



Laboratory Procedure Manual

Analyte: anti-Müllerian Hormone
Matrix: Serum and Plasma
Method: anti-Müllerian Hormone Immunoassay

Method No: 1046

Revised:

As performed by:

Clinical Biomarkers Research Laboratory
Clinical Chemistry Branch
Division of Laboratory Sciences
National Center for Environmental Health

Contact: Hubert W. Vesper, Ph.D.
Phone: 770-488-4191
Fax: 404-638-5393
Email: HVesper@cdc.gov

James Pirkle, M.D., Ph.D.
Director
Division of Laboratory Sciences, NCEH

Important Information for Users

CDC periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

	VARIABLE NAME	SAS LABEL (and SI units)
TST_K_R	LBXAMH	Anti-Mullerian hormone (ng/mL)
	LBDAMHSI	Anti-Mullerian hormone (pmol/L)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Clinical and Public Health Relevance

Anti-Müllerian hormone (AMH) or Müllerian-inhibiting substance, a dimeric glycoprotein secreted by granulosa cells of the preantral and small antral ovarian follicles, has been implemented in various clinical applications including assisted reproduction, reproductive disorders, and the assessment of ovarian health since the development of the first AMH immunoassay in 1990.¹ AMH is also involved in ovarian folliculogenesis.^{2,3} Follicle development in the ovaries is composed of two distinct stages: [1] initial recruitment or the maturation of primordial follicles, and [2] cyclic recruitment, which leads to the cohort growth of small antral follicles.⁴ AMH expression begins in the primary follicles and is at the highest levels in the granulosa cells of preantral and small antral follicles (ranging 2-6 mm in diameter)⁵. AMH expression supports the inhibitory role of AMH at two distinct stages of folliculogenesis.^{6,7} First, AMH has an important role in regulating the number of follicles left in the primordial pool by inhibiting the transition of follicles from primordial into maturation stages. Second, AMH has a role in follicular inhibitory effects by influencing follicular sensitivity to follicle-stimulating hormone (FSH).⁸

Serum AMH levels are initially very low for females after birth and highest after puberty.⁸ AMH levels progressively decrease after puberty until levels become undetectable at menopause.^{9, 10} Studies suggest the utility of AMH for assessing ovarian reserve and antral follicle count as serum levels remain stable during menstruation.^{1, 11, 12} Serum AMH levels have been reported to decrease upon the use of contraceptives.¹³ Recent clinical findings highlight the applicability of AMH to predict the onset of menopause, serve as a marker for ovarian reserve¹⁴, and monitor disruptions in ovarian function (e.g., polycystic ovarian syndrome and ovarian toxicity)¹. AMH has also been proposed as a biomarker to assess child development. In addition, the measurement of serum AMH levels has been clinically used to assess ovarian reserve through correlations with the number of antral and preantral follicles, also referred to as the antral follicle count (AFC).⁸

Test Principle

This test is based on the reaction of AMH with immuno-antibodies and chemo-luminescence measurements of the reaction products. It consists of 2 incubation steps and a chemiluminescent measurement obtained with a photomultiplier tube that spans 18 minutes.¹⁵ The first incubation period begins by sandwiching the sample of AMH containing serum or plasma between a biotinylated monoclonal AMH-specific antibody and a monoclonal AMH-specific antibody that is labeled with ruthenium. The second incubation entails the addition of streptavidin-coated microparticles to the sample mixture. The microparticles bind to the solid phase via biotin and streptavidin interactions. The resulting sample mixture is then aspirated into a measuring cell that is subjected to a magnetic field. This captures the microparticles on an electrode. The remains of the sample mixture are subsequently washed out of the measuring cell. A voltage is applied to the electrode causing a chemiluminescent reaction that is measured by a photomultiplier tube. The readings are compared to an instrument- and lot-specific calibration curve.

Scope

The measurement procedure described in this document is intended for quantitatively measuring AMH in human serum and plasma. It addresses all aspects related to the measurement process (specimen collection, storage, processing, analysis, and reporting). This method was evaluated for AMH measurements in human serum and plasma matrices only.

Specific details related to equipment maintenance and operations are provided in the Roche/Hitachi **cobas e 411 Analyzer Operator's Manual**.¹⁶ Furthermore, this document is not intended to provide information on data interpretation.

2. SAFETY PRECAUTIONS

General Safety

All serum specimens should be considered potentially positive for infectious agents including HIV and the hepatitis B virus. Hepatitis B vaccination series are required for all analysts performing this measurement procedure.

Universal precautions should be observed: protective gloves, laboratory coats, and safety glasses must be worn at all times during all tasks of this measurement procedure.

Disposable bench covers must be used during sample preparation and sample handling and must be discarded after use. All non-metal work surfaces must be wiped with 10% bleach solution after work is finished. Metal work surfaces in the biological safety cabinet and the **cobas e 411** analyzer must be wiped with a non-bleach disinfectant.

Chemical Hazards

All chemical products used in this measurement procedure must be handled while wearing personal protective equipment and with extreme care.

For additional information about specific reagents provided by Roche as part of the reagent kit consult Roche Diagnostics' Material Safety Data Sheets available in the laboratory and online at <http://www.mylabonline.com>.¹⁷

The safety precautions for specific products are as follows:

BlankCell: (Roche product# 11729306122)

Eye contact: Contact with eyes may cause irritation

Skin contact: Substance may cause slight skin irritation

Ingestion: Ingestion may cause gastrointestinal irritation, nausea, vomiting, and diarrhea

Inhalation: Avoid breathing vapors or mists. Irritating to respiratory system.

Sensitization or Odor threshold: None

Medical conditions aggravated by exposure: None

Additional information: None

CleanCell: (Roche product# 11662970122)

Eye contact: Severe eye irritation. This chemical may cause eye pain, redness, blurred vision, and/or burns.

Skin contact: May cause irritation on contact with skin. Prolonged skin contact causes burns.

Ingestion: Ingestion causes burns of the upper digestive and respiratory tracts

Inhalation: May cause irritation of respiratory tract. May cause coughing.

Sensitization or Odor threshold: None

Medical conditions aggravated by exposure: Persons with pre-existing skin disorders, impaired liver, or pulmonary function may be more susceptible to the effects of this material.

Additional information: None

Diluent Universal: (Roche product# 03183971)

Eye contact: Contact with eyes may cause irritation

Skin contact: None

Ingestion: Ingestion may cause gastrointestinal irritation, nausea, vomiting, and diarrhea

Inhalation: None

Sensitization or Odor threshold: None

Medical conditions aggravated by exposure: None

Additional information: None

PreciControl AMH: (Roche product# 06709966190)

Eye contact: Contact with eyes may cause irritation

Skin contact: None

Ingestion: Ingestion may cause gastrointestinal irritation, nausea, vomiting, and diarrhea

Inhalation: None

Sensitization or Odor threshold: None

Medical conditions aggravated by exposure: None

Additional information: It is recommended to handle this product in accordance with normal precautions required for handling all laboratory reagents. Disposal of all waste material should be in accordance with local guidelines.

Procell: (Roche product# 11662988122)

Eye contact: Severe eye irritation

Skin contact: Substance may cause slight skin irritation

Ingestion: Ingestion may cause gastrointestinal irritation, nausea, vomiting and diarrhea

Inhalation: May cause irritation of respiratory tract

Sensitization or Odor threshold: None

Medical conditions aggravated by exposure: None

Additional information: The toxicological properties of Oxaban A have not been investigated. Oxaban A, an anti-bacterial preservative, is present at less than or equal to 0.1%. Oxaban A degrades to release formaldehyde at a pH below 6. If all of the Oxaban A degraded to formaldehyde in the analyzer's waste, the total theoretical maximum concentration of formaldehyde in the waste would be 30.0 to 32.6 mg/L.

Elecsys AMH: (Roche product# 06331076160)

Eye contact: Contact with eyes may cause irritation

Skin contact: May cause irritation on contact with skin

Ingestion: Ingestion may cause gastrointestinal irritation, nausea, vomiting, and diarrhea

Inhalation: None

Sensitization or Odor threshold: The following chemical may cause sensitization, be absorbed via skin, and/or have an odor threshold for detection:

1. 3(2H)-Isothiazolone, 2-methyl-, hydrochloride (1:1)- skin sensitizer

Medical conditions aggravated by exposure: Persons with pre-existing skin disorders may be more susceptible to the effects of this material.

AMH CalSet: (Roche product# 06331084190)

Eye contact: Contact with eyes may cause irritation

Skin contact: May cause sensitization by skin contact. Substance may cause slight skin irritation

Ingestion: Ingestion may cause gastrointestinal irritation, nausea, vomiting, and diarrhea

Inhalation: Avoid breathing vapors or mists. May cause irritation of respiratory tract

Sensitization or Odor threshold: The following chemical may cause sensitization, be absorbed via skin, and/or have an odor threshold for detection:

1. 2-methyl-2H-isothiazol-3-one hydrochloride – skin sensitizer

Medical conditions aggravated by exposure: None

Additional information: None

AMH CalCheck 5: (Roche product# 06331092190)

Eye contact: Contact with eyes may cause irritation

Skin contact: May cause irritation in contact with skin

Ingestion: Ingestion may cause gastrointestinal irritation, nausea, vomiting, and diarrhea

Inhalation: Avoid breathing dust. Dust irritating to respiratory tract

Sensitization or Odor threshold: None

Medical conditions aggravated by exposure: None

Additional information: Exercise the normal precautions required for handling all reagents.

SysWash: (Roche product# 11930346122)

Eye contact: May cause irritation

Skin contact: Substance may cause slight skin irritation

Ingestion: Ingestion may cause gastrointestinal irritation, nausea, vomiting, and diarrhea

Inhalation: Avoid breathing vapors or mists. May cause irritation of respiratory tract

Sensitization or Odor threshold: The following chemical may cause sensitization, be absorbed via skin, and/or has an odor threshold for detection:

1. Methylisothiazolone (MIT)- skin sensitizer

Medical conditions aggravated by exposure: None

Additional information: None

Additional information: All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods applied were approved by the FDA. All human material should be considered potentially infectious. It is recommended to handle this product in accordance with Universal Precautions and the OSHA Bloodborne Pathogen Standard.

Radioactive Hazards

There are no radioactive hazards associated with this measurement procedure.

Mechanical Hazards

There are only minimal mechanical hazards when performing this procedure using standard safety practices. Analysts must read and follow the manufacturer's information regarding safe operation of the equipment. Generally, mechanical and electronic maintenance and repair must only be performed by qualified technicians or trained personnel. Follow the instructions in the Roche/Hitachi **cobas e 411 Analyzer Operator's Manual**¹⁶ located in the Clinical Chemistry Branch Service Laboratory.

Waste Disposal

All solid waste used in the sample preparation process (e.g., disposable plastic pipette tips, gloves, bench covers, caps, etc.) as well as any residual sample material must be placed into the appropriate biohazard autoclavable bags and waste pans until sealed and autoclaved.

All sharps (e.g., broken glass) must be placed in appropriate sharps containers along with any other metal containing materials.

All liquid waste must be disposed of in accordance with CDC policies using the appropriate waste management and chemicals tracking systems.

All waste disposals must be performed in compliance with CDC policies and regulations. The CDC Safety Policies and Practices Manual are located in the laboratory and can be accessed at http://isp-v-ehp-asp/dlsintranet/safety_manual/.

Training

Analysts performing this measurement procedure must, at a minimum, successfully complete the following safety courses as required by Centers for Disease Control and Prevention and Division of Laboratory Sciences (DLS).

- Safety Survival Skills Parts 1 and 2
- Bloodborne Pathogens
- Hazardous Chemical Waste Management
- Information Security Awareness Training
- Records Management Overview
- Occupant Emergency Plan

Furthermore, analysts must receive training on the specific instrumentation used with this measurement procedure from designated staff or the instrument manufacturer.

At a minimum, analysts performing this measurement procedure must be familiar with the

- Lab-specific Chemical Hygiene Plan
- Lab-specific Exposure Control Plan
- Relevant MSDS

- DLS Policies and Procedures Manual
- DLS After-Hours Work Policy
- Policy on confidentiality, data security and release of information
- Proper use of biological safety cabinets

3. COMPUTERIZATION AND DATA-SYSTEM MANAGEMENT

Software and Knowledge Requirements

The **cobas e 411** instrument is computer operated. Please refer to the Roche/Hitachi *cobas e 411 Analyzer Operator's Manual*¹⁶ for instructions on use of the software. Specific training to operate this software by a Roche Diagnostics instructor, or a qualified staff member, is required to ensure appropriate and safe instrument use.

Data obtained from the **cobas e 411** instrument are transferred to the Division of Laboratory Sciences (DLS) Laboratory Data Information System (STARLIMS), which was created and is maintained by DLS staff. Data transfer is performed by specifically trained and assigned staff.

Assessment of bench quality control (QC) results is performed using a program created with Statistical Analysis System (SAS) Inc. software, and it is integrated into STARLIMS. The SAS software is maintained by DLS staff.

Sample Information

All sample vials must be labeled as described in the latest version of the DLS Policies and Procedures Manual and according to the work instructions set in place in the PBL laboratory. No personal identifiers are used and all samples are referenced to a blind coded identifier.

Information about samples and related analytical data are checked prior to being entered into the STARLIMS system for transcription errors and overall validity. Filing of electronic and physical files and their maintenance is the responsibility of designated staff in the Protein Biomarker Laboratory. STARLIMS is routinely backed up by CDC Information Technology Services Office (ITSO). ITSO must be contacted for emergency assistance via email (itservice@cdc.gov) or by phone (404-639-6000).

Information Security

Information security is managed at multiple levels. Information management systems that contain the final reportable results are restricted through user ID and password security access. Computers and instrument systems that contain the raw and processed data files require specific knowledge of software manipulation techniques and physical location. Site security is provided through restricted access to the individual laboratories, buildings, and offices. Confidentiality of results is protected by referencing results to blind coded sample IDs (no names or personal identifiers).

4. PREPARATION OF REAGENTS, CALIBRATION MATERIALS, CONTROL MATERIALS, AND ALL OTHER MATERIALS; EQUIPMENT AND INSTRUMENTATION.

The chemicals, equipment, and other materials described below, or equivalents, are used in this measurement procedure. It is recommended to only use reagents, calibrators and other solutions that are suitable, as stated by the material provider, for the instrumentation used in this procedure. The reagents for this assay are provided as kits by Roche Diagnostics.

Equipment, Instrumentation, and Supplies Used For Sample Analysis

1. Roche/Hitachi **cobas e 411** analyzer (immunoassay analysis). Roche Diagnostics, 9115 Hague Road, Indianapolis, IN 46250. For instrument settings see the most recent version of the instructions provided by the manufacturer.
2. Ovation BioNatural 20-200 µL pipette (VistaLab Technologies, Brewster, NY)
3. Rotator to homogenize samples: Adams Nutator (model# 1105, serial# 0586030) and Fisher Scientific hematology mixer (model# MIXER, serial# 2291M003)
4. Sample cups (standard): Roche Diagnostics, product# 10394246001
5. Purified water supplied via a combination reverse osmosis and DI system installed and maintained by Aqua Solutions, Inc.
6. Calibrator: AMH CalSet, Roche Diagnostics, product# 06331084190
7. Control: PreciControl AMH, Roche Diagnostics, product# 06709966190
8. SysWash: Roche Diagnostics, product# 11930346122
9. Assay Tips: Roche Diagnostics, product# 1706799
10. Assay Cups: Roche Diagnostics, product# 1706802

Preparation of Reagents Used For Sample Analysis

Roche reagents are ready to use. Do not mix or invert the reagent bottles due to the microparticles needing to remain on the bottom of the containers. If there are residual microparticles near the mouth of any of the containers, Roche should be contacted to order a replacement. Reagent bottles may be stored on-board (in the climate controlled reagent well of the **cobas e 411**) or recapped and stored at 2-8°C until the expiration date determined by Roche (see package insert).

The lot number, expiration date, and date opened is documented for the reagents used in this method.

Reagents are loaded in the instrument as described by the instrument manufacturer (see the Roche/Hitachi *cobas e 411 Analyzer Operator's Manual*¹⁶ for directions).

Preparation of Calibration Materials Used For Sample Analysis

Elecsys AMH CalSet is a bi-level material used for the calibration of quantitative Elecsys AMH assays. The CalSet kit provided by Roche includes lyophilized equine (Cal1) and bovine (male and female) (Cal2) serum. A particular lot of calibrator can be used for any lot of reagent.

Each calibrator concentration is lot-specific and each lot is encoded in the barcodes as well as printed on the package insert. The calibrators are designed to provide 1.0 mL of working solution. Calibrator 1 has an AMH concentration of approximately 0.04 ng/mL and Calibrator 2 has an AMH concentration of approximately 8 ng/mL.

Dissolve the contents of each bottle of calibrator by adding exactly 1.0 mL of DI water to the bottle. Allow the mixture to stand in the closed container for 15 minutes followed by gentle swirling for 1 minute, being careful to avoid the formation of foam. Transfer aliquots of reconstituted calibrator into empty labeled CalSet Vials (snap-cap vials) and immediately store in the CCB Service Lab's -70 °C freezer. Calibrators are stable for 4 weeks (28 days) at -20 °C and for one day once thawed.

Preparation of Quality Control Materials Used for Sample Analysis

Quality control (QC) materials are used to aid in the detection and correction of problems in a laboratory's analytical processes in order to assure quality results are reported by the laboratory. QC materials should be the exact matrix as the samples being analyzed or as close as possible to the sample matrix under investigation. QC materials are run at the beginning of each analytical run, after every 50 samples, and at the end of the run to monitor the performance of the instrument throughout the entire analytical run.

LSP and SHBG pools, previously collected from individual human donors, are prescreened on the Roche **cobas** e 411 for AMH content. The LSP and SHBG pools were prepared from units that were combined, aliquoted into individual cryovials in 1 mL increments, and frozen at -70 °C until use. LSP and SHBG pools were assigned to each level of the AMH pools (e.g., low, mid, and high levels).

Commercially prepared control materials such as PreciControl AMH can also be used. The lot numbers, expiration dates, and date opened are documented for all commercial controls used.

Records with certificates of analysis for the human serum used to prepare in-house QCs, as well as package inserts for commercially prepared QCs, are maintained in the laboratory.

The desired concentration ranges for the low, mid, and high in-house AMH QC pools are 0.08 – 0.54, 1.0 – 2.0, and 3.03 – 5.63 ng/mL, respectively.¹⁸

Control values for the bench QC materials should be entered into the cobas e 411 before analyzing a new lot number of control. See the Roche/Hitachi cobas e 411 Analyzer Operator's Manual⁷ for directions on how to enter values for control materials.

Quality control materials are characterized following guidance provided in the DLS Policies and Procedures Manual and Clinical and Laboratory Standards Institute (CLSI) documents¹⁹, EP5-A3 (Evaluation of Precision Performance of Quantitative Measurement Methods)²⁰, EP10-A3 (Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures)²¹, and

EP15-A3 (User Verification of Performance for Precision and Trueness)²². Records with package inserts for the commercial control material are maintained in the laboratory.

Preparation of In-House Quality Control (QC) Serum Pools

LSP and SHBG pools were previously prepared and used as AMH pools. To achieve concentrations within the desired ranges, pools were selected with enough volume to sustain the low, medium, and high AMH bench QCs for 2 years. For further details on the preparation of the SHBG QC pools and LSP pools used for this method, please refer to work instruction PBLW10SH20 (Preparing In-House Bench Quality Control Pools for the Measurement of SHBG) and the CLSI C37A guideline²³ by Solomon Park Research Laboratories, respectively.

Each LSP and SHBP pool was prescreened (for sample preparation and instrument operation, please refer to section 6 below) on the **cobas e 411** to obtain AMH content. Following the prescreening analysis, a suitable SHBG pool was chosen to create the low and high AMH bench QC serum pools. A suitable LSP pool was chosen to create the medium AMH bench QC serum pool.

5. PROCEDURE FOR COLLECTING, STORING, AND HANDLING SPECIMENS; CRITERIA FOR SPECIMEN REJECTION

General Specimen Requirements

A minimum of 230 µL of plasma/serum is needed for measurement of serum AMH on the **cobas e 411**. A sample volume of 460 µL is preferred in order to allow for repeat analyses. Serum collected using standard sampling tubes or tubes containing separating gel, or lithium heparin plasma are considered acceptable by the test manufacturer. EDTA plasma should not be used. Samples containing precipitates should be centrifuged prior to testing. Because of possible evaporation effects, all samples, calibrators, and QCs on the analyzer should be measured within 2 hour.

Specimens should be transported in 2.0-mL cryogenic vials with external screw-caps. These cryovials should be labeled in accordance with CDC and DLS policies and regulations. Other specimen handling conditions are outlined in the Policies and Procedures Manual of the Division of Laboratory Sciences (DLS). Barcodes are scanned upon receipt of the samples, during the process of sample preparation, and during sample transfer in order to ensure that individual samples can be tracked throughout the process. The instructions for creating barcode labels are in work instruction PBLW00LT01_Procedure for barcode labeling. Additional specimen handling instructions are outlined in the DLS Policies and Procedures Manual.

Based on the experiments performed in Appendix A (Method Performance Documentation), specimens collected in the field are frozen and then shipped on dry ice by overnight mail. Samples received cooled at 4 °C ± 1 °C after overnight shipment are also acceptable.

Receiving Specimen

Sample information and related study information are entered in the laboratory information system upon receipt of samples.

Acceptability of samples is assessed upon receiving in the laboratory.

Acceptable samples are registered by scanning the sample ID barcode and estimating the specimen volume.

Specimen Storage

Samples should be stored frozen at -70 °C upon receipt unless they are to be analyzed immediately.

Samples should be analyzed immediately after thawing and reaching room temperature.

The unopened PreciControl AMH is stable at -70 °C until the expiration date specified on the package. Once opened, reconstituted, and aliquoted, (400 µL into each cryovial) controls are stable for 4 weeks at -70 °C. Controls should be allowed to come to room temperature before analyzing.

The manufacturer suggests that the PreciControl AMH is stable for up to 3 hours at 15 - 25°C.

While multiple freeze thaw cycles should be avoided, samples are stable up to three freeze-thaw cycles.

Unacceptable Specimens

Specimens that do not meet the above mentioned criteria (e.g., minimum sample volume, were transported at room temperature, have evidence of leakage, and/or are in a condition that may compromise sample integrity) are not acceptable.

6. PROCEDURES FOR OPERATION, INSTRUCTIONS, CALCULATIONS, AND INTERPRETATION OF RESULTS

All instruments are checked before use for correct function using the manufacturer's acceptance criteria.

Specimen Storage and Handling during Testing

1. Specimens are allowed to reach room temperature for sample preparation. The volume of each sample must be recorded prior to withdrawing an aliquot from the original sample

vial, and the amount withdrawn must be recorded for inventory purposes. Alternatively, samples in cryovials can be placed directly into 3D-printed cryovial inserts and transferred to the Roche barcoded cups, which are then loaded onto the analyzer for analysis. Details on the alternative approach using the cryovial inserts can be found in work instruction PBLW24MS01 (Cobas e 411 Basic Operating Procedures for AMH Testing).

2. After aliquoting, samples in the original sample vials may be stored at -70°C until it is determined that the final QCs for the run have passed and no samples need to be retested. If no samples require re-testing, the sample may be stored long-term at -70°C . If samples need to be retested at a later time, a fresh aliquot must be used from the original sample vial. If the cryovial is sampled directly using the 3D-printed cryovial holder, the sample can be reused for future sample analysis.
3. Due to possible evaporation effects, samples, calibrators, and controls on the analyzer should be analyzed/measured within two hours once placed in the instrument. Samples that have been aliquoted into sample cups for use on the analyzer cannot be reused for future sample analysis.

Preparation of Samples for Analysis

All patient samples are tested with two sets of QC samples per 50 samples (one set in the beginning and another set at the end of the 50 samples). A total of 100 patient samples can be processed in one run. Therefore, a total of 4 sets of QC samples should be included. Samples are transferred into Roche sample cups using the PerkinElmer JANUS Automated Liquid Handling System. Details on preparation of samples for processing can be referenced from the work instruction PBLW10SH22 (Serum Sample Preparation via JANUS Automated Liquid Handling System). Alternatively, special custom cryovial holders can be used to place original sample vial directly on the clinical analyzer.

Operation of the cobas e 411 analyzer

The **cobas e 411** analyzer must be used for AMH quantification of serum samples. Operation of the **cobas e 411** analyzer must only be performed by trained personnel. The operation of this analyzer consists of preliminary maintenance procedures, calibration procedures, QC verification procedures, and routine sample processing procedures. Details on operation of the **cobas e 411** analyzer are found in work instruction PBLW24MS01 (**Cobas e 411** Basic Operating Procedures for AMH Testing).

7. CALIBRATION AND CALIBRATION VERIFICATION

Manufacturer recommended calibration procedures are followed for AMH testing using manufacturer reagent packs. Details on calibration of the **cobas e 411** analyzer are found in work instruction PBLW10RP23 (**Cobas e 411** Calibration Procedures).

8. METHOD PERFORMANCE CHARACTERISTICS

Method performance documentation for this method including accuracy, precision, sensitivity, specificity and stability is provided in Appendix A of this method documentation.

The signatures of the branch chief and director of the Division of Laboratory Sciences on the first page of this procedure denote that the method performance is fit for the intended use of the method.

Reportable Range of Results and Linearity limits

The measurement range for this method is 0.03-23.0 ng/mL (defined by the manufacturer as the Limit of Quantitation and the maximum of the master curve). Values below the Limit of Quantitation are reported as < 0.03 ng/mL. Values above the measurement range are automatically 2-fold diluted as recommended by the manufacturer. Thus, the reportable range of the assay is 0.03-46.0 ng/mL. Results from samples diluted by the rerun function are automatically multiplied by the dilution factor used when ordering the test.

Limit of detection (LOD)

The limit of detection (LOD), as determined by the assay manufacturer, is 0.01 ng/mL. The limit of Quantitation (LOQ), as determined by the assay manufacturer, is 0.03 ng/mL and the Limit of Blank (LOB) is 0.007 ng/mL.⁸ According to the manufacturer, the LOD and LOQ were determined in accordance with the requirements of the CLSI (Clinical Laboratory Standards Institute) EP17-A2.²⁴ The LOD is determined based on the LOB and the standard deviation of low concentration samples. The LOD corresponds to the lowest analyte concentration which can be detected (value above the LOB with a probability of 95%). The LOB was the 95th percentile value from $n \geq 60$ measurements of analyte-free samples over several independent series. The LOB corresponds to the concentration below which analyte-free samples were found with a probability of 95%. For the AMH assay, the LOQ was determined as the lowest concentration of the analyte that could be quantified with an intermediate precision of $\leq 20\%$ as determined by the manufacturer.

Accuracy (Trueness and Precision)

Imprecision of the method was determined according to CLSI guide EP5-A3²⁰ by analyzing 3 levels of QC materials in duplicate on 20 different days, and in addition, according to the DLS Policy and Procedure Manual by analyzing 2 levels of bench QC materials in duplicate on 10 different days.

Within-run, between-run and total precision were calculated. Total precision using DLS method was 2.94% and 3.10% for two levels of material and did not exceed 15% relative standard deviation (coefficient of variation (CV). Please refer to Appendix A for more details.

	Quality Material 1	Quality Material 2
Within Run	2.35%	2.11%

Between Run	1.77%	2.26%
Total	2.94%	3.10%

The imprecision of the method is reflected in the variance of QC materials data with different concentration levels analyzed over time. Records of the method's precision are maintained on the network drive for the PBL laboratory. The evaluation of within and among run imprecision using both methods are very similar. The concentration of the QC ranges are applicable to the method.

No commercially-available reference materials exist for AMH. Due to the unavailability of a well-characterized pure AMH material, which is a large glycoprotein, spiking with the pure compound material would lead to non-commutability of the sample. Therefore, accuracy was assessed by performing a mixing experiment. This was done by mixing the low and high in-house QC materials at different volume ratios (25%, 50%, and 75%). For further details on the accuracy by mixing experiment, please refer to work instruction PBLW24CL01 (Test Procedure for Accuracy of Assay by Mixing Using Roche Cobas e411 Analyzer). Recovery was calculated from the expected values.²⁵ The results of the accuracy test for the measurement of serum AMH using the Roche **cobas** e 411 are shown in the table below.

Accuracy Mix	Measured Value (ng/mL)	Expected Value (ng/mL)	Recovery (%)
25%	1.37	1.46	94%
50%	2.37	2.46	96%
75%	3.42	3.47	98%

The accuracy, as determined by these recovery experiments, is found on average to be 96% for AMH. The accuracy was found to be acceptable for the mixing experiment.

Limitations of Method, Interfering Substances and Conditions

Analytical specificity was verified by testing the effect of potential interfering substances in method development by commercial assay manufacturer.

- The assay is unaffected below the concentrations measured by icterus (bilirubin $\leq 1129 \mu\text{mol/L}$ or $\leq 66 \text{ mg/dL}$), hemoglobin (Hb $\leq 0.621 \text{ mmol/L}$ or $\leq 1.0 \text{ g/dL}$), lipemia (Intralipid $\leq 1000 \text{ mg/dL}$), biotin ($\leq 143 \text{ nmol/L}$ or $\leq 30 \text{ ng/mL}$), Rheumatoid factors ($\leq 1000 \text{ IU/mL}$), IgG ($\leq 2.5 \text{ g/dL}$), IgA ($\leq 1.8 \text{ g/dL}$), and IgM ($\leq 0.5 \text{ g/dL}$)
- *In vitro* tests were performed on 20 commonly used pharmaceuticals. No interference with the assay was found.
- Samples should not be taken from patients receiving therapy with high biotin doses (i.e., $> 5 \text{ mg/day}$) until at least 8 hours following the last biotin administration.
- No interference was observed from rheumatoid factors up to a concentration of 1000 IU/mL.
- There is no high-dose hook effect at AMH concentrations up to 1400 ng/mL.
- The monoclonal antibodies have a high specificity for AMH. No cross-reactivities were found for inhibin A (100 ng/mL), activating A (100 ng/mL), LH (500 mIU/mL), and FSH (500 mIU/mL).

Although measures were taken to minimize interference caused by human anti-mouse antibodies, erroneous findings may be obtained from samples taken from patients who have been treated with monoclonal mouse antibodies or have received them for diagnostic purposes.

As this assay must be performed according to the manufacturer's specifications, none of the parameters can be altered. Therefore, ruggedness testing cannot be performed for this assay.

8.5 Stability

Sample stability was evaluated using two levels of QC material. Three replicates of each QC material were freshly prepared for the evaluation. Stability was evaluated using three replicates of each QC material. The following stability assessments were performed: freeze-thaw stability, bench-top stability, and processed sample stability. The mean values from the replicates in the freeze/thaw stability experiment were within 15% of the established values for each QC material after 3 freeze/thaw cycles. Bench top stability at room temperature was assessed after 2 h and 24 h, and was within 15% of the established value. Processed sample stability was evaluated at 1 h, 2 h, and 24 h. The samples were stable on board (ready for analysis) for 1 hour. At the 2 hour mark the bias was deviating 8% from the established value, and at 4 h it was 13%. Therefore, the samples have to be processed within 4 h of being placed in the instrument.

Long term stability could not be evaluated for AMH, as the assay is being established on the Cobas e411 system.

9. QUALITY ASSESSMENT AND PROFICIENCY TESTING

Quality assessment activities for this measurement procedure follow the requirements outlined in the DLS Policies and Procedures Manual.

Quality Control Procedures

Quality Control Materials

Bench QC materials (tri-level) are used in this measurement procedure. The intent of QC material is for the analyst to evaluate the performance of the analytical system. The QC materials are inserted in each sample batch and processed the same as the patient samples. When each component part of the bench QC material, or commercially purchased QC material, is run as a respective set on the **cobas e 411** analyzer, the concentrations span the low to high ranges for serum AMH. For further details about the bench QC materials see section 4.4.

Establishing QC Limits and Quality Control Evaluation

Acceptance criteria for values obtained with the bench QC materials ("QC limits") are established according to the procedure described by Caudill et al.²⁶

The rules described in the most recent version of the DLS Policies and Procedures Manual⁹ together with the acceptance criteria are applied to measurement results obtained with the QC materials. Sample runs are rejected, if:

- one bench QC result is beyond the characterization mean $\pm 4SD$
- one bench QC result is outside a 3SD limit
- current and previous bench QC results are outside the same 2SD limit
- current and previous 9 run results are on same side of the characterization mean
- current and previous run results differ by more than 4SD

For further details, see the DLS Policies and Procedures Manual. Quality control evaluation is performed using a SAS program developed and maintained by DLS.¹⁹

Remedial Action if Calibration or QC Systems Fail to Meet Acceptable Criteria.

When results of control or calibration materials fail to meet the laboratory's established criteria for acceptability, all patient test results obtained in the unacceptable test run and since the last acceptable test run must be considered adversely affected, and thus cannot be reported. Specimen processing and analysis is stopped and will only resume when corrective actions have been performed that ensure the reporting of accurate and reliable patient test results.

Activities typically performed when calibration or QC systems fail to meet acceptable criteria are:

1. Check to make sure that the hardware is functioning properly.
2. Recalibrate the instrument.
3. Test reference material.
4. If the steps outlined above do not result in correction of the "out of control" values for QC materials, consult the supervisor for other appropriate corrective actions.
5. Call Roche Diagnostics Technical Support.
6. Do not report analytical results for runs not in statistical control.

Proficiency Testing

Proficiency testing is performed by participation in a program offered by the College of American Pathologists (anti-Müllerian Hormone Survey).

10. REFERENCE RANGES (NORMAL VALUES)

The results below were obtained from 718 healthy female human donors of reproductive age (20-44) and the mean measured using Elecsys AMH immunoassay at the 95% confidence interval.⁸ The results are based on the AMH cutoff (1.77 ng/mL). Two antral follicle count (AFC) groups were defined: $AFC \leq 15$ and $AFC > 15$.

Estimated Quantiles of Elecsys AMH

Healthy Women (years)	N	2.5%-q (ng/mL)	5%-q (ng/mL)	Median (ng/mL)	95%-q (ng/mL)	97.5%-q (ng/mL)
20 – 24	150	1.22	1.52	4.00	9.95	11.7
25 – 29	150	0.890	1.20	3.31	9.05	9.85
30 – 34	138	0.576	0.711	2.81	7.59	8.13
35 – 39	138	0.147	0.405	2.00	6.96	7.49
40 – 44	142	0.030	0.059	0.882	4.44	5.47

The reference values reported below were obtained from the Mayo Clinic Laboratories Rochester 2019 Pediatric Test Catalog with Clinical and Interpretive Information based on clinical references from Broer et al.²⁷ and Dewailly et al.²⁸

AMH Reference Range (ng/mL)	
Males	
< 24 months	14 – 466
24 months – 12 years	7.4 – 243
> 12 years	0.7 – 19
Females	
< 24 months	< 4.7
24 months – 12 years	< 8.8
13-45 years	0.9 – 9.5
> 45 years	< 1.0

11. CRITICAL CALL RESULTS (“PANIC VALUES”)

No critical call values exist for AMH.

12. ALTERNATE METHODS FOR PERFORMING TEST AND STORING SPECIMENS IF TEST SYSTEM FAILS

If the analytical system fails, the specimens will be stored at -70°C until the analytical system is restored to functionality.

13. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Not applicable.

14. TRANSFER OR REFERRAL OF SPECIMENS

Transfer or referral of specimens will follow the procedures outlined in the most recent version of the DLS Policies and Procedures Manual.

15. PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

Following successful completion of analysis, the remaining samples will be retained until all results have been reported and sufficient time has passed for review of the results. After this time, samples are either returned to the contact person who requested the analysis or are treated according to DLS and CDC policy.

Standard record keeping (e.g., database, notebooks, and data files) is used to track specimens. Records (including related QA/QC data) are maintained for 3 years, and duplicate records are kept off-site in electronic format. The laboratory does not keep any personal identifiers.

16. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

Samples are identified by their sample ID. The sample ID is a number that is unique to each sample that links the laboratory information to demographic data recorded by those who collected the sample.

Calculation of AMH concentration is performed by the software on the Roche/Hitachi **cobas e 411** control unit, and generated data are saved on a dedicated, access-restricted network space and transferred to the DLS Laboratory Information System.

17. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

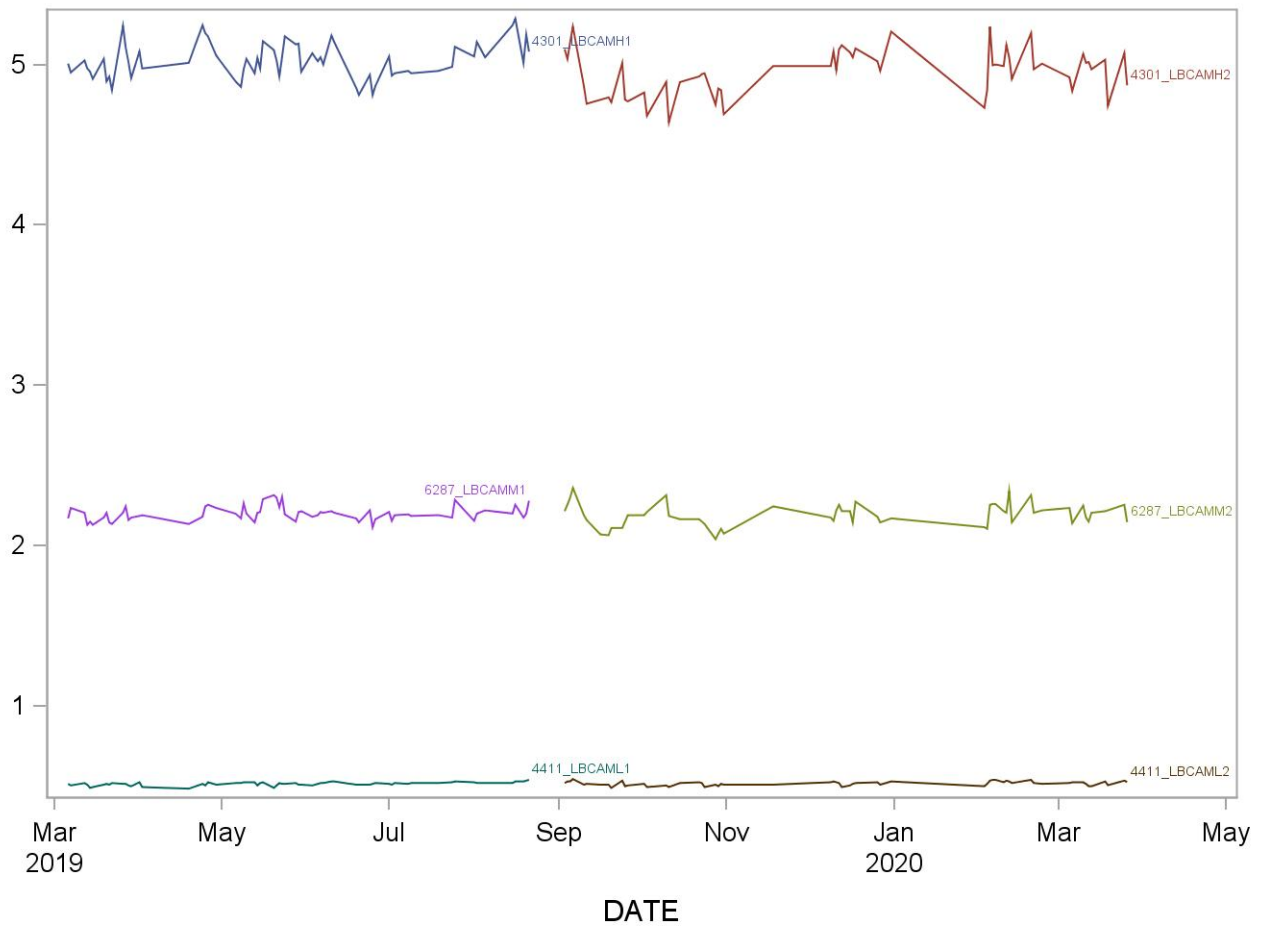
Not applicable for this procedure.

18. SUMMARY STATISTICS AND QC GRAPHS

See following pages.

2019-2020 Summary Statistics and QC Chart LBXAMH (Anti-Mullerian hormone (ng/mL))

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
4301_LBCAMH1	64	06MAR19	21AUG19	5.02367	0.11177	2.2
4411_LBCAML1	64	06MAR19	21AUG19	0.51555	0.01081	2.1
6287_LBCAMM1	64	06MAR19	21AUG19	2.19992	0.04533	2.1
4301_LBCAMH2	58	03SEP19	26MAR20	4.94997	0.14563	2.9
4411_LBCAML2	58	03SEP19	26MAR20	0.51747	0.01344	2.6
6287_LBCAMM2	58	03SEP19	26MAR20	2.18986	0.06992	3.2



19. REFERENCES

1. Iwase A, Osuka S, Goto M, Murase T, Nakamura T, Takikawa S, et al. Clinical application of serum anti-Müllerian hormone as an ovarian reserve marker: A review of recent studies. *Journal of Obstetrics and Gynaecology Research*. 2018;44(6):998-1006.
2. Broekmans FJ, Visser JA, Laven JS, Broer SL, Themmen AP, Fauser BC. Anti-Mullerian hormone and ovarian dysfunction. *Trends in endocrinology and metabolism: TEM*. 2008;19(9):340-7.
3. Wilson CA, di Clemente N, Ehrenfels C, Pepinsky RB, Josso N, Vigier B, et al. Mullerian inhibiting substance requires its N-terminal domain for maintenance of biological activity, a novel finding within the transforming growth factor-beta superfamily. *Molecular endocrinology (Baltimore, Md)*. 1993;7(2):247-57.
4. Weenen C, Laven JSE, von Bergh ARM, Cranfield M, Groome NP, Visser JA, et al. Anti-Müllerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. *MHR: Basic science of reproductive medicine*. 2004;10(2):77-83.
5. Panchal S, Nagori C. Comparison of anti-mullerian hormone and antral follicle count for assessment of ovarian reserve. *Journal of human reproductive sciences*. 2012;5(3):274-8.
6. Nilsson EE, Schindler R, Savenkova MI, Skinner MK. Inhibitory actions of Anti-Müllerian Hormone (AMH) on ovarian primordial follicle assembly. *PloS one*. 2011;6(5):e20087-e.
7. Xu J, Bishop CV, Lawson MS, Park BS, Xu F. Anti-Müllerian hormone promotes pre-antral follicle growth, but inhibits antral follicle maturation and dominant follicle selection in primates. *Human reproduction (Oxford, England)*. 2016;31(7):1522-30.
8. Package Insert: Elecsys AMH. Cobas. Indianapolis, IN: Roche Diagnostics; 2017. p. 1.
9. Kelsey TW, Anderson RA, Wright P, Nelson SM, Wallace WH. Data-driven assessment of the human ovarian reserve. *Molecular human reproduction*. 2012;18(2):79-87.
10. Nelson SM, Iliodromiti S, Fleming R, Anderson R, McConnachie A, Messow CM. Reference range for the antimullerian hormone Generation II assay: a population study of 10,984 women, with comparison to the established Diagnostics Systems Laboratory nomogram. *Fertil Steril*. 2014;101(2):523-9.
11. Kruszynska A, Slowinska-Srzednicka J. Anti-Mullerian hormone (AMH) as a good predictor of time of menopause. *Przegląd menopauzalny = Menopause review*. 2017;16(2):47-50.
12. La Marca A, Stabile G, Artenisio AC, Volpe A. Serum anti-Mullerian hormone throughout the human menstrual cycle. *Human Reproduction*. 2006;21(12):3103-7.
13. Kallio S, Puurunen J, Ruokonen A, Vaskivuo T, Piltonen T, Tapanainen JS. Antimullerian hormone levels decrease in women using combined contraception independently of administration route. *Fertil Steril*. 2013;99(5):1305-10.
14. Lee JY, Ahn S, Lee JR, Jee BC, Kim CH, Seo S, et al. Reference Values for the Revised Anti-Müllerian Hormone Generation II Assay: Infertile Population-based Study. *Journal of Korean Medical Science*. 2017;32(5):825-9.
15. Electrochemiluminescence immunoassay (ECLIA) for the in vitro quantitative determination of anti-Müllerian Hormone in human serum and plasma. Roche Diagnostics International Ltd. Indianapolis, IN: Roche; 2016. p. 1-2.
16. Roche/Hitachi cobas e 411 Analyzer Operator's Manual Roche Diagnostics International Ltd.
17. Material Safety Data Sheet: Roche Diagnostics MyLabOnline®; [Available from: www.mylabonline.com].

18. Salmassi A, Mettler L, Hedderich J, Jonat W, Deenadayal A, von Otte S, et al. Cut-Off Levels of Anti-Mullerian Hormone for The Prediction of Ovarian Response, In Vitro Fertilization Outcome and Ovarian Hyperstimulation Syndrome. *International journal of fertility & sterility*. 2015;9(2):157-67.
19. DLS Policies and Procedures Manual and Clinical and Laboratory Standards Institute (CLSI) documents [Available from: \\cdc.gov\project\CCEHIP_NCEH_DLS_CCB_PBL_Steroids\CLIA_Method_1046_AMH\CLIA\DLS P&PM.
20. EP5-A3: Evaluation of Precision Performance of Quantitative Measurement Procedures. 3 ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
21. EP10-A3: Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures. 3 ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
22. EP15-A3: User Verification of Precision and Estimation of Bias. 3 ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
23. C37-A: Preparation and validation of commutable frozen human serum pools as secondary reference materials for cholesterol measurement procedure. Wayne, PA: Clinical and Laboratory Standards Institute; 1999.
24. EP17-A2: Evaluation of Determination Capability for Clinical Laboratory Measurement Procedures. 2 ed. Wayne, PN: Clinical and Laboratory Standards Institute; 2012.
25. Skoog DA, Holler FJ, Nieman TA. Principles of instrumental analysis. Philadelphia; Orlando, Fla.: Saunders College Pub. ; Harcourt Brace College Publishers; 1998.
26. Caudill SP, Schleicher RL, Pirkle JL. Multi-rule quality control for the age-related eye disease study. *Statistics in medicine*. 2008;27(20):4094-106.
27. Broer SL, Broekmans FJM, Laven JSE, Fauser BCJM. Anti-Müllerian hormone: ovarian reserve testing and its potential clinical implications. *Human Reproduction Update*. 2014;20(5):688-701.
28. Dewailly D, Andersen CY, Balen A, Broekmans F, Dilaver N, Fanchin R, et al. The physiology and clinical utility of anti-Müllerian hormone in women. *Human Reproduction Update*. 2014;20(3):370-85.

Appendix A: Method Performance Documentation

Precision

Quality material 1: +0316244SA						
Run	Result 1 (ng/mL)	Result 2 (ng/mL)	Mean (ng/mL)	SS 1	SS 2	2*mean^2
1	0.47	0.48	0.47	0.00	0.00	0.45
2	0.46	0.47	0.47	0.00	0.00	0.44
3	0.49	0.50	0.49	0.00	0.00	0.49
4	0.50	0.50	0.50	0.00	0.00	0.50
5	0.49	0.48	0.49	0.00	0.00	0.47
6	0.49	0.49	0.49	0.00	0.00	0.48
7	0.50	0.49	0.49	0.00	0.00	0.49
8	0.49	0.49	0.49	0.00	0.00	0.48
9	0.52	0.50	0.51	0.00	0.00	0.52
10	0.49	0.48	0.49	0.00	0.00	0.47
Grand sum	9.77	Grand mean	0.49			

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.0013	0.0001	0.0115	2.35
Between Run	0.0025	0.0003	0.0086	1.77
Total	0.0039		0.0144	2.94

Quality material 2: +0116242SA						
Run	Result 1 (ng/mL)	Result 2 (ng/mL)	Mean (ng/mL)	SS 1	SS 2	2*mean^2
1	4.50	4.76	4.63	0.02	0.02	42.87
2	4.70	4.67	4.69	0.00	0.00	43.90
3	4.95	5.00	4.98	0.00	0.00	49.50
4	4.95	5.00	4.98	0.00	0.00	49.50
5	4.88	4.94	4.91	0.00	0.00	48.22
6	4.80	4.87	4.84	0.00	0.00	46.75
7	4.99	4.77	4.88	0.01	0.01	47.63
8	4.94	4.79	4.87	0.01	0.01	47.34
9	5.05	5.09	5.07	0.00	0.00	51.41
10	4.99	4.75	4.87	0.01	0.01	47.43
Grand sum	97.39	Grand mean	4.87			

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.1061	0.0106	0.1030	2.11
Between Run	0.3136	0.0348	0.1101	2.26
Total	0.4197		0.1508	3.10

Accuracy by Mixing

Concentration	Replicate	Expected value (ng/mL)	Measured concentration			Average Recovery (%)
			Value (ng/mL)	Mean (ng/mL)	Recovery (%)	
Low (100)	1	0.487	0.444	0.45		96.2%
Low (100)	2		0.453			
Low/High (75:25)	1	1.46	1.36	1.37	93.8	
Low/High (75:25)	2		1.37			
Low/High (50:50)	1	2.46	2.35	2.37	96.3	
Low/High (50:50)	2		2.38			
Low/High (25:75)	1	3.47	3.46	3.42	98.6	
Low/High (25:75)	2		3.37			
High (100)	1	4.85	4.47	4.48		
High (100)	2		4.49			

Freeze and Thaw Stability

Freeze and thaw stability assesses the stability of samples after 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions. Samples were frozen three times at -70°C and then thawed (3 freeze-thaw cycles).

Quality material 1: A46QC2L1			
	Initial measurement	After two freeze-thaw cycles	After three freeze-thaw cycles
Replicate 1, ng/mL	1.88	1.92	2.01
Replicate 2, ng/mL	1.93	1.93	1.99
Replicate 3, ng/mL	1.94	1.94	2.02
Mean, ng/mL	1.92	1.93	2.01
% difference from initial measurement	--	0.70	4.70

Quality material 2: +0116242SA			
	Initial measurement	After two freeze-thaw cycles	After three freeze-thaw cycles
Replicate 1, ng/mL	4.34	4.31	4.49
Replicate 2, ng/mL	4.3	4.34	4.46
Replicate 3, ng/mL	4.43	4.34	4.43
Mean, ng/mL	4.36	4.33	4.5
% difference from initial measurement	--	-0.6	2.4

Bench-Top Stability

Bench-top stability assesses short-term stability for the length of time needed to handle study samples (at room temperature).

Original samples (not yet prepared for instrument analysis) were stored at room temperature for 2 hours and 24 hours.

Quality material 1: A46QC2L1			Bench-top stability
	Initial measurement	After 2 hours on rotator	After 24h on rotator
Replicate 1, ng/mL	1.88	1.96	2.04
Replicate 2, ng/mL	1.93	1.94	2.11
Replicate 3, ng/mL	1.94	1.91	2.07
Mean, ng/mL	1.92	1.94	2.1
% difference from initial measurement	--	1.04	8.2

Quality material 2: +0116242SA			Bench-top stability
	Initial measurement	After 2 hours on rotator	After 24h on rotator
Replicate 1, ng/mL	4.34	4.31	4.49
Replicate 2, ng/mL	4.3	4.34	4.46
Replicate 3, ng/mL	4.43	4.34	4.43
Mean, ng/mL	4.36	4.33	4.46
% difference from initial measurement	--	-0.61	2.37

Processed Sample Stability

Processed sample stability assesses the short-term stability of processed samples, including resident time in autosampler. The processed samples (ready for instrument analysis) were stored on the instrument for 1 hour, 2 hours, and 4 hours.

Quality material 1: A46QC2L1			Processed sample stability	
	Initial measurement	After one hour onboard	After two hours onboard	After 4 hours onboard
Replicate 1, ng/mL	1.93	2.03	2.09	2.2
Replicate 2, ng/mL	1.94	2.06	2.08	2.19
Replicate 3, ng/mL	1.91	2.03	2.07	2.15
Mean, ng/mL	1.93	2.04	2.08	2.18
% difference from initial measurement	--	5.91	8.00	13.15

Quality Material 2: +0116242SA			Processed sample stability	
	Initial measurement	After one hour onboard	After two hours onboard	After 4 hours onboard
Replicate 1, ng/mL	4.29	4.61	4.61	4.76
Replicate 2, ng/mL	4.35	4.7	4.7	4.66
Replicate 3, ng/mL	4.34	4.62	4.62	4.84
Mean, ng/mL	4.33	4.64	4.64	4.75
% difference from initial measurement	--	7.32	7.32	9.86

LOD, Specificity, and Fit for Intended Use

Analytes	Limit of Detection (LOD)	Interferences successfully checked in at least 50 human samples	Accuracy, precision, LOD, specificity and stability meet performance specifications for intended use
AMH	0.01 ng/mL	yes	yes