Analytical Procedure Manual

Analyte: Sex Hormone-Binding Globulin

Matrix: Serum and Plasma

Method: Sex Hormone-Binding Globulin Immunoassay

Method No: 1031

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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:

	VARIABLE NAME	SAS LABEL
TST_L	LBXSHBG	Sex Hormone Binding Globulin (SHBG) (nmol/L)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Clinical and Public Health Relevance

Sex Hormone-Binding Globulin (SHBG) is a glycoprotein that is produced mainly in the liver of humans. The purpose of SHBG in serum is the transportation of steroids to target tissues.[1] SHBG has a high affinity for dihydrotestosterone (DHT), moderate affinity for testosterone and estradiol, and low affinity for estrone and androstenedione. Only steroid hormones in blood that are not bound to SHBG are available to certain cell receptors. Therefore, only free, unbound steroid hormones are considered biologically active. Thus, SHBG in blood affects the amount of biologically active steroid hormones.

SHBG levels in blood can be affected by age and certain diseases.[2,3] In elderly men, patients with cirrhosis of the liver and hyperthyroidism, SHBG levels are usually found to be high.[4,5] Low levels of SHBG are most often accompanied by polycystic ovarian syndrome, acromegaly, obesity, hirsutism, and hypothyroidism. Thus, the correct interpretation of steroid hormone levels in blood requires knowledge of the blood SHBG levels, i.e., to calculate the amount of biologically active steroid hormones. In clinical practice, SHBG levels are determined when total testosterone levels are inconsistent with clinical signs and symptoms.[7]

Research studies suggest an inverse correlation between SHBG and high sensitivity C-reactive protein, a biomarker for inflammation and cardiovascular health risks.[6] Furthermore, some research studies found SHBG levels in blood to be associated with diabetes and other chronic diseases independently from steroid hormone levels.

Test Principle

This test is based on the reaction of SHBG with immuno-antibodies and chemo-luminescence measurements of the reaction products. It consists of 2 incubation steps and a chemiluminescent measurement via photomultiplier tube that spans 18 minutes.[5] The first incubation period begins by sandwiching the sample of SHBG containing serum between a biotinylated monoclonal SHBG-specific antibody and a monoclonal SHBG-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 SHBG 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 SHBG 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*.[8] 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.

All secondary containers need to be labeled in accordance with the CDC policies, including those mentioned in the Chemical Hygiene Plan. For labeling requirements for secondary containers see https://intranet.cdc.gov/olss/media/2022/01/OLSS_MAN_ChemicalHygienePlan_CDC-001-00354_rev3_final.pdf.

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.[9]

The safety precautions for specific products are as follows:

BlankCell: (Roche product# is 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# is 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# is 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 Universal: (Roche product# is 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.

Procell: (Roche product# is 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.

SHBG: (Roche product# is 03052001160)

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. Methylisothiazolone (MIT)- skin sensitizer

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

SHBG CalSet: (Roche product# is 03052028190)

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. Methylisothiazolone (MIT)- skin sensitizer

Medical conditions aggravated by exposure: None

Additional information: None

SHBG CalCheck 5: (Roche product# is 06269915160)

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: 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.

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*[8] 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

Further, 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*[8] 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 pipette (VistaLab Technologies, Brewster, NY), 20-200uL
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. Calibrators: SHBG Calset, Roche Diagnostics' product# 03052028190
7. Controls: PreciControl Universal, Roche Diagnostics' product# 11731416160
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*[7] for directions).

Preparation of Calibration Materials Used for Sample Analysis

Elecsys SHBG CalSet is a bi-level material used for the calibration of quantitative Elecsys SHBG assays. The CalSet kit provided by Roche includes lyophilized equine (Cal1) and human (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 a SHBG concentration of approximately 0 nmol/L and Calibrator 2 has a SHBG concentration of approximately 40 nmol/L.

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 -40 to -80 °C freezer. Calibrators are stable for 2 months at -15 to -40 °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 blood bank serum units, previously collected from individual human donors, are prescreened on the Roche **cobas** e 411 for SHBG content. Once suitable donor units are identified to prepare each level of QC pools (e.g., the low, mid, and high levels), those units are combined, aliquoted into individual cryovials in 1 mL increments, and frozen at -70 ºC until they are ready for use.

Commercially prepared control materials 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 16.87 – 30.43 (5^{th} – 25^{th} percentile), 30.43 – 71.78 (25^{th} – 75^{th} percentile), and 71.78 – 144.01 (75^{th} – 95^{th} percentile) nmol/L, respectively.

Control values for the bench QC materials should be entered into the cobas e 411 before analyzing a new lot number of control material. 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,[10] EP5-A3 (Evaluation of Precision Performance of Quantitative Measurement Methods),[11] EP10-A3 (Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures),[12] and EP15-A3 (User Verification of Performance for Precision and Trueness).[13] Records with package inserts for the commercial control material are maintained in the laboratory.

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

Following the prescreening analysis on the **cobas e** 411, suitable units with concentrations within the desired ranges were chosen to create the low, medium, and high SHBG bench QC serum pools. Units were selected with enough volume to sustain the low, medium, and high SHBG bench QCs for 2 years. For the SHBG low and medium QC pools, suitable LSP pools that were previously prepared were adopted. For the SHBG high QC, individual serum units, which were ordered from commercial sources, were combined. For further details on the preparation of the bench QC pools for this method, please refer to work instruction PBLW31SH01 (Preparing In-House Bench Quality Control Pools for the Measurement of SHBG and AMH).

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 SHBG 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 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 QC on the analyzer should be measured within 2 hours.

It is recommended that specimens are transported in 2.0-mL cryogenic vials with external screw-caps. Collected specimens can be shipped at refrigerated temperatures (2-8 ºC) or frozen on dry ice. Specimens can be stored at refrigerated temperatures (2-8 ºC) for up to 3 days prior to analysis. For long-term storage, samples must be stored at ultra-low temperatures (-40 to -80 ºC). 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), it is recommended that specimens collected in the field be frozen and then shipped on dry ice by overnight mail. Samples received cooled at 2 to 8°C are also acceptable.

Receiving Specimen

The following procedure is used for all study samples received in the laboratory:

1. Inform sample logistics team about sample handling requirements as outlined in APM section 5.
2. After receiving samples in the laboratory, inspect samples for
    a. Meeting requirements as outlined in section 5 of the APM
    b. Verify number of samples and sample IDs for consistency with expected samples
    c. Email sample logistics and study coordinator findings from the inspection. The email contains:
        i. the Name or ID # of the study
        ii. the date when samples were received in the laboratory
        iii. the number of samples received
        iv. the sample batch ID, if applicable
        v. statement that samples met criteria as outlined in the APM
        vi. a list of samples that do not meet acceptance criteria with a brief description which criteria was not met
        vii. a list of all samples IDs received
        viii. the name of the person who received the samples in the laboratory
3. After inspection, store samples at conditions described in section 5 of the APM

The inspection must be conducted in a manner that frozen samples do not thaw.

Specimen Storage

Samples should be stored frozen at ultra-low temperatures -40 to -80 °C upon receipt unless they are to be analyzed immediately.

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

The unopened PreciControl Universal is stable at 2 to 8 °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 2 – 8 °C. Controls should be allowed to come to room temperature before analyzing.

The manufacturer suggests that samples are stable for 5 days at 15 – 25 °C, 7 days at 2-8 °C and 12 months at -15 to -40°C.

While multiple freeze thaw cycles should be avoided, samples are stable up to three freeze-thaw cycles. The manufacturer suggests that samples should only be frozen once.

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

Specimens are allowed to reach room temperature for sample preparation. 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 approach using the cryovial inserts can be found in work instruction PBLW31MS01 (Cobas e 411 Basic Operating Procedures for SHBG Testing).

1. After aliquoting, samples in the original sample vials may be stored at -40 to -80 °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 -40 to -80 °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.
2. Due to possible evaporation effects, samples, calibrators, and controls on the analyzer should be analyzed/measured within two hours. Samples that have been aliquoted into sample cups for use on the analyzer cannot be reused for future sample analysis. If samples need to be retested at a later time, a fresh aliquot must be used from the original sample vial.

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 50 patient samples can be processed in one run. Samples, in cryovials, are transferred into special custom cryovial holders, which are directly loaded onto the clinical analyzer according to work instruction PBLW31MS01 (Cobas e 411 Basic Operating Procedures for SHBG Testing).

Operation of the cobas e 411 analyzer

The **cobas e** 411 analyzer must be used for quantification of SHBG in 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 PBLW31MS01 (Cobas e 411 Basic Operating Procedures for SHBG Testing).

Data Calculation and Reporting

Calculations are performed as described in the user manual and assay documentation from the manufacturer.

Calculation of SHBG concentration is determined by the calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode. The SHBG concentration is then determined by comparing the measured intensity of the sample to the calibration curve. The result is expressed in nmol/L. A 1:10 dilution can be programmed directly on the instrument, and all calculations will be done automatically by the instrument software.

Data is transferred from the instrument in a csv file, which is then processed by an R script to separate analyte specific data into different excel workbooks. This process was tested and approved by the QA officer.

7. CALIBRATION AND CALIBRATION VERIFICATION

According to the manufacturer, CalCheck 5 is an “assayed control for use in calibration verification and for use in the verification of the assay range established by the Elecsys SHBG reagent”.[14] Manufacturer recommended calibration procedures are followed for SHBG testing using manufacturer reagent packs. Details on calibration of the cobas e 411 analyzer are found in work instruction PBLW31RP01 (Cobas e 411 Calibration Procedures for SHBG).

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 analytical measurement range for this method is 0.800 – 200 nmol/L (defined by the manufacturer as the lower detection limit and the maximum of the master curve). The reportable range for this method is 0.800 – 2000 nmol/L (defined by the lower detection limit and the maximum of the master curve with 10-fold dilution). Values below the detection limit are reported as < 0.800 nmol/L. The maximum of the master curve, that defined the measuring range, is 200 nmol/L. Samples measuring above 200 nmol/L are diluted 10-fold on the instrument using Elecsys Diluent MultiAssay and repeated. Values above the measuring range are reported up to 2000 nmol/L for 10-fold diluted samples. 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 0.350 nmol/L. 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[11] by analyzing 3 levels of QC materials in duplicate on 20 different runs, and in addition, according to the DLS Policy and Procedure Manual by analyzing 3 levels of bench QC materials in duplicate on 10 different days.

Within-run, between-run, and total precision were calculated. Total precision using the DLS method was 3.49%, 4.22%, and 5.64% for three 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	Quality Material 3
Within Run	2.42%	2.74%	2.84%
Between Run	2.52%	3.21%	4.87%
Total	3.49%	4.22%	5.64%

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 is applicable to the method.

Certified reference materials are not available for SHBG. Therefore, the NIBSC International Standard (08/266) was used to calibrate the method and to assess the method's accuracy. The NIBSC international reference reagent (08/266) for SHBG has a reported geometric mean estimate of 180 pmol/amp (95% CI: 176.16 – 194.14 ng/amp, n=14, GCV 3.92%). Therefore, the percent recovery was determined using the geometric mean of 180 pmol/amp. In this experiment, NIBSC 08/266 was reconstituted in 500 µL and spiked in synthetic serum at 4 different concentrations across the reportable range. Samples at four different levels were analyzed in triplicate across two analytical runs that spanned two separate days. For further details on the accuracy by NIBSC material, please refer to work instruction PBLW31LC02 (Accuracy Test Procedure for Measurement of the NIBSC Sex Hormone-Binding Globulin International Standard Using a Roche Cobas e411 Analyzer). The average percent recovery based on dilutions of the NIBSC 08/266 international standard for SHBG was 98.92%. Therefore, the accuracy was found to be acceptable for the NIBSC standard spiking experiment.

Due to the unavailability of well-characterized pure SHBG material, which is a large glycoprotein, spiking with pure compound material would lead to non-commutability of the sample. Therefore, accuracy was also 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%) at concentrations within the reportable range. For further details on the accuracy by mixing experiment, please refer to work instruction PBLW31CL01 (Accuracy by Mixing Test Procedure for Measurement of Sex Hormone-Binding Globulin Using a Roche Cobas e411 Analyzer). The recovery was calculated from the expected values.[15] The results of the accuracy test for the measurement of serum SHBG using the Roche **cobas** e 411 are shown in the table below.

Accuracy Mix	Average Measured Value (nmol/L)	Expected Value (nmol/L)	Recovery in %
25%	57.7	59.8	96.4
50%	85.7	89.2	96.1
75%	116.5	118.5	98.3

The accuracy, as determined by these mixing experiments, is found on average to be 96.9% for SHBG. 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 < 1026 µmol/L or < 60 mg/dL), hemolysis (Hb < 1.8 mmol/L or < 2.9 g/dL), lipemia (Intralipid < 2700 mg/dL), and biotin (< 246 nmol/L or < 60 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 1160 IU/mL.
- There is no high-dose hook effect at SHBG concentrations up to 1000 nmol/L
- In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

- 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.
- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

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 three 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, processed sample stability, and long-term stability (where applicable). The mean values from the replicates in 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 24h and was within 15% of the established value. Processed sample stability was evaluated at 4h. The samples were stable on board (ready for analysis) for 4 hours. Therefore, the samples have to be processed within 4h of being placed in the instrument.

The long-term stability of quality material 3 was evaluated as this pool has been stored at -40 to -80 °C for 5 years. The long-term stability could not be evaluated for quality material 1 & 2, as these LSP pools were recently adopted and are being assessed for long-term stability on the cobas e 411 system. The values of quality material 3, a serum–based material, was within 15% of the established value, confirming the long-term stability of material. Please refer to Appendix A for further details.

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 SHBG.

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

The results from the QC pools are assessed after each run by the QA Officer in collaboration with a statistician using the DLS SAS-based QC evaluation program. Each assessment includes

a. Verification that results meet acceptance criteria as indicated the SAS-QC report
b. A visual inspection of the graphs (performance of QCs over time) included in the SAS report to identify concerning trends (trends that are within the 10X rule but may have the potential to lead to failure of criteria).

When the SAS-QC report indicates failure to meet acceptance criteria, then actions described in the section below are initiated. Concerning trends are reported to the technical supervisor who will conduct further investigations as needed.
Acceptance criteria for values obtained with the bench QC materials (i.e., QC limits) are established according to the procedure described by Caudill et al.[16] and DLS P&PM Section 8.7.[10] This includes statistical assessment of trends. (10x rule). Each time the quality control of a runs is evaluated the trends is also evaluated.

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 SHBG immunoassay. The data was taken from the Tietz N.W. Clinical Guide to Laboratory Tests.[17]

SHBG Reference Range (nmol/L)	
Adult Male	10 – 80
Female (Non-pregnant)	20 – 130

11. CRITICAL CALL RESULTS ("PANIC VALUES")

No critical call values exist for SHBG.

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

If the analytical system fails, the specimens will be stored at -40 to -80°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, 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. Study subject confidentiality is protected by providing personal identifiers only to the medical officer, if needed, or remain with the contact person who requested the analyses.

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 SHBG 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

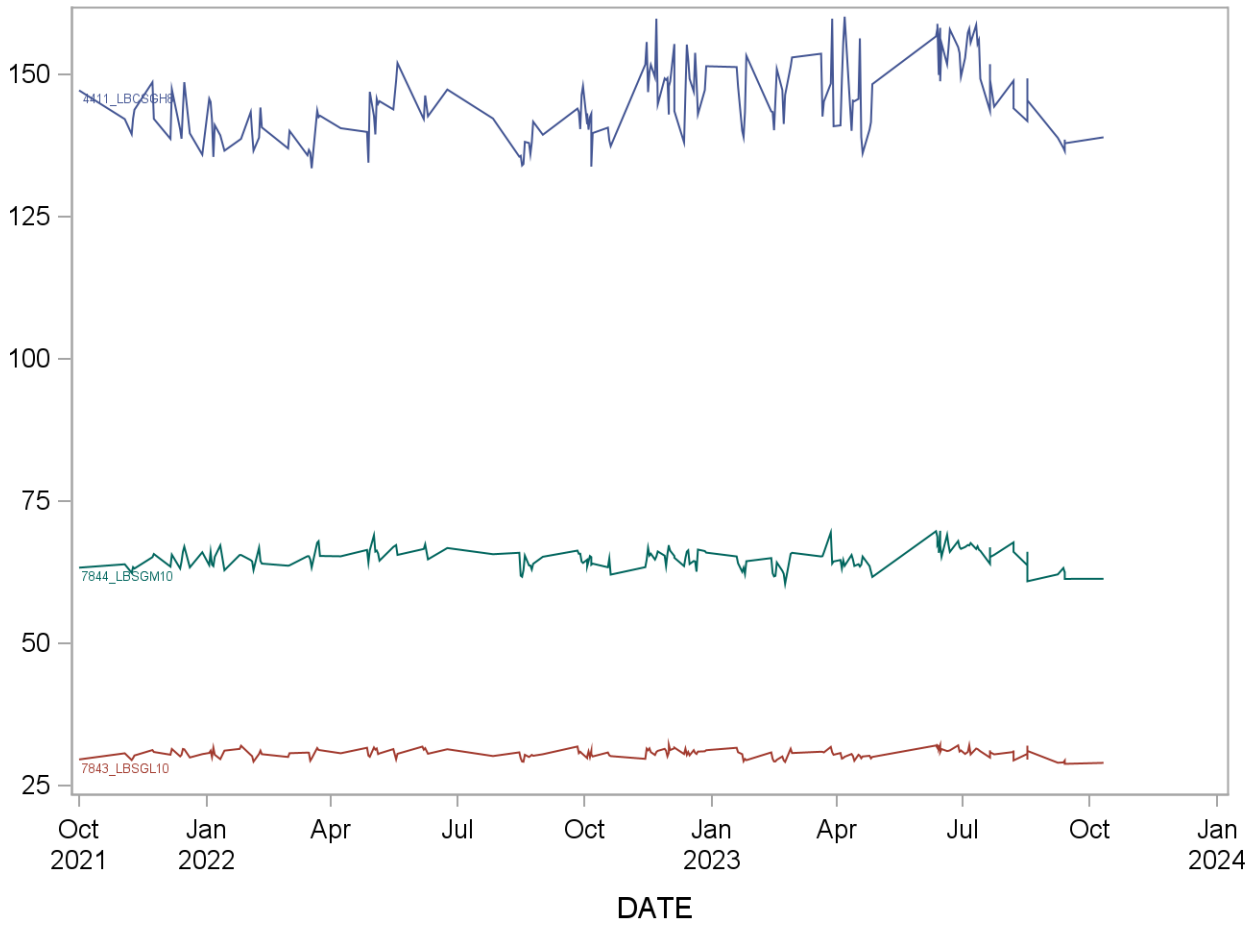
Not applicable for this procedure.

18. SUMMARY STATISTICS AND QC GRAPH

Please see following page.

August 2021 – August 2023 Summary Statistics and QC Chart
LBXSHBG (SHBG (nmol/L))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
4411_LBCSGH8	185	01OCT21	11OCT23	145.319	6.511	4.5
7843_LBSGL10	185	01OCT21	11OCT23	30.653	0.777	2.5
7844_LBSGM10	185	01OCT21	11OCT23	65.017	1.801	2.8



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Appendix A: Method Performance Documentation

Precision

Quality material 1: PHL31QC1L1 (LSP 205)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	28.53	30.68	29.61	1.155625	1.155625	1752.91205
2	29.41	29.78	29.60	0.034225	0.034225	1751.72805
3	29.87	30.21	30.04	0.0289	0.0289	1804.8032
4	29.29	29.52	29.41	0.013225	0.013225	1729.30805
5	28.73	30.66	29.70	0.931225	0.931225	1763.58605
6	27.82	29.75	28.79	0.931225	0.931225	1657.15245
7	29.73	28.77	29.25	0.2304	0.2304	1711.125
8	30.9	31.82	31.36	0.2116	0.2116	1966.8992
9	30.59	31.11	30.85	0.0676	0.0676	1903.445
10	31.16	29.7	30.43	0.5329	0.5329	1851.9698
11	29.47	30.28	29.88	0.164025	0.164025	1785.03125
12	31.4	31.46	31.43	0.0009	0.0009	1975.6898
13	30.28	30.73	30.51	0.050625	0.050625	1861.11005
14	31.62	31.33	31.48	0.021025	0.021025	1981.35125
15	30.35	29.89	30.12	0.0529	0.0529	1814.4288
16	31.07	30.84	30.96	0.013225	0.013225	1916.42405
17	30.4	32.31	31.36	0.912025	0.912025	1966.27205
18	31.78	32.3	32.04	0.0676	0.0676	2053.1232
19	31.11	31.22	31.17	0.003025	0.003025	1942.51445
20	31.72	31.51	31.62	0.011025	0.011025	1999.01645
Grand sum	1219.10	**Grand mean**	30.4775			

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	10.8666	0.54333	0.737109219	2.42
Between Run	32.76995	1.724734211	0.76857147	2.52
Total	43.63655		1.064909435	**3.49**

Quality material 2: PHL31QC2L1 (LSP 303)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	65.50	64.53	65.02	0.235225	0.235225	8453.90045
2	66.33	63.2	64.77	2.449225	2.449225	8389.01045
3	64.95	65.92	65.44	0.235225	0.235225	8563.47845
4	61.75	63.88	62.82	1.134225	1.134225	7891.44845
5	61.07	63.85	62.46	1.9321	1.9321	7802.5032
6	61.03	62.07	61.55	0.2704	0.2704	7576.805
7	57.67	60.92	59.30	2.640625	2.640625	7031.79405
8	63.52	61.06	62.29	1.5129	1.5129	7760.0882
9	71.10	66.29	68.70	5.784025	5.784025	9438.00605
10	66.92	65.71	66.32	0.366025	0.366025	8795.35845
11	65.78	68	66.89	1.2321	1.2321	8948.5442
12	63.78	65.99	64.89	1.221025	1.221025	8420.12645
13	67.59	68.53	68.06	0.2209	0.2209	9264.3272
14	64.05	64.02	64.04	0.000225	0.000225	8200.96245
15	65.28	68.83	67.06	3.150625	3.150625	8992.74605

16	66.91	62.96	64.94	3.900625	3.900625	8433.10845
17	67.91	66.51	67.21	0.49	0.49	9034.3682
18	68.05	66.17	67.11	0.8836	0.8836	9007.5042
19	64.56	68.65	66.61	4.182025	4.182025	8872.45205
20	67.46	67.14	67.30	0.0256	0.0256	9058.58
Grand sum	2605.44	**Grand mean**	65.136			

	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**
Within Run	63.7334	3.18667	1.785124646	2.74
Between Run	227.17216	11.95642947	2.094010443	3.21
Total	290.90556		2.751644915	**4.22**

Quality material 3: +0316244SA

Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	149.60	150.30	149.95	0.1225	0.1225	44970.005
2	143.9	153.1	148.50	21.16	21.16	44104.5
3	143.4	155.6	149.50	37.21	37.21	44700.5
4	142.8	140.9	141.85	0.9025	0.9025	40242.845
5	132.6	135.8	134.20	2.56	2.56	36019.28
6	138.5	135.5	137.00	2.25	2.25	37538
7	132.1	131.8	131.95	0.0225	0.0225	34821.605
8	135.2	138.8	137.00	3.24	3.24	37538
9	153.00	150.80	151.90	1.21	1.21	46147.22
10	151	155.9	153.45	6.0025	6.0025	47093.805
11	147.3	141.3	144.30	9	9	41644.98
12	158	158	158.00	0	0	49928
13	152.7	152.4	152.55	0.0225	0.0225	46543.005
14	153.8	158.3	156.05	5.0625	5.0625	48703.205
15	150.4	139.8	145.10	28.09	28.09	42108.02
16	147.4	150	148.70	1.69	1.69	44223.38
17	151.8	156.4	154.10	5.29	5.29	47493.62
18	157.5	153	155.25	5.0625	5.0625	48205.125
19	148.9	162.6	155.75	46.9225	46.9225	48516.125
20	152.2	152	152.10	0.01	0.01	46268.82
Grand sum	5914.40	**Grand mean**	147.86			

	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**
Within Run	351.66	17.583	4.193208795	2.84
Between Run	2306.856	121.4134737	7.205222886	4.87
Total	2658.516		8.336560252	**5.64**

Accuracy by Mixing

Mean concentration should be within ±15% of the nominal value except at 3*LOD, where it should be within ± 20%

	Measured concentration						
	Concentration	**Replicate**	**Expected value (nmol/L)**	**Value (nmol/L)**	**Mean (nmol/L)**	**Recovery (%)**	**Average Recovery (%)**
Day 1	Low (100)	1		30.4	30.2		
	Low (100)	2	30.5	30.2			
	Low (100)	3		30.2			
	Low/High (75:25)	1		57.4	56.8	94.9	96.5
	Low/High (75:25)	2	59.8	56.1			
	Low/High (75:25)	3		56.8			
	Low/High (50:50)	1		85.6	85.7	96.1	
	Low/High (50:50)	2	89.2	86.0			
	Low/High (50:50)	3		85.4			
	Low/High (25:75)	1		114.7	116.9	98.7	
	Low/High (25:75)	2	118.5	118.7			
	Low/High (25:75)	3		117.4			
	High (100)	1		150.8	150.6		
	High (100)	2	147.9	151.7			
	High (100)	3		149.4			
Day 2	Low (100)	1		31.5	30.9		
	Low (100)	2	30.5	30.4			
	Low (100)	3		31.0			
	Low/High (75:25)	1		58.6	58.6	98.0	97.3
	Low/High (75:25)	2	59.8	58.9			
	Low/High (75:25)	3		58.4			
	Low/High (50:50)	1		87.1	85.7	96.1	
	Low/High (50:50)	2	89.2	82.7			
	Low/High (50:50)	3		87.2			
	Low/High (25:75)	1		114.6	116.0	97.9	
	Low/High (25:75)	2	118.5	118.6			
	Low/High (25:75)	3		114.7			
	High (100)	1		155.9	153.7		
	High (100)	2	147.9	151.9			
	High (100)	3		153.3			
						Total Average Recovery	**96.9**

Accuracy compared to Reference Material

Mean concentration should be within ±15% of the nominal value except at 3*LOD, where it should be within ± 20%.

Reference Material: NIBSC 08/266

Certified reference materials are not available for this analyte. Therefore, the NIBSC International Standard (08/266), which is one level, was used to calibrate the method and to assess the method's accuracy.

Analyte	Dilution level (v/v)	Day 1	Day 2	Total
		Average Measured Value (nmol/L)	Average Measured Value (nmol/L)	Average Percent Recovery (%)
		%CV	%CV	
SHBG	1:10, v/v (Instrument assigned)	313.67	337.97	90.50
		1.60	2.83	
	1:20, v/v (Manual dilution)	310.07	373.53	94.94
		1.55	2.52	
	1:30, v/v (Manual dilution)	381.10	379.90	105.7
		2.13	0.36	
	1:40, v/v (Manual dilution)	382.13	370.40	104.5
		3.75	2.28	

The average percent recovery based on dilutions of the NIBSC 08/266 international standard for SHBG is **98.92%.**

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 -40 to -80°C and then thawed (3 freeze-thaw cycles)

Quality material 1: PHL31QC1L1 (LSP 205)		
	Initial measurement	**After three freeze-thaw cycles**
Replicate 1, nmol/L	29.67	30.96
Replicate 2, nmol/L	30.1	30.68
Replicate 3, nmol/L	29.96	29.32
Mean, nmol/L	29.91	30.32
% difference from initial measurement	--	**1.4**

Quality material 2: PHL31QC2L1 (LSP 303)		
	Initial measurement	**After three freeze-thaw cycles**
Replicate 1, nmol/L	60.26	63.22
Replicate 2, nmol/L	66.23	64.9
Replicate 3, nmol/L	66.13	64.77
Mean, nmol/L	64.21	64.3
% difference from initial measurement	--	**0.1**

Quality material 3: +0316244SA		
	Initial measurement	**After three freeze-thaw cycles**
Replicate 1, nmol/L	142.3	144.5
Replicate 2, nmol/L	141.2	152.2
Replicate 3, nmol/L	160.9	165.3
Mean, nmol/L	148.1	154
% difference from initial measurement	--	**4.0**

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 24 hours.

Quality Material 1: PHL31QC1L1 (LSP-205)		
	Initial measurement	**After 24h on rotator**
Replicate 1, nmol/L	29.97	30.05
Replicate 2, nmol/L	30.58	29.89
Replicate 3, nmol/L	30.38	29.75
Mean, nmol/L	30.31	29.9
% difference from initial measurement	**--**	**-1.4**

Quality Material 2: PHL31QC2L1 (LSP-303)		
	Initial measurement	**After 24h on rotator**
Replicate 1, nmol/L	63.73	59.91
Replicate 2, nmol/L	67.5	63.55
Replicate 3, nmol/L	66.52	63.61
Mean, nmol/L	65.92	62.4
% difference from initial measurement	**--**	**-5.4**

Quality Material 3: +0316244SA		
	Initial measurement	**After 24h on rotator**
Replicate 1, nmol/L	146.7	142
Replicate 2, nmol/L	150.4	143.9
Replicate 3, nmol/L	149	145
Mean, nmol/L	148.7	143.6
% difference from initial measurement	**--**	**-3.4**

Processed Sample Stability

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

Quality Material 1: PHL31QC1L1 (LSP-205)		
	Initial measurement	**After 4 hours onboard**
Replicate 1, nmol/L	28.87	28.57
Replicate 2, nmol/L	30.7	30.46
Replicate 3, nmol/L	29.32	31.42
Mean, nmol/L	29.63	30.15
% difference from initial measurement	**--**	**1.8**

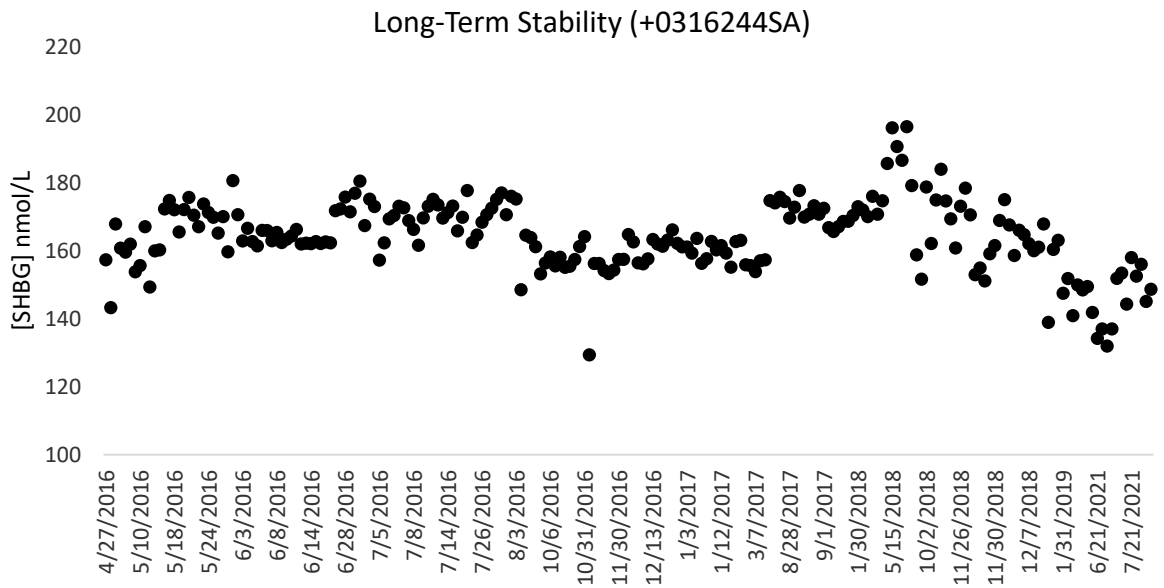
Quality Material 2: PHL31QC2L1 (LSP-303)		
	Initial measurement	**After 4 hours onboard**
Replicate 1, nmol/L	62.79	58.65
Replicate 2, nmol/L	63.55	66.45
Replicate 3, nmol/L	65.32	66.88
Mean, nmol/L	63.89	63.99
% difference from initial measurement	**--**	**0.2**

Quality Material 3: +0316244SA		
	Initial measurement	**After 4 hours onboard**
Replicate 1, nmol/L	143.8	144.5
Replicate 2, nmol/L	152.7	157.4
Replicate 3, nmol/L	149.7	150.1
Mean, nmol/L	148.7	150.7
% difference from initial measurement	**--**	**1.3**

Long-Term Stability

Long-term stability assesses long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis.

Quality material 3 has been stored at -40 to -80 °C for 5 years. The long-term stability could not be evaluated for quality material 1 & 2, as these LSP pools were recently adopted and are being assessed for long-term stability on the Cobas e411 system.



Mean (nmol/L)	164.06
%CV	6.37
STDEV	10.45
2STDEV	20.9
days	201
n	430

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
SHBG	0.350 nmol/L	yes	yes