

For the Quantitative Measurement of Sex Hormone Binding Globulin in Human Serum and Plasma

The concentration of Sex Hormone Binding Globulin in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the Sex Hormone Binding Globulin assay method used. Values obtained with different assay methods should not be used interchangeably.

INTENDED USE

FastPack® IP SHBG is a chemiluminescent immunoassay intended for the quantitative determination of Sex Hormone Binding Globulin in human serum and plasma on the FastPack® System. The FastPack® IP SHBG assay is intended for use as an aid in the diagnosis of androgen disorders.

SUMMARY

Sex Hormone Binding Globulin (SHBG) is a dimeric glycoprotein (MW 90,000 Da) comprising of 373 amino acids responsible for blood transport of testosterone and estradiol. SHBG is synthesized in the liver and its production is controlled by certain hormonal as well as physiological and pathological conditions.^{1,2,3} It has a high binding affinity for 17-hydroxysteroid hormones. Less than 2% of biologically active steroids are free in the circulation with the remainder being bound mostly to SHBG and albumin. SHBG has a high binding affinity to the 17-hydroxysteroid hormones while albumin has a low binding affinity. Initially, the free portion or unbound hormone fraction was believed to be the only biologically active form.⁴ It is now recognized that the portion of hormone that is weakly bound to albumin is also available to the tissues. The free hormone plus the albumin bound portion of hormones represents the "bioavailable" hormone.⁵

The measurement of SHBG can be an important indicator of a chronic or excessive androgenic activity where clinical symptoms would seem to indicate androgen in excess, but androgen levels are normal. Elevated SHBG levels can be seen in persons with androgen insensitivities, hyperthyroidism, cirrhosis of the liver and is found in patients on oral contraceptives or antiepileptic drugs. Decreased concentrations of SHBG are often seen in men with hypothyroidism and androgen replacement therapy; where women with hirsutism, virilism, polycystic ovarian syndrome (PCOS), elevated androgen levels, obesity and acromegaly will also see a decrease in SHBG levels.^{5,6,7}

SHBG production is regulated by the androgen/estrogen balance, thyroid hormones, insulin, and dietary factors. The concentration of SHBG is increased by estrogens and decreased by androgens.⁵ Therefore, SHBG production is stimulated by estradiol and suppressed by testosterone.⁸ As a result, SHBG concentrations are higher in women versus men. Pregnant women have markedly higher SHBG serum concentrations due to their increased estrogen production.^{9,10}

Free testosterone can be measured directly by equilibrium dialysis. Alternatively, the non-SHBG-bound fraction may be obtained by precipitation of SHBG-bound testosterone with ammonium sulfate. As both methods are not routinely performed in most laboratories, an indirect method calculation can be utilized to estimate free testosterone. Calculating the Free Testosterone Index (FTI) or the Free Androgen Index (FAI) requires the measurement of total testosterone and SHBG concentrations when used in assessing a patient androgen status.¹¹

The FAI is generally considered useful in estimating free testosterone in women with hirsutism or hyperandrogenism.

The FastPack® IP Sex Hormone Binding Globulin Immunoassay is intended for use in exactly the same manner as the predicate device identified in this submission (Beckman Coulter Access SHBG reagent) and other assays with similar or identical intended uses.

TEST PRINCIPLE

The FastPack® IP Sex Hormone Binding Globulin Immunoassay is a paramagnetic particle chemiluminescence immunoassay based on the "sandwich" immunoassay principle.

- Endogenous SHBG in a patient sample, calibrator, or control is dispensed into a FastPack® IP reagent pack.
- In the reagent pack, the sample binds with a monoclonal anti-SHBG antibody covalently linked to alkaline phosphatase (ALP) and a different monoclonal anti-SHBG antibody linked to biotin.
- After incubation, immunoreacted complex (monoclonal anti-SHBG antibody-ALP conjugate and anti-SHBG antibody linked to biotin reacted with SHBG in the sample) is mixed with streptavidin coated paramagnetic particles.
- After washing steps (using a Tris buffer containing detergents) to separate bound from unbound anti-SHBG monoclonal antibody-ALP, a chemiluminogenic substrate mixture is added to the system. This mixture contains indoxyl-3-phosphate, a substrate for ALP, and lucigenin (N,N'-dimethyl-9,9'-biacridinium dinitrate).

- ALP dephosphorylates indoxyl-3-phosphate to indol-3-ol, which subsequently undergoes oxidation. As a result, lucigenin is reduced to form a dioxetane structure that is cleaved to yield N-methylacridone. This compound produces a sustained luminescent glow following excitation.
- The raw relative luminescence units (RLUs) generated are measured by a photomultiplier tube in the FastPack® Analyzer and are directly proportional to the concentration of SHBG in the sample.
- The entire reaction sequence takes place at 37 ± 0.5 °C and is protected from external light.

Kit – Content and Concentration

Each FastPack® IP Sex Hormone Binding Globulin Immunoassay Kit - Cat. No. 25000080 contains:

- 60 FastPack® IP Sex Hormone Binding Globulin Reagent Packs
- 64 Sample diluent vials, 0.9 mL each
- 1 Vial FastPack® SHBG Calibrator, 2.0 mL
- 1 Vials FastPack® SHBG Control 1, 2.0 mL
- 1 Vials FastPack® SHBG Control 2, 2.0 mL
- 1 Calibration Card
- 1 Control Range Card

Each FastPack® IP Sex Hormone Binding Globulin Immunoassay Kit - Cat. No. 25000081 contains:

- 30 FastPack® IP Sex Hormone Binding Globulin Reagent Packs
- 32 Sample diluent vials, 0.9 mL each
- 1 Vial FastPack® SHBG Calibrator, 2.0 mL
- 1 Vials FastPack® SHBG Control 1, 2.0 mL
- 1 Vials FastPack® SHBG Control 2, 2.0 mL
- 1 Calibration Card
- 1 Control Range Card

Each FastPack® IP Sex Hormone Binding Globulin Reagent Pack contains:

- Paramagnetic Particles coated with streptavidin-monoclonal anti-SHBG antibody linked to covalently biotin, 150 µL
- Monoclonal anti-SHBG antibody covalently linked to alkaline phosphatase and Monoclonal anti-SHBG antibody covalently linked to biotin, 100 µL
- Wash Buffer, 2.0 mL
Tris buffer containing surfactants
- Substrate, 145 µL
ImmuGlow™ Plus: Indoxyl-3-phosphate and lucigenin in buffer containing preservatives

Materials required but not provided

- FastPack® System

WARNINGS AND PRECAUTIONS

- For *in-vitro* diagnostic use only.
- Do not pipette by mouth.
- Do not eat, drink or smoke in designated work areas.
- Wash hands thoroughly after handling specimen.
- HAMA Interference: some individuals have antibodies to mouse protein (HAMA), which can cause interference in immunoassays that employ antibodies derived from mice.
- FastPack® IP reagents are stable until the expiration date on the label when stored and handled as directed. Do not use FastPack® IP reagents beyond the expiration date.
- Discard used FastPacks into a Biohazard container.
- Proclin 300 is an irritant. The following are appropriate Risk (R) and Safety (S) phrases for Proclin 300:
 - R43 May cause sensitization by skin contact
 - S28-37 After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

STORAGE INSTRUCTIONS

Store at 2 – 8 °C.








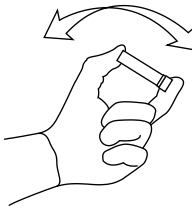
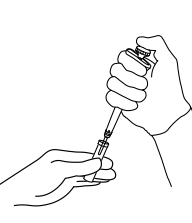
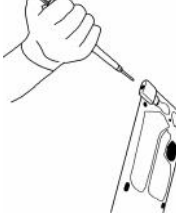


SPECIMEN COLLECTION/PREPARATION

1. Serum or lithium-heparin plasma samples can be used for the FastPack® IP Sex Hormone Binding Globulin Immunoassay.
2. The Clinical and Laboratory Standards Institute (CLSI) provides the following recommendations for handling, processing and storing blood.¹²
 - A. Collect all blood samples observing routine precautions for venipuncture.

- B. For serum samples:
 - a. Serum should be separated from the cells by centrifugation within 3 hours from time of collection and stored at 2-8 °C. Transfer the serum from the original tube for storage.
 - b. If not tested within 24 hours, store the sample at 2-8°C.
 - c. Don not freeze samples.
- C. For plasma samples:
 - a. Collect samples in a lithium-heparin (green top) tube.
 - b. Mix the tube immediately after collection by gently inverting it several times.
 - c. Plasma should be separated from the cells by centrifugation within 3 hours from time of collection and stored at 2-8 °C. Transfer the plasma from the original tube for storage.
 - d. If not tested within 24 hours, store the sample at 2-8°C.
 - e. Don not freeze samples.
- D. Samples should be free of red blood cells, or other particulate material for optimal results.
- E. Samples showing particulate matter should be centrifuged prior to use.
- F. Samples showing turbidity (high lipid content) should not be used.
- G. Ensure the samples are free of bubbles.

ASSAY PROCEDURE

See the QA Manual or the FastPack® System Procedure Manual for detailed instructions for running FastPack® IP SHBG assays.

 <p>1. Write the Patient's Name/ID # and the operator's initials on the FastPack® label.</p>	 <p>2. Press and hold the pipette plunger down completely so that the metal grippers are extended and open.</p>	 <p>3. While holding the plunger down, firmly press the pipette into the pipette tip until it snaps in place, then release the plunger.</p>	 <p>4. Be sure the pipette tip is seated properly on the end of the pipette.</p>	 <p>5. Verify that the pipette tip is properly seated by gently pressing the plunger down to the first stop and releasing. An audible "Click" may occur if the piston is not seated properly.</p>	 <p>6. Gently press the pipette plunger down to the first stop and hold. Place the pipette tip into the sample tube; withdraw sample by slowly releasing the plunger. Inspect the pipette tip to confirm there are no air bubbles in the sample.</p>
 <p>7. All samples must be diluted 1:10 with FastPack® SHBG Sample Diluent (1 part sample, 9 parts sample diluent). Eject sample into the tube (pre-loaded with 900 µL Sample Diluent) by pressing down on the pipette plunger to the first stop. Replace the screw cap tightly onto the Sample Diluent tube.</p>	 <p>8. Invert the buffer tube at least 3 times to thoroughly mix together the sample and buffer.</p>	 <p>9. Withdraw the sample from the diluted sample tube using the same pipette tip as before and following the same technique as in Step 6 above.</p>	 <p>10. With your finger off the pipette plunger, fully insert the filled pipette tip into the FastPack® Injection Port. It should fit tightly.</p>	 <p>11. Be sure the pipette tip is seated properly into the injection port.</p>	 <p>12. In one continuous motion, quickly press the pipette plunger all the way down. This action will simultaneously inject the sample into the FastPack® and automatically eject the pipette tip.</p>

INSTRUMENTATION

FastPack® System

DETAILS OF CALIBRATION

During the FastPack production process, Qualigen generates a master standard curve and places this information in the barcode of each FastPack[®] label, where it can be read by the FastPack Analyzer during the testing sequence. The FastPack Analyzer must be calibrated by the user to ensure that it is properly adjusted for the particular lot of FastPacks that is being used. Separate calibrations must be run for each type of test, i.e. Total PSA, Testosterone, Vitamin D, TSH, free T4, High Sensitivity C-Reactive Protein, Sex Hormone Binding Globulin, etc. The frequency of calibration varies for each test type. For the FastPack[®] IP Sex Hormone Binding Globulin Immunoassay, the FastPack[®] Analyzer must be calibrated once every 28 days or whenever a new lot of Sex Hormone Binding Globulin FastPacks are to be used.

Whenever the user performs an initial calibration for a particular lot of FastPacks or uses a new lot of calibrator, 2 FastPacks must be run for calibration (duplicates). Whenever recalibration is performed with the same lot of FastPacks and calibrator, 2 FastPacks must be run for calibration. See FastPack[®] System Procedure Manual for "Running a Calibration".

Use FastPack[®] IP Sex Hormone Binding Globulin Kit – Cat. No. 25000080 or 25000081

RESULTS

The FastPack[®] Analyzer uses the information from the barcode to construct a lookup table of x,y values that represent the standard curve and estimates the concentration of unknown samples by linear interpolation.

QUALITY CONTROL

Quality control materials simulate real specimens and are essential for monitoring the system performance of assays. Good Laboratory Practices (GLP) include the use of control specimens to ensure that all reagents and protocols are performing properly. See FastPack[®] System Procedure Manual for "Control Testing". At least two levels of quality control materials should be used.

Users should follow the appropriate federal, state and local guidelines concerning the running of external quality controls.

LIMITATION OF PROCEDURE

- Plasma samples to be collected using lithium-heparin as the anticoagulant.
- Do not use lipemic samples because lipemic samples will generate a falsely low result.
- Specimens can be measured within the reportable range of the limit of quantitation (0.80 nmol/L) and the upper end of the calibration range, 174 nmol/L.
- Samples < 0.80 nmol/L should be reported as such.
- Samples >174 nmol/L should be reported as such.
- Specimen from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits employing mouse monoclonal antibodies.
- Heterophilic antibodies in a sample have the potential to cause interference in immunoassay systems. Infrequently, SHBG levels may appear depressed due to heterophilic antibodies present in the patient's sample or to nonspecific protein binding. If the SHBG level is inconsistent with clinical evidence, additional SHBG testing is suggested to confirm the result.^{13,14}
- For diagnostic purposes, the FastPack[®] IP Sex Hormone Binding Globulin Immunoassay should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.
- The FastPack[®] IP Sex Hormone Binding Globulin Immunoassay has not been evaluated in Point of Care settings and should not be used at Point of Care settings.

EXPECTED VALUES/REFERENCE INTERVALS

Each laboratory should determine ranges for their local population. A reference interval study employing serum samples from 237 apparently healthy subjects with no known pre-existing endocrine disorders was performed. The non-parametric 2.5th - 97.5th percentiles (central 95%) were determined for reference partitions as shown below.

Partition	N	Median (nmol/L)	Reference Interval (nmol/L)
Males 13 – 50 years	149	26.6	9.4 – 61.8
Males > 50 years	155	35.9	13.0 – 86.4
Females 12 – 46 years	151	39.6	9.2 – 134.4
Females > 46 years	158	49.8	12.2 – 121.2

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

Precision was evaluated following the CLSI EP5-A3 guidance. Seven serum patient samples with concentrations of ~5, 30, 60, 90, 100 and 150 nmol/L SHBG were tested in duplicate determinations in each of two runs per day on each of three FastPack® IP reagent lots, one FastPack® analyzer per reagent lot (total of three Analyzers), one FastPack® Calibrator per reagent lot (total of three Calibrator lots) over a period of 20 non-consecutive days to yield 240 replicate determinations of each sample. Within-run, between-run, between-day, and total imprecision were calculated using a fully nested 2-way random factor analysis of variance (ANOVA) model. The following three tables present the results by combination of reagent lot, analyzer, and calibrator lot:

Reagent lot 1, analyzer 1, calibrator lot 1

Sample	Mean nmol/L	Within-Run		Between-Run		Between-Day		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	4.85	0.27	5.55	0.00	0.00	0.21	4.37	0.34	7.06
2	12.56	0.60	4.82	0.68	5.46	0.28	2.19	0.95	7.60
3	25.59	1.40	5.46	1.83	7.14	0.87	3.40	2.46	9.61
4	59.49	3.67	6.16	1.80	3.03	2.95	4.96	5.04	8.47
5	91.33	5.99	6.56	7.12	7.79	0.00	0.00	9.30	10.18
6	102.26	7.11	6.95	3.85	3.76	4.76	4.66	9.38	9.18
7	154.79	4.24	2.74	0.62	0.40	2.51	1.62	4.96	3.21

Reagent lot 2, analyzer 2, calibrator lot 2

Sample	Mean nmol/L	Within-Run		Between-Run		Between-Day		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	5.02	4.88	4.88	0.00	0.00	0.16	3.20	0.29	5.83
2	16.74	0.74	4.40	0.89	5.29	0.88	5.24	1.45	8.65
3	29.97	1.58	5.27	1.97	6.56	0.37	1.25	2.55	8.51
4	63.53	2.99	4.71	3.36	5.29	2.57	4.04	5.18	8.15
5	94.47	7.31	7.74	5.33	5.64	4.44	4.70	10.07	10.66
6	107.81	8.31	7.70	4.59	4.26	8.03	7.44	12.43	11.53
7	150.43	4.16	4.16	0.00	0.00	4.28	2.85	7.58	5.04

Reagent lot 3, analyzer 3, calibrator lot 3

Sample	Mean nmol/L	Within-Run		Between-Run		Between-Day		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	4.90	0.21	4.19	0.00	0.00	0.10	2.02	0.23	4.66
2	14.26	0.67	4.69	0.51	3.56	0.45	3.13	0.95	6.67
3	25.47	1.22	4.78	1.75	6.86	0.91	3.58	2.32	9.10
4	58.56	3.30	5.64	4.22	7.21	2.25	3.85	5.81	9.93
5	91.40	8.78	9.60	3.70	4.05	5.53	6.05	11.02	12.05
6	105.25	7.42	7.05	5.21	4.95	0.00	0.00	9.07	8.61
7	148.44	5.04	3.40	0.81	0.55	2.97	2.00	5.91	3.98

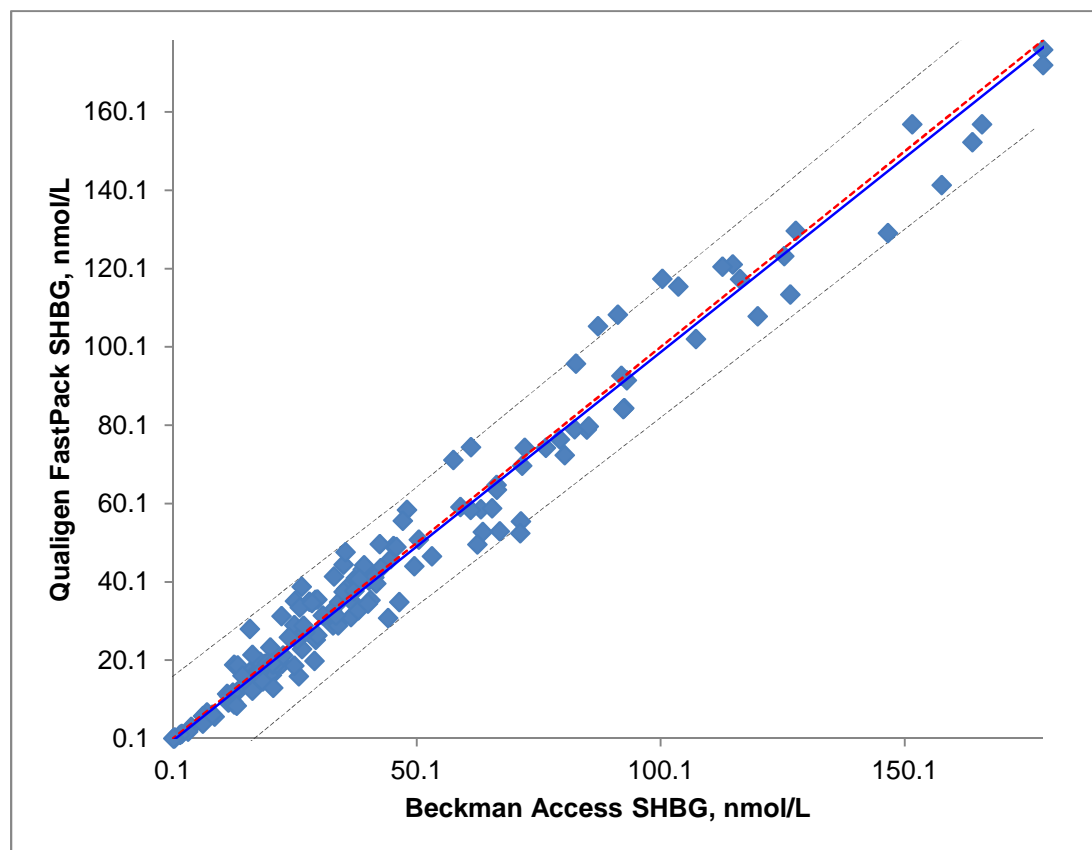
Range of Linearity

For SHBG as tested by the FastPack® IP Sex Hormone Binding Globulin Immunoassay, the method has been demonstrated to be linear from the LOQ (0.80 nmol/L) to 174 nmol/L SHBG.

Method Comparison

Clinical serum samples (n=158) were used to compare the values obtained using the FastPack® IP Sex Hormone Binding Globulin Immunoassay method and the values obtained using the Beckman-Coulter Access SHBG assay. The values were evaluated for agreement using Passing-Bablok regression analysis, with the associated correlation coefficient.

n	Range of Observation (nmol/L)	Intercept (mg/L)	Slope	R
158	5.7 – 176.0	-0.614	0.993	0.985



Interfering Substances

The effect of endogenous interferences on quantification of SHBG was investigated by preparation of two serum samples with differing SHBG concentrations (a low and high) with known concentrations of conjugated bilirubin, unconjugated bilirubin, hemoglobin, lipids, and d-biotin. The value obtained for the sample with each interfering substance was compared to the value obtained for the sample without the interfering substance and the percentage recovery in nmol/L SHBG determined. These compounds did not show interference at the levels indicated in the following table. Higher levels may cause interference.

Compound	Highest level demonstrating no interference
Conjugated Bilirubin	40 mg/dL
Unconjugated Bilirubin	30 mg/dL
Hemoglobin	1.0 g/dL
Lipid	1000 mg/dL
d-Biotin	0.2 mg/dL

The effect of potentially cross-reacting substances on quantification of SHBG was investigated. Again, two serum samples with differing SHBG concentrations (a low and high) with known concentrations of spiked cross-reactants were prepared. The value obtained for the sample with each potentially cross-reacting substance was compared to

the value obtained for the sample without the substance and the percentage recovery in nmol/L SHBG determined. These compounds did not show cross-reactivity at the levels indicated in the following table. Higher levels may cause cross-reaction.

Compound	Highest level demonstrating no cross-reaction
Transferrin	0.5 g/dL
Heparin	10,000 U/dL
Low Molecular Weight Heparin (LMWH)	0.6 U/dL
Ibuprofen	60 mg/dL
Human Albumin	Endogenous in samples + 8.0 g/dL
Human IgG	1.0 g/dL
Thyroxine Binding Globulin (TBG)	20 mg/dL
Thyroglobulin	300 µg/L
Testosterone	2.5. mg/dL
Laminin	6,000 µg/L
GAS6	250 µg/L
Protein S	30 mg/L
Estradiol	4.0 mg/dL
11-deoxycortisol	0.5 mg/dL
5 -dihydrotestosterone	2.0 mg/dL
Cortisol	10 mg/dL
AFP	500 µg/L
Acetaminophen	10 mg/dL
Acetylsalicylic acid	80 mg/dL

Rheumatoid Factor, Human Anti-Mouse Reactions, and Heterophile Interference

Rheumatoid factor at up to 1000 IU/mL and human anti-mouse IgG at up to 4 µg/mL do not cross-react in the FastPack® Sex Hormone Binding Globulin Immunoassay. Additionally, six known heterophile samples did not generate detectable interference in the assay.

Hook Effect

The FastPack® IP Sex Hormone Binding Globulin Immunoassay displays no hook effect up to and including 1000 nmol/L SHBG.

Limit of Blank (LOB), Limit of Detection (LOD), and Limit of Quantitation (LOQ)

The limit of blank (LOB, the highest measurement likely to be observed for a blank sample), limit of detection (LOD, the lowest amount of analyte in a sample that can be detected with type I and II error rates set to 5%), and limit of quantitation (LOQ, the lowest amount of analyte in a sample that can be reliably detected) were determined according to CLSI EP17-A2 for the FastPack® IP Sex Hormone Binding Globulin Immunoassay. In this study, the limit of blank was determined from 180 replicate determinations of a blank sample tested on six different FastPack® analyzers using three reagent lots. Raw RLUs from the assays were converted to apparent nmol/L based on the calibration curve for each assay. The LOB was determined as the 171.5th rank of the sorted distribution of values. This value was 0.08 nmol/L SHBG.

The LOD was estimated from 180 replicate determinations of four low level samples. Per the CLSI EP17-A2 guideline, the parametric LOD calculation was utilized and yielded 0.20 nmol/L SHBG.

The LOQ was determined as the lowest sample which provided 20% CV. The LOQ was set to 0.80 nmol/L SHBG.

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