 10 and 99.9 pg/mL is 3.64 times more likely to be < 5 years away from her FMP than to be > 5 years away from her FMP.

a. *Clinical Sensitivity:*

Not applicable.

b. *Clinical specificity:*

Not applicable.

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

Not applicable.

4. Clinical cut-off:

The sponsor specified two clinical cut-offs for the pico AMH ELISA assay: < 10 pg/mL to distinguish women at or later than their final menstrual period (FMP) and > 100 pg/mL to distinguish women > 5 years from FMP.

5. Expected values/Reference range:

A reference range was determined by analyzing 644 serum samples from 644 apparently healthy women enrolled in the SWAN study. The picoAMH test results for these samples was then stratified into four different age groups and percentiles calculated. The results are as follows:

Age (years)	N	picoAMH ELISA test result centile					CI for 95th centile
		5th	25th	50th	75th	95th	
42.9 to 44.9	159	<6	100	390	1,200	2,900	2,200 – 3,500
45.0 to 49.9	175	<6	3.1	75	280	640	700 – 1,500
50.0 to 54.9	175	<6	<6	<6	16	98	160 – 350
55.0 to 62.4	135	<6	<6	<6	<6	28	6 – 110

M. Proposed Labeling:

The labeling supports the decision to grant the De Novo request for this device.

N. Identified Risks and Identified Mitigations:

Identified Risks to Health	Identified Mitigations
Incorrect test results	General controls and special controls (1) and (2)
Incorrect interpretation of test results	General controls and special controls (1) and (2)

O. Benefit/Risk Analysis:

Summary of the Assessment of Benefit
For the Proposed Indications for Use

Natural menopause is determined after a women has experienced 12 months of amenorrhea without any other obvious pathological or physiological cause. Knowing in what timeframe a woman may become menopausal may prompt preventive approaches for loss in bone mineral density and cardiovascular disease, which are known to increase after menopause.

Summary of the Assessment of Risk
For the Proposed Indications for Use

The main risks associated with the test are a falsely low or falsely high AMH level. A falsely low value may incorrectly indicate that a woman was post-menopausal or closer to her final menstrual period (FMP). In this case, a clinician may have a lower threshold to discontinue contraception, and thus may increase the risk of pregnancy. However, this risk is only as high

as the differential likelihood of pregnancy, considering age and other factors contributing to pregnancy risk for the individual. A falsely low value may also increase the risk of prescribing hormone replacement therapy in a woman who is not yet menopausal. However, hormone replacement therapy may be prescribed based on menopausal symptoms which may occur during the menopausal transition and would be prompted based on the severity of the symptoms, more so than post-menopausal status. According to the American College of Obstetricians and Gynecologists Practice Bulletin on the management of Menopausal Symptoms, "the decision to continue [systemic hormone therapy] should be individualized and be based on a woman's symptoms and the risk-benefit ratio, regardless of age."

A falsely high AMH value may incorrectly indicate that a woman was not post-menopausal or farther from her final menstrual period. In a woman with irregular bleeding which may be confused with menopause transition bleeding, a falsely high AMH value may delay the evaluation of endometrial cancer. However, the character of bleeding (e.g., increased bleeding or more frequent bleeding) is the main determination for suspecting endometrial cancer. If the bleeding described by a patient is concerning for features of endometrial cancer, the AMH test result would not likely impact the standard of care for the work up of endometrial cancer. Additionally, continued symptoms of irregular bleeding would prompt a clinician to perform endometrial cancer evaluation without further delay.

There is a risk for incorrect interpretation of test results. The clinical study described above was designed to detect menopause rather than predict menopause since the study analysis categorized women by the outcome (FMP) first, then correlated an AMH value, instead of predicting a clinical outcome based on a measured AMH value. Therefore, the use of the test is limited to aiding in detecting a women's menopausal category (more than 5 years from her expected FMP, within 5 years from FMP, or at FMP or later) and cannot be used to predict an individual woman's FMP or fertile lifespan.

Summary of the Assessment of Benefit-Risk
For the Proposed Indications for Use

Given that there are possible risks associated with an incorrect test result and the incorrect interpretation of test results, the benefit-risk balance of this device is undetermined and requires additional mitigations in the form of limitations and special controls, beyond general controls.

Summary of the Assessment of Benefit-Risk, considering risk mitigation strategies
For the Proposed Indications for Use

Overall, the likelihood of benefit of the picoAMH test to detect postmenopausal status and prompt preventive strategies for conditions associated with menopause outweighs the likelihood of pregnancy due to a falsely low AMH level or the likelihood of delaying an opportunity for endometrial cancer evaluation due to a falsely high AMH level, when considering the mitigations provided by the limitations and special controls, beyond general controls.

P. Conclusion:

The information provided in this de novo submission is sufficient to classify this device into class II under regulation 21 CFR 862.1093. FDA believes that the special controls, in combination with the general controls provide a reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code: QDH
Device Type: Menopause test system
Class: II (special controls)
Regulation: 21 CFR 862.1093

a) Identification: A menopause test system is an in vitro diagnostic device intended to measure hormones or other analytes in human clinical specimens as an aid in the determination of menopausal status in women.

b) Classification: Class II (special controls). A menopause test system must comply with the following special controls:

(1) Design verification and validation must include the following:

(i) An appropriate traceability plan to minimize the risk of drift in the menopause test system results over time.

(ii) Detailed documentation of a 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 appropriate clinical performance relative to a well-accepted and appropriate comparator.

(B) Data must demonstrate accuracy of device output for each indicated specimen type throughout the device measuring range as appropriate for the intended use population.

(2) The 21 CFR 809.10 labeling must include the following:

(i) A statement in the intended use that the device is intended to be used for the determination of menopausal status only in conjunction with other clinical and laboratory findings prior to any diagnostic or treatment decisions.

(ii) A limiting statement that the device is intended to be used for the determination of menopausal status only in conjunction with other clinical and laboratory findings prior to any diagnostic or treatment decisions.

(iii) A limiting statement appropriately describing the risks of false test results and that test results should not be relied upon in clinical decision making

(e.g., to discontinue contraceptive use and/or to evaluate patients for the presence of endometrial cancer) without other clinical and laboratory findings.