Laboratory Procedure Manual

Analyte: Luteinizing Hormone (LH)

Matrix: Serum and Plasma

Method: Luteinizing Hormone Electrochemiluminescence Immunoassay

Method No: 1048

Revised:

As performed by:

CCB Service Laboratory
Clinical Chemistry Branch
Division of Laboratory Sciences
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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.

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table for **TST_K_R**:

VARIABLE NAME	SAS LABEL (and SI units)
LBXLUH	Luteinizing Hormone (mIU/mL)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Clinical and Public Health Relevance

Luteinizing Hormone (LH) is a heterodimeric glycoprotein that consists of one alpha and one beta subunit. LH is produced mainly in gonadotropic cells in the anterior pituitary gland. LH works in conjunction with follicle stimulating hormone (FSH) to control the menstrual cycle. The LH surge triggers ovulation and the development of corpus luteum in females. LH promotes testosterone production by Leydig cells of testes in males.[1]

LH levels in blood can be affected by dysfunctions within the hypothalamus-pituitary-gonad system.[1-3] Together with FSH, LH is used as a biomarker for congenital diseases with chromosome aberrations (e.g., Turner's syndrome) and polycystic ovaries (PCO). In addition, LH is used to investigate suspected Leydig cell insufficiency as well as causes of amenorrhea and menopausal syndrome.

Test Principle

This test is based on the reaction of LH with immuno-antibodies and chemo-luminescence measurements for the reaction products. It consists of 2 incubation steps and a chemiluminescent measurement obtained with a photomultiplier tube that spans 18 minutes.[4, 5] The first incubation period begins by sandwiching the sample of LH containing serum or plasma between a biotinylated monoclonal LH-specific antibody and a monoclonal LH-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 LH 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 LH 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*.[6] 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.[7]

The safety precautions for specific products are as follows:

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.

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

PreciControl Universal: (Roche product# 11731416160)
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: Using FDA approved methods, the human source material in this product has been tested and shown to be free from HBsAg and antibodies to HCV and HIV. 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.

LH: (Roche product# 11732234122)
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: May cause irritation if inhaled
Sensitization or Odor threshold: The following chemicals may cause sensitization, be absorbed via skin, and/or have an odor threshold for detection:
1. alpha-D-Glucopyranoside – skin sensitizer with acute oral toxicity
2. beta-D-Fructofuranosyl – skin sensitizer with acute oral toxicity

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

LH CalSet II: (Roche product# 03561097190)
Eye contact: Contact with eyes may cause irritation
Skin contact: Contaminated work clothing must not be allowed out of the workplace. In case of inadequate ventilation wear respiratory protection. Wear protective gloves. Extremely corrosive and destructive to tissue. Causes burns. May cause an allergic skin reaction.
Ingestion: Acute oral toxicity
Inhalation: Avoid breathing dust/ fume/ gas/ mist/ vapors/ spray. May cause allergy or asthma symptoms or breathing difficulties if inhaled.
Additional information: Extremely corrosive and destructive to tissue.
Ingredients:
1. 3(2H)-Isothiazolone, 2-methyl-, hydrochloride (1:1): Causes burns.
2. Benzenemethanesulfonyl fluoride: Causes burns.

Elecsys 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*[6] 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-ehip-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*[6] 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 Diagnostic, product# 10394246001
5. Purified water supplied via a combination reverse osmosis and DI system installed and maintained by Aqua Solutions, Inc.
6. Calibrator: LH Calset II, Roche Diagnostics, product# 03561097190
7. Control: PreciControl Universal, Roche Diagnostics, product# 11731416160
8. Elecsys 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*[6] for directions).

Preparation of Calibration Materials Used For Sample Analysis

This method has been standardized against the 2nd NIBSC International Standard 80/552. Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

LH CalSet II is a bi-level material used for the calibration of quantitative Elecsys LH assays. The CalSet kit provided by Roche includes lyophilized human serum matrix with added human LH in two concentration ranges.

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 a LH concentration of approximately 1 mIU/mL and Calibrator 2 has a LH concentration of approximately 45 mIU/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 -70 °C freezer. Calibrators are stable for 3 months at -20 °C and for one day once thawed.

Renewed calibration is recommended as follows:

- After 1 month (28 days) when using the same reagent lot
- After 7 days (when using the same reagent kit on the analyzer)
- As required (e.g., quality control findings outside the defined limits)

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 LH 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 the low and mid-level LH pools. For the high LH pool, serum units, collected from individual post-menopausal female donors, were prescreened on the Roche **cobas** e 411 for LH content. Once suitable donor units are were identified, those units were combined, aliquoted into individual vials, and frozen at -70 ºC until use.

Commercially prepared control materials such as PreciControl Universal 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 QC pools are 0.3 – 8 mIU/mL, 10 – 20 mIU/mL, and 25 - 135 mIU/mL, respectively.[8-10]

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 Manual[7] 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) documents[11], EP5-A3 (Evaluation of Precision Performance of Quantitative Measurement Methods)[12], EP10-A3 (Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures)[13], and EP15-A3 (User Verification of Performance for Precision and Trueness)[14]. Records with package inserts for the commercial control material are maintained in the laboratory.

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

SHBG and LSP pools were previously prepared and used as the low and mid-bench LH QC pool, respectively. To achieve concentrations within the desired ranges, pools were selected with enough volume to sustain the low and medium LH 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 guideline[15] by Solomon Park Research Laboratories, respectively.

To create the LH high QC pool, individual post-menopausal serum units were ordered from commercial sources. Units were stored at -70 °C freezer until analysis. Each unit was prescreened (for sample preparation and instrument operation please refer to section 6 below) on the **cobas e** 411 to obtain LH content.

Following the prescreening analysis, suitable units were chosen to create high bench QC serum pools. To achieve concentrations within the desired ranges, the volumes needed from each unit to obtain the high pool was determined. The volume of this QC pool created should be sufficient for 2 years. For further details on the preparation of the bench QC pools for this method, please refer to work instruction PBLW24RP01.

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 LH on the **cobas e** 411. A sample volume of 460 µL is preferred in order to allow for single sample repeat analyses and entire run repeat analysis in case of QC failure. Serum collected using standard sampling tubes or tubes containing separating gel, or Li^-, Na^-, NH_4^+ heparin, K_3-EDTA and sodium fluoride/potassium oxalate plasma are considered acceptable by the test manufacturer. When sodium citrate is used, the results must be corrected by +10%. 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 hours. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide. Ensure the samples, calibrators and controls are at 20-25°C prior to measurement.

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).[11] 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.

Unopened commercial controls are 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 1 month at -70 °C. Controls should be allowed to come to room temperature before analyzing.

The manufacturer suggests that the PreciControl Universal is stable up to 5 hours at 20-25°C, 3 days at 2-8°C, and 1 month at -20°C. Freeze only once.

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 PBLW24MS03 (Cobas e 411 Basic Operating Procedures for LH 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 LH 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 PBLW24MS03 (**Cobas e** 411 Basic Operating Procedures for LH Testing).

7. CALIBRATION AND CALIBRATION VERIFICATION

Manufacturer recommended calibration procedures are followed for LH 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 reportable range for this method is 0.100-200 mIU/mL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 0.100 mIU/mL. Values above the measuring range are reported as > 200 mIU/mL.

Limit of detection (LOD)

The limit of detection (LOD), as determined by the assay manufacturer, is 0.100 mIU/mL. According to the manufacturer, the LOD is based on the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Accuracy (Trueness and Precision)

Imprecision of the method was determined according to CLSI guide EP5-A3[12] 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 4.76% and 4.43% 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	1.31	0.62
Between Run	4.58	4.39
Total	4.76	4.43

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.

Due to the unavailability of a well-characterized pure LH material, which is a large glycoprotein, spiking with 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. The results of the accuracy test for the measurement of serum LH using the Roche **cobas** e 411 are shown in the table below.

Accuracy Mix	Measured Value (mIU/mL)	Expected Value (mIU/mL)	Recovery (%)
25%	18.43	18.75	98.3
50%	31.23	31.54	99.0
75%	44.93	44.32	101.4

The accuracy, as determined by these recovery experiments, is found on average to be 99.6% for LH. 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 by icterus (bilirubin < 1129 µmol/L or < 66 mg/dL), hemolysis (Hb < 0.621 mmol/L or < 1 g/dL), lipemia (Intralipid < 1900 mg/dL) and biotin (< 205 nmol/L or < 50 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.
- No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.
- There is no high-dose hook effect at LH concentrations up to 1150 mIU/mL.
- *In vitro* tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found.
- Samples of neonates have not been tested with the Elecsys LH assay.
- In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

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. 3 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 14% 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 4% 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 3% from the established value, and at 4 h it was 7%. Therefore, the samples have to be processed within 4 h of being placed in the instrument.

Long term stability could not be evaluated for LH, 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 LH.

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.[16]

The rules described in the most recent version of the DLS Policies and Procedures Manual[9] 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 ± 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.[11]

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 (Ligand Assay Special Survey).

10. REFERENCE RANGES (NORMAL VALUES)

The results below were obtained from healthy human donors and measured using Elecsys LH immunoassay. The data was taken from Elecsys LH Assay package insert.[4]

Test subjects	N	LH mIU/mL		
		Percentile		
		50th	5th	95th
Men	322	4.0	1.7	8.6
Women				
• Folicular phase	316	5.9	2.4	12.6
• Ovulation phase	56	30.8	14.0	95.6
• Luteal phase	280	4.3	1.0	11.4
• Postmenopause	132	29.1	7.7	58.5

LH/FSH quotient: Quotients have been calculated from the results obtained with the Elecsys LH assay and the Elecsys FSH assay in the samples of healthy women of child-bearing age. The following medians have been calculated:

- Follicular phase: 0.82 (n = 315)
- Luteal phase: 1.12 (n = 279)

11. CRITICAL CALL RESULTS (“PANIC VALUES”)

No critical call values exist for LH.

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 LH 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

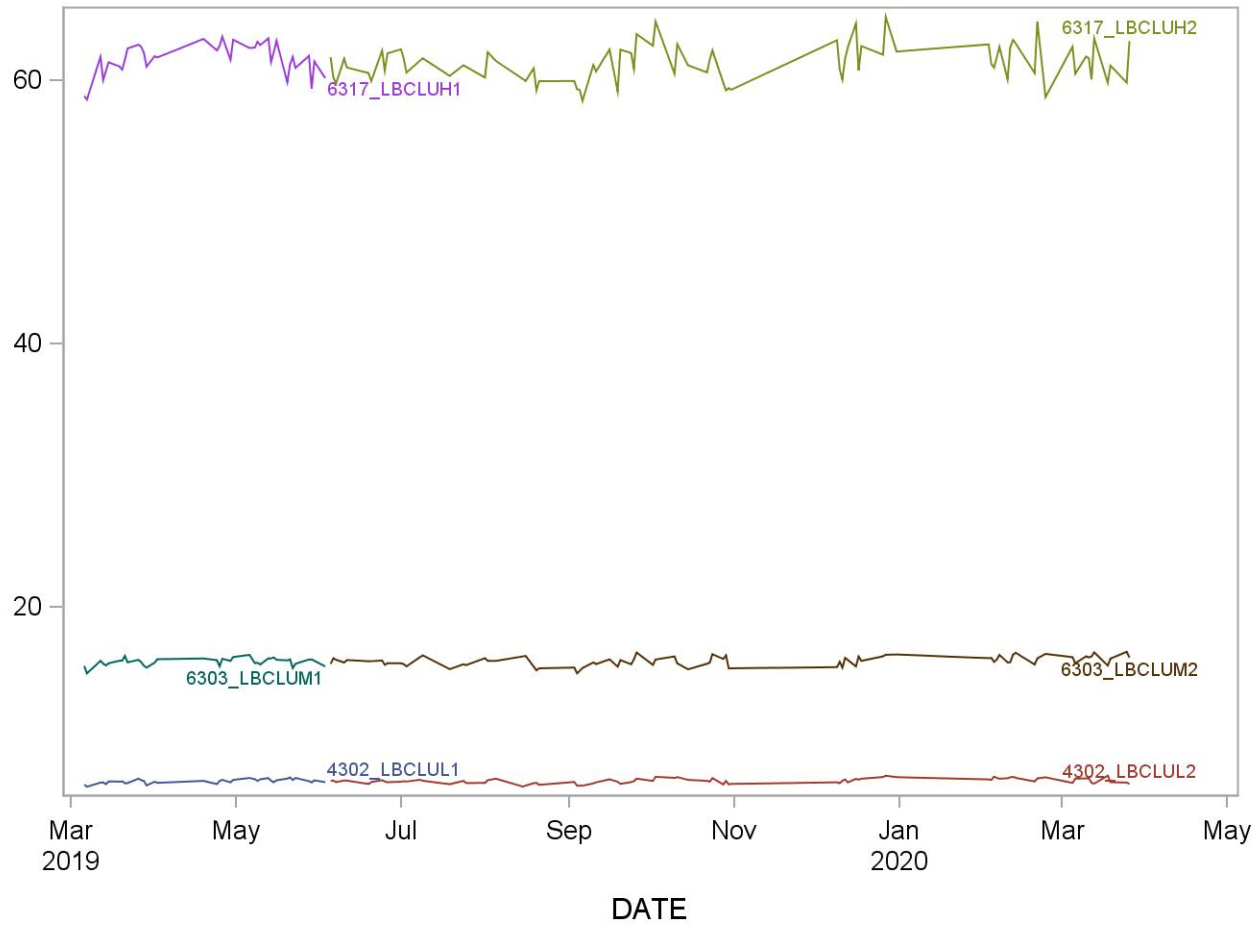
Not applicable for this procedure.

18. SUMMARY SATISTICS AND QC GRAPH

Please see following page.

2019-2020 Summary Statistics and QC Chart
LBXLUH (Luteinizing Hormone (mIU/mL))

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
6317_LBCLUH1	38	06MAR19	03JUN19	61.60447	1.20677	2.0
4302_LBCLUL1	38	06MAR19	03JUN19	6.77596	0.16332	2.4
6303_LBCLUM1	38	06MAR19	03JUN19	15.84215	0.28574	1.8
6317_LBCLUH2	83	05JUN19	26MAR20	61.25566	1.39012	2.3
4302_LBCLUL2	83	05JUN19	26MAR20	6.80225	0.19816	2.9
6303_LBCLUM2	83	05JUN19	26MAR20	15.89321	0.37503	2.4



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Appendix A: Method Performance Documentation

Precision

Quality material 1: +0216243SA						
Run	**Result 1 (mIU/mL)**	**Result 2 (mIU/mL)**	**Mean (mIU/mL)**	**SS 1**	**SS 2**	**2*mean^2**
1	6.75	6.56	6.66	0.01	0.01	88.58
2	6.62	6.51	6.57	0.00	0.00	86.20
3	6.56	6.48	6.52	0.00	0.00	85.02
4	6.07	5.99	6.03	0.00	0.00	72.72
5	5.94	5.84	5.89	0.00	0.00	69.38
6	6.12	5.98	6.05	0.00	0.00	73.21
7	6.00	6.04	6.02	0.00	0.00	72.48
8	6.20	6.01	6.11	0.01	0.01	74.54
9	5.92	5.90	5.91	0.00	0.00	69.86
10	5.98	6.02	6.00	0.00	0.00	72.00
Grand sum	123.49	**Grand mean**	6.17			

	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**
Within Run	0.0652	0.0065	0.0807	1.31
Between Run	1.4983	0.1665	0.2828	4.58
Total	1.5635		0.2941	**4.76**

Quality material 2: A4748QC3L1						
Run	**Result 1 (mIU/mL)**	**Result 2 (mIU/mL)**	**Mean (mIU/mL)**	**SS 1**	**SS 2**	**2*mean^2**
1	61.04	61.48	61.26	0.05	0.05	7505.58
2	60.76	60.21	60.49	0.08	0.08	7316.87
3	60.78	61.09	60.94	0.02	0.02	7426.15
4	58.53	57.79	58.16	0.14	0.14	6765.17
5	55.17	55.03	55.10	0.00	0.00	6072.02
6	57.35	57.76	57.56	0.04	0.04	6625.16
7	55.77	56.01	55.89	0.01	0.01	6247.38
8	55.14	55.65	55.40	0.07	0.07	6137.21
9	55.81	56.38	56.10	0.08	0.08	6293.30
10	55.04	54.30	54.67	0.14	0.14	5977.62
Grand sum	1151.09	**Grand mean**	57.55			

	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**
Within Run	1.2589	0.1259	0.3548	0.62
Between Run	116.0440	12.8938	2.5266	4.39
Total	117.3029		2.5514	**4.43**

Accuracy by Mixing

			Measured concentration			
Concentration	**Replicate**	**Expected value (mIU/mL)**	**Value (mIU/mL)**	**Mean (mIU/mL)**	**Recovery (%)**	**Average Recovery (%)**
Low (100)	1	6.17	5.91	5.97		99.6
Low (100)	2		6.03			
Low/High (75:25)	1	18.75	18.1	18.43	98.3	
Low/High (75:25)	2		18.76			
Low/High (50:50)	1	31.54	31.17	31.23	99.0	
Low/High (50:50)	2		31.29			
Low/High (25:75)	1	44.32	45.28	44.93	101.4	
Low/High (25:75)	2		44.57			
High (100)	1	57.55	56.82	57.10		
High (100)	2		57.38			

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: A4748QC2L1			
	Initial measurement	**After two freeze-thaw cycles**	**After three freeze-thaw cycles**
Replicate 1, mIU/mL	14.16	14.72	16.23
Replicate 2, mIU/mL	14.22	14.38	16.14
Replicate 3, mIU/mL	14.16	14.49	16.08
Mean, mIU/mL	14.18	14.53	16.15
% difference from initial measurement	--	**2.47**	**13.89**

Quality material 2: A4748QC3L1			
	Initial measurement (mIU/mL)	**After two freeze-thaw cycles (mIU/mL)**	**After three freeze-thaw cycles (mIU/mL)**
Replicate 1, mIU/mL	61.44	55.44	62.86
Replicate 2, mIU/mL	55.61	55.96	62.07
Replicate 3, mIU/mL	55.68	55.83	61.71
Mean, mIU/mL	57.58	55.74	62.2
% difference from initial measurement	--	**3.2**	**8.1**

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: A4748QC2L1			**Bench-top stability**
	Initial measurement	**After 2 hours on rotator**	**After 24h on rotator**
Replicate 1, mIU/mL	14.49	14.52	14.6
Replicate 2, mIU/mL	14.55	14.22	14.46
Replicate 3, mIU/mL	14.52	13.82	14.62
Mean, mIU/mL	14.52	14.19	14.6
% difference from initial measurement	--	**2.30**	**0.3**

Quality Material 2: A4748QC3L1			**Bench-top stability**
	Initial measurement	**After 2 hours on rotator**	**After 24h on rotator**
Replicate 1, mIU/mL	56.77	55.18	57.2
Replicate 2, mIU/mL	57.17	55.54	56.46
Replicate 3, mIU/mL	58.33	54.82	55.49
Mean, mIU/mL	57.42	55.18	56.38
% difference from initial measurement	--	**3.91**	**1.81**

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: A4748QC2L1			**Processed sample stability**	
	Initial measurement	**After one hour onboard**	**After two hours onboard**	**After 4 hours onboard**
Replicate 1, mIU/mL	14.87	14.84	14.76	15.7
Replicate 2, mIU/mL	14.32	14.62	14.61	15.47
Replicate 3, mIU/mL	14.37	14.7	14.88	15.43
Mean, mIU/mL	14.52	14.72	14.75	15.53
% difference from initial measurement	--	**1.38**	**1.58**	**6.98**

Quality Material 2: A4748QC3L1			**Processed sample stability**	
	Initial measurement	**After one hour onboard**	**After two hours onboard**	**After 4 hours onboard**
Replicate 1, mIU/mL	56.32	57.4	56.12	58.71
Replicate 2, mIU/mL	55.23	57.04	57.59	59.89
Replicate 3, mIU/mL	55.9	57.12	58.3	60.81
Mean, mIU/mL	55.82	57.19	57.34	59.80
% difference from initial measurement	--	**2.45**	**2.72**	**7.14**

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**
LH	0.100 mIU/mL	yes	yes