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EU rev. CL-SHBG-020324

CL AIA-PACK® SHBG TEST CUP

For Quantitative Measurement of Sex Hormone-Binding Globulin (SHBG) in Serum or Heparinised Plasma

NAME AND INTENDED USE

CL AIA-PACK SHBG TEST CUP is a single use reagent, designed for *IN VITRO* DIAGNOSTIC USE ONLY, by healthcare professionals, for the quantitative measurement of SHBG in human serum or heparinised plasma, using the automated Tosoh AIA®-CL Analyzers, to aid to clinical management of androgen status. Measurement of SHBG is useful in the evaluation of mild disorders of androgen metabolism and enables identification of those women with hirsutism who are more likely to respond to oestrogen therapy. Testosterone/SHBG ratios correlate well with both measured and calculated values of free testosterone and help to discriminate subjects with excessive androgen activity from normal individuals.

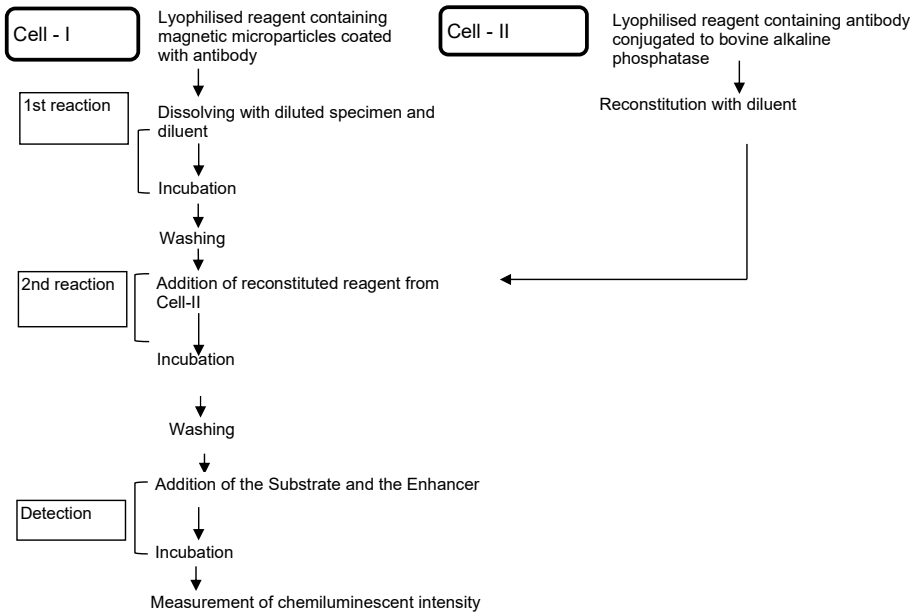
SUMMARY AND EXPLANATION OF TEST

Sex hormone-binding globulin (SHBG), one of blood transport proteins, is a large homodimeric glycoprotein that especially binds testosterone and estradiol *(1)*. It is mostly synthesized in the liver and is released into the bloodstream. The biological activities of these sex steroids in plasma are regulated by plasma SHBG, since its high binding affinity to the steroids limits their access to target cells *(2)*.
It is suggested that SHBG concentrations are influenced by various factors, such as thyroid hormones, oestrogens, insulin, and several growth factors. Increases in SHBG levels are found in hyperthyroidism, hepatic cirrhosis, and pregnancy. High SHBG values are also seen with oral oestrogen treatment, tamoxifen administration, and Klinefelter's syndrome. Decreases in SHBG levels are observed in female patients with hirsutism, hyperandrogenism, or insulin resistance syndrome. In hypothyroidism, SHBG is often lowered *(3, 4)*.
SHBG is widely measured together with testosterone assays to calculate the "Free Androgen Index" (FAI), the ratio of total testosterone level divided by SHBG level. The FAI is known as a useful indicator to determine abnormal androgen status *(5, 6)*.

PRINCIPLE OF THE ASSAY

The CL AIA-PACK SHBG assay is a two-step chemiluminescence enzyme immunoassay (CLEIA) kit. The assay is performed in the CL AIA-PACK SHBG TEST CUP. SHBG present in a test sample is bound to anti-SHBG mouse monoclonal antibody immobilized on the magnetic microparticles in one cell (Cell-I). After first incubation, the magnetic microparticles are washed to remove unbound materials and then a specific volume of the enzyme-labelled anti-SHBG mouse monoclonal antibody that has been reconstituted in another cell (Cell-II) is dispensed into Cell-I. After second incubation, the magnetic microparticles are washed again to remove unbound enzyme-labelled monoclonal antibody and are incubated with a chemiluminescent substrate, DIFURAT®(*). The amount of enzyme-labelled antibodies that bind to the magnetic microparticles is directly proportional to the SHBG concentration in the test sample. A standard curve is constructed, unknown sample concentrations are calculated by using this curve.

(*) DIFURAT: 3-(5-*tert*-Butyl-4,4-dimethyl-2,6,7-trioxabicyclo[3.2.0]hept-1-yl) phenylphosphate disodium salt



MATERIAL PROVIDED

Cat. No. 0029139
3 trays x 32 test cups CL AIA-PACK SHBG TEST CUP
Plastic test cups (twin-cup, Cell-I and Cell-II) containing the following:

- Cell-I: Lyophilised reagent containing magnetic microparticles coated with anti-SHBG mouse monoclonal antibodies.
- Cell-II: Lyophilised reagent containing anti-SHBG mouse monoclonal antibody conjugated to bovine alkaline phosphatase.

MATERIALS REQUIRED BUT NOT PROVIDED

The following materials are required to perform SHBG analysis using the CL AIA-PACK SHBG TEST CUP (Cat. No. 0029139) on the Tosoh AIA-CL Analyzers. They are available from Tosoh separately.

Materials			Cat. No.
AIA-CL2400 ST			0023650
AIA-CL2400 LA			0023651
AIA-CL1200 ST			0024130
AIA-CL1200 LA			0024131
AIA-CL300			0024450
CL AIA-PACK SUBSTRATE SET (for 50 mL)			0029701
CL AIA-PACK SUBSTRATE/CL AIA-PACK ENHANCER			
CL AIA-PACK SUBSTRATE SET (for 100 mL)			0029702
CL AIA-PACK SUBSTRATE/CL AIA-PACK ENHANCER			
CL AIA-PACK SUBSTRATE SET (CUPS)			0029709
CL AIA-PACK SUBSTRATE/CL AIA-PACK ENHANCER			
CL AIA-PACK SHBG ADJUSTER SET			0029239
CL AIA-PACK SHBG ADJUSTER (1)	0.30	nmol/L (approx.)	
CL AIA-PACK SHBG ADJUSTER (2)	6.25	nmol/L (approx.)	

AIA-PACK SHBG CONTROL SET	0025438
AIA-PACK SHBG CONTROL LEVEL 1	
AIA-PACK SHBG CONTROL LEVEL 2	
CL AIA-PACK SAMPLE DILUTING REAGENT A	0029401
CL AIA-PACK WASH CONCENTRATE	0029703
CL AIA-PACK DILUENT CONCENTRATE (100 mL: for Automatic Dilution)	0029704
CL AIA-PACK DILUENT CONCENTRATE (60 mL: for Manual Dilution)	0029708
SAMPLE CUPS	0018581
CL AIA-PACK DETECTOR STANDARDIZATION CUP	0029705
PIPETTE TIPS	0019215
TIP RACK SET (for AIA-CL2400 and AIA-CL1200)	0023709
TIP RACK SET (for AIA-CL300)	0023306

Only materials obtained from Tosoh should be used. Materials obtained elsewhere should not be substituted since assay performance is characterized based strictly on Tosoh materials.

WARNINGS AND PRECAUTIONS

1. This Instructions For Use must be combined with the Operators' manual of the Tosoh AIA-CL Analyzers in use.
2. The CL AIA-PACK SHBG TEST CUP is intended for *in vitro* diagnostic use only.
3. Inspect the packaging and the exterior of the aluminium pouch for any sign of damage before use. If any damages are visible, contact your local Tosoh sales representative.
4. Test cups from different lots or for different analytes shall not be mixed within a tray.
5. The CL AIA-PACK SUBSTRATE SET, the CL AIA-PACK WASH CONCENTRATE, and the CL AIA-PACK DILUENT CONCENTRATE contain sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
6. No human-derived material is used in the preparation of this product; however, since human specimens will be used as samples and other quality control products used in the lab may be derived from human sources, please use standard laboratory safety procedures in handling all products, specimens and quality controls.
7. Do not use beyond the expiry date.
8. For safe waste disposal, it is recommended that each laboratory complies with the established laboratory procedures and local, state, and federal regulations.
9. Please place the CL AIA-PACK SUBSTRATE SET on the instrument in a clean environment. Once placed, do not remove the bottles until they are replaced with new ones. Contamination by blood, body fluid, dust, metal, or microorganism may lead to erroneous results because of degradation of the substrate.
10. When replacing the bottles of the CL AIA-PACK SUBSTRATE SET with new ones, discard both old bottles and remaining solution. Do not mix the remaining solution with a new one, not even when they are both the same lot.t.
11. Tosoh recommends that a new pouch of the Test Cups be used for Master curve adjustment.
12. Do not report patient samples if controls are out of range.
13. Any serious incident that has occurred in relation to the device should be reported to the manufacturer and the regulatory authority (e.g. EU competent authorities) in the country where the user and/or patient is established.
14. Handle the cups with care and do not use any cups that have been dropped, or that have fallen down, which could cause erroneous results.

STORAGE AND STABILITY

All unopened materials will remain stable until the expiry date on the label when stored at the specified temperature.

Materials	**Cat. No.**
2-8 °C:	
CL AIA-PACK SHBG TEST CUP	0029139
CL AIA-PACK SHBG ADJUSTER SET	0029239
CL AIA-PACK SAMPLE DILUTING REAGENT A	0029401
AIA-PACK SHBG CONTROL SET	0025438
CL AIA-PACK SUBSTRATE SET (for 50 mL)	0029701
CL AIA-PACK SUBSTRATE SET (for 100 mL)	0029702
CL AIA-PACK SUBSTRATE SET (CUPS)	0029709
CL AIA-PACK WASH CONCENTRATE	0029703
CL AIA-PACK DILUENT CONCENTRATE (100 mL: for Automatic Dilution)	0029704
CL AIA-PACK DILUENT CONCENTRATE (60 mL: for Manual Dilution)	0029708
1-30 °C:	
CL AIA-PACK DETECTOR STANDARDIZATION CUP	0029705

- Once the aluminium pouch is opened, the CL AIA-PACK SHBG TEST CUP can be left on-board in the test cup sorter of the Tosoh AIA-CL Analyzers (kept at 2°-15 °C) or stored refrigerated for up to 30 days. If leaving the CL AIA-PACK SHBG TEST CUP at 18°-25 °C, the test cups should be used within 10 days (10 x 24 hours). When stored refrigerated over night at 2°-8 °C, the test cups can be used for up to 30 days (30 cycles of 8 hours at 18°-25 °C and 16 hours in the refrigerator).
- Once the aluminium pouch is opened, the CL AIA-PACK SHBG ADJUSTER SET can be left on-board in the test cup sorter of the Tosoh AIA-CL Analyzers (kept at 2°-15 °C) for up to 180 days. After opening the aluminium pouch, if the tray or remaining strips of the CL AIA-PACK SHBG ADJUSTER SET are stored refrigerated at 2°-8 °C, the product will remain stable until the expiry date. If leaving the CL AIA-PACK SHBG ADJUSTER SET at 18°-25 °C, the cups should be used within 24 hours.
- Once the aluminium pouch is opened, the CL AIA-PACK SAMPLE DILUTING REAGENT A can be left on-board in the test cup sorter of the Tosoh AIA-CL Analyzers (kept at 2°-15 °C), or stored refrigerated for up to 180 days. If leaving the CL AIA-PACK SAMPLE DILUTING REAGENT A at 18°-25 °C, the cups should be used within 10 days (10 x 24 hours). When stored refrigerated overnight at 2°-8 °C, the cups can be used for up to 30 days (30 cycles of 8 hours at 18°-25 °C and 16 hours in the refrigerator).
- The AIA-PACK SHBG CONTROL SET must be kept tightly sealed and refrigerated at 2°-8 °C. After opening or reconstituting, the controls should be used within 7 days.
- After opening the bottles, the CL AIA-PACK SUBSTRATE SET (for 50 mL) can be left on-board of the Tosoh AIA-CL Analyzers (kept at 2°-15 °C) for up to 60 days.
- After opening the aluminium pouch, unused cups of the CL AIA-PACK SUBSTRATE SET (CUPS) will remain stable until the expiry date provided that they are stored refrigerated at 2°-8 °C. If leaving the CL AIA-PACK SUBSTRATE SET (CUPS) at 18°-25 °C, the cups should be used within 3 days.
- After opening, the CL AIA-PACK WASH CONCENTRATE and the CL AIA-PACK DILUENT CONCENTRATE (100 mL per bottle / Cat. No. 0029704) can be left on-board of the Tosoh AIA-CL Analyzers (at 18°-25 °C) for up to 30 days.
- The manually-diluted Wash Solution and Diluent will remain stable for 30 days at 18°-25 °C.
- Even when stored as stated above, the reagents cannot be used beyond their expiry date.

SPECIMEN COLLECTION AND HANDLING

1. Serum or heparinised plasma is required for the assay. EDTA or citrated plasma SHOULD NOT BE USED.
2. When serum is used, a venous blood sample is collected aseptically without additives. Store at 18-25 °C until a clot has formed (usually 15-45 minutes), then centrifuge to obtain the serum specimen for assaying.

3. When using heparinised plasma, a venous blood sample is collected aseptically with designated additive. The blood sample should be collected according to the instructions by the specimen collection tube manufacturer as well as this Instructions For Use in order to avoid false results. Centrifuge and separate plasma from the packed cells as soon as possible.
4. An inadequate clotting or centrifugation, presence of fibrin or particulate matter in the sample may cause an erroneous result.
5. Samples containing inhibitors of alkaline phosphatase may cause erroneous results.
6. Inspect all samples for air bubbles and foam. Remove any air bubbles before the assay.
7. Specimen types should not be used interchangeably during serial monitoring of an individual patient. Measured concentrations may vary slightly between sample types in certain patients.
8. All patient specimens require dilution. Samples are diluted automatically by 20-fold with the CL AIA-PACK SAMPLE DILUTING REAGENT A on the Tosoh AIA-CL Analyzers. For further information regarding instrument operation, consult the specific Tosoh AIA-CL Analyzer Operators' manual. The AIA-PACK SHBG CONTROL SET does not require dilution before assay.
9. Samples may be stored at 2-8 °C for up to 7 days before analysis. If the analysis cannot be done within 7 days, the sample should be frozen and stored at -20 °C or below for up to 60 days.
10. Repeated freeze-thaw cycles should be avoided. Turbid samples or samples containing particulate matter should be centrifuged before the testing. Before the assay, bring frozen samples slowly to 18-25 °C and mix gently.
11. The amount of the diluted sample required for the analysis is 10 µL.

PROCEDURE

Refer to the Tosoh AIA-CL Analyzer Operators' manual for detailed instructions.

I. Reagent Preparation

A. Test Cup

The CL AIA-PACK SHBG TEST CUP is provided ready for use.

B. Substrate

<Bottles>

1. The bottles of the CL AIA-PACK SUBSTRATE SET are provided ready for use.
2. Uncap the bottles of the CL AIA-PACK SUBSTRATE SET and place them in the appropriate positions on the instrument according to the Operator's Manual.
3. When replacing the CL AIA-PACK SUBSTRATE SET, replace both CL AIA-PACK SUBSTRATE and CL AIA-PACK ENHANCER at the same time.

<Cups>

1. The cups of the CL AIA-PACK SUBSTRATE SET are provided ready for use.
2. Take out the necessary quantity of the cups from the tray and place them on the instrument according to the Operator's Manual. The remaining cups on the tray should be stored at 2°-8°C.

C. Wash Solution

<Automatic Dilution>

Uncap the bottle of the CL AIA-PACK WASH CONCENTRATE and place it in the appropriate position on the instrument. The CL AIA-PACK WASH CONCENTRATE is diluted automatically by the instrument to prepare the Wash Solution.

<Manual Dilution>

Add the entire content in one bottle of the CL AIA-PACK WASH CONCENTRATE to approximately 2.0 L of clinical laboratory reagent water, adjust the final volume to 3.0 L and mix well.

D. Diluent

<Automatic Dilution>

1. Use the CL AIA-PACK DILUENT CONCENTRATE (100 mL per bottle / Cat. No. 0029704).

2. Uncap this bottle and place it in the appropriate position on the instrument. The CL AIA-PACK DILUENT CONCENTRATE is diluted automatically by the instrument to prepare the Diluent.

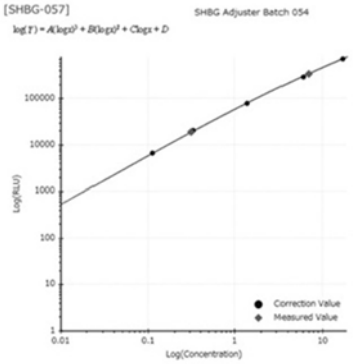
<Manual Dilution>

1. Use the CL AIA-PACK DILUENT CONCENTRATE (60 mL per bottle / Cat. No. 0029708).
2. Add the entire content in one bottle of the CL AIA-PACK DILUENT CONCENTRATE (60 mL) to approximately 2.0 L of clinical laboratory reagent water, adjust the final volume to 3.0 L and mix well.

II. Calibration Procedure

A. Calibration Curve

The calibration curve for use with the CL AIA-PACK SHBG assay has been standardized against the WHO 2nd International Standard for SHBG, NIBSC code 08/266.
Information on the Master curve is provided by the QR code® on the box label of the CL AIA-PACK SHBG TEST CUP for each lot. The Tosoh AIA-CL Analyzers establish the calibration curve by reading the QR code and measuring the CL AIA-PACK SHBG ADJUSTER SET.
Since the adjusted calibration curve for the CL AIA-PACK SHBG TEST CUP will remain stable for up to 90 days, it should be readjusted with the CL AIA-PACK SHBG ADJUSTER SET before expiry of the curve. Calibration stability is monitored by quality control performance and is dependent on proper reagent handling and the Tosoh AIA-CL Analyzer maintenance according to the manufacturer's instructions.
Readjustment of the Master curve may be necessary more frequently if controls are out of the established range for this assay or when certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, maintenance of the wash probe, or detector lamp adjustment or change).
Readjustment of the Master curve with the CL AIA-PACK SHBG ADJUSTER SET should be performed for each Assay Module on the AIA-CL2400.
For further information regarding instrument operation, consult the Tosoh AIA-CL Analyzer Operators' manual.
A sample calibration curve from the AIA-CL2400 follows and shows the algorithm used for calculating results.



B. Calibration Procedure

1. Refer to the appropriate Tosoh AIA-CL Analyzer Operators' manual for the procedural instructions.
2. Place a sufficient quantity of the CL AIA-PACK SHBG TEST CUP and the CL AIA-PACK SHBG ADJUSTER SET on the instrument.
3. When using a new lot of the Test Cups, scan the QR code printed on the box label to enter the Master curve and the lot number of the Test Cups into the Tosoh AIA-CL Analyzers.

4. When using a new lot of the Adjuster Set, scan the QR code printed on the box label to enter the concentration values and the lot number of the Adjuster Set into the Tosoh AIA-CL Analyzers.
5. Select START. The Adjuster Set is automatically reconstituted and measured as samples on the Tosoh AIA-CL Analyzers.

C. Calibration Acceptability Criteria

The replicate values should be within a 10 % range.

D. Calibration Review and Acceptance

1. Review the calibration curve carefully by using the criteria.
2. Confirm the calibration values, and then accept the calibration.
3. Tosoh recommends that the Adjuster Set be run in triplicate. Alternatively, users should validate the calibration procedure in accordance with their own standard operating procedures.

For further information regarding calibration by using a Master curve, consult the Tosoh AIA-CL Analyzer Operators' manual.

III. Quality Control Procedure

A. Commercially Available Controls

Commercially available controls shall be run at least once per day. It is recommended that at least two levels of controls, normal and abnormal, be used. Laboratory policy for this particular assay designates the following:

Control Material: _____
Frequency: _____

The lot number of control material, acceptable limits, and corrective action to be taken if controls do not meet the laboratory criteria will be found in a separate quality control document maintained by the laboratory.

B. Quality Control Procedure

1. Assay quality control specimens as instructed in the specific Operators' manual for your analyser. In addition, refer to the Tosoh AIA-CL Analyzer Operators' manual for detailed instructions on defining and editing the files.
2. Quality control material to be run with this assay is defined by individual laboratory policy.

IV. Specimen Processing

A. Preparation

All patient specimens require dilution. Samples are diluted automatically by 20-fold with the CL AIA-PACK SAMPLE DILUTING REAGENT A on the Tosoh AIA-CL Analyzers. Following the specific instructions in the Operators' manual for the analyser, place samples on the instrument appropriately. Barcoded primary tubes as well as sample cups can be run on the Tosoh AIA-CL Analyzers.

B. Assay Procedure

1. Provide a sufficient quantity of the CL AIA-PACK SHBG TEST CUP for the number of samples to be run.
2. Load patient samples as instructed in the Operators' manual and proceed with analysis.

PROCEDURAL NOTES

1. Ligand assays performed by the Tosoh AIA-CL Analyzers require the clinical laboratory reagent water defined by CLSI GP40-A4-AMD guideline. For further information, consult the CLSI document GP40-A4-AMD, Preparation and Testing of Reagent Water in the Clinical Laboratory; Approved Guideline-Fourth Edition.
2. If the SHBG concentration in a specimen is found to be greater than the upper limit of the measuring range, being 250 nmol/L, the specimen should be diluted with the CL AIA-PACK SAMPLE DILUTING REAGENT A and reassayed according to the Assay Procedure. The recommended dilution for specimens containing greater than 250 nmol/L is a 200-fold dilution. It is desirable to dilute the specimen so that the diluted specimen reads between 0.1 and 250 nmol/L. The dilution factor should be entered into the software so that the specimen is automatically reassayed with the designated dilution factor. The Tosoh AIA-CL Analyzers will report the result for the specimen before dilution. For further information on the dilution of specimens, refer to the Tosoh AIA-CL Analyzer Operators' manual.
3. If the assay specifications for this test are not ready in the system software, the specifications must be entered under test code **124**.

CALCULATION OF RESULTS

The Tosoh AIA-CL Analyzers perform all sample and reagent handling operations automatically. The Tosoh AIA-CL Analyzers measure the chemiluminescent intensity as counts per second (cps) produced by the reaction proportionally and automatically convert the cps to SHBG concentration by nmol/L. For samples requiring dilution, the Tosoh AIA-CL Analyzers will automatically perform dilutions and calculate results if the dilution factors are entered into the software. For detailed information regarding programmed dilutions, consult the appropriate Tosoh AIA-CL Analyzer Operators' manual.

EVALUATION OF RESULTS

Quality Control

In order to monitor and evaluate the precision of the analytical performance, it is recommended that commercially available control samples shall be assayed according to the local regulations.

The minimum recommendations for the frequency of running internal control material are:

After calibration, two levels of the internal control are run in order to accept the calibration curve.

After certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, maintenance of the wash probe, or detector lamp adjustment or change), the two levels of controls should be repeated.

After daily maintenance, two levels of the control shall be run in order to verify the overall performance of the Tosoh AIA-CL Analyzers.

If one or more control sample value(s) is out of the acceptable range, it is necessary to investigate the validity of the calibration curve before reporting patient results.

The end user should follow the recommendations of local, state, and federal regulatory agencies as well as laboratory policies.

LIMITATIONS OF THE PROCEDURE

1. For diagnostic purposes, the results obtained from this assay should be used in conjunction with other data (e.g. symptoms, results of other tests, clinical impressions, therapy, etc.).
2. When the CL AIA-PACK SHBG TEST CUP is used, the highest measurable concentration of SHBG by 20-fold dilution with the CL AIA-PACK SAMPLE DILUTING REAGENT A is 12.5 nmol/L, which corresponds to 250 nmol/L in specimens, and the lowest measurable concentration is 0.005 nmol/L, which corresponds to 0.1 nmol/L in specimens.
3. Although haemolysis has an insignificant effect on the assay, a haemolyzed sample might indicate mistreatment of the specimen before the assay and the result should be interpreted with caution.
4. Lipemia has an insignificant effect on the assay except in the case of gross lipemia where spatial interference may occur.
5. Specimens from patients taking medicines and/or undergoing medical treatment may show erroneous results.
6. Specimens containing fibrin may exhibit false results. Fibrin must be eliminated from the sample before assay begins.

7. Specimens from the patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show false results.
8. For a more complete understanding of the limitations of this procedure, please refer to the SPECIMEN COLLECTION AND HANDLING, WARNINGS AND PRECAUTIONS, STORAGE AND STABILITY, and PROCEDURAL NOTES sections in this Instructions For Use.

EXPECTED VALUES

Each laboratory should determine a reference interval which corresponds to the characteristics of the population being tested. As with all diagnostic procedures, clinical results must be interpreted with regard to concomitant medications administered to the patient.

I. Reference Ranges

The intervals for females during each phase were determined in serum samples from 128 apparently healthy American females. The interval for males given here was determined in serum samples from 130 apparently healthy American males.

Category(n)	Number of Samples (nmol/L)	Reference Interval
Premenopausal females	66	25.2 – 138.9
Postmenopausal females	62	13.5 – 139.8
Males	130	14.9 – 74.8

PERFORMANCE CHARACTERISTICS

ACCURACY

a. Recovery: Three serum samples and three heparinised plasma samples were spiked with three different levels of SHBG and assayed before and after spiking.

Sample	Initial	SHBG	Expected	Measured	Percent of
	Value (nmol/L)	Added (nmol/L)	Value (nmol/L)	Value (nmol/L)	Recovery (%)
Serum A1	17.0	38.2	55.2	52.4	93
	17.0	76.4	93.4	86.5	91
	17.0	153	170	159	93
Serum B1	53.7	38.2	91.9	91.6	99
	53.7	76.4	130	127	96
	53.7	153	207	192	91
Serum C1	54.6	38.2	92.8	93.1	101
	54.6	76.4	131	126	94
	54.6	153	207	196	92
Plasma A1	15.6	30.8	46.4	45.1	96
	15.6	61.6	77.2	74.2	95
	15.6	123	139	137	98
Plasma B1	64.1	30.8	94.9	94.8	100
	64.1	61.6	126	120	92
	64.1	123	187	190	102
Plasma C1	19.2	24.0	43.2	42.1	95
	19.2	48.0	67.2	65.7	97
	19.2	96.0	115	112	97

b. Dilution: Three serum samples and three heparinised plasma samples containing high concentrations of SHBG which had been diluted by 20-fold were serially diluted with the CL AIA-PACK SAMPLE DILUTING REAGENT A and assayed.

Sample	Dilution Factor	Expected Value (nmol/L)	Measured Value (nmol/L)	Percent of Recovery (%)
Serum A2	none		18.6	
	5-fold	3.73	3.69	99
	10-fold	1.86	1.92	103
Serum B2	none	57.9		
	5-fold	11.6	11.3	98
	10-fold	5.79	5.80	100
Serum C2	none		164	
	5-fold	32.8	31.7	96
	10-fold	16.4	15.7	96
Plasma A2	none		17.1	
	5-fold	3.43	3.48	101
	10-fold	1.71	1.76	103
Plasma B2	none		71.3	
	5-fold	14.3	13.4	94
	10-fold	7.13	6.93	97
Plasma C2	none		150	
	5-fold	29.9	28.2	94
	10-fold	15.0	14.1	94

c. Linearity: The linearity for CL AIA-PACK SHBG TEST CUP was determined based on the guidance from CLSI Protocol EP6-A. The linearity was measured on the AIA-CL2400 and has been demonstrated to be linear from 0.1 to 250 nmol/L.

PRECISION

a. Repeatability was determined using six controls in a total of 20 runs. Within each run, one set of duplicates per control was assayed. The mean of each duplicate was used to obtain the pooled standard deviation (SD), which was then used to calculate the coefficient of variation (CV).

Sample	Mean (nmol/L)	Pooled SD (nmol/L)	CV (%)
Serum A3	20.0	0.483	2.4
Serum B3	62.8	1.51	2.4
Serum C3	174	4.29	2.5
Plasma A3	18.4	0.356	1.9
Plasma B3	74.2	1.71	2.3
Plasma C3	151	3.95	2.6

b. Within-device precision was determined by the duplicate assay of six controls in 20 separate runs. The means of each run were used to calculate the pooled standard deviation (SD) and coefficient of variation (CV).

Sample	Mean (nmol/L)	Pooled SD (nmol/L)	CV (%)
Serum A3	20.0	0.637	3.2

Sample	Mean	Pooled SD	CV
	(nmol/L)	(nmol/L)	(%)
Serum B3	62.8	2.25	3.6
Serum C3	174	6.04	3.5
Plasma A3	18.4	0.562	3.1
Plasma B3	74.2	2.56	3.5
Plasma C3	151	5.25	3.5

CORRELATION

The correlation between serum (x) and heparinised plasma (y) on CL AIA-PACK SHBG assay was carried out using 151 patient specimens.

Slope	0.979
y-Intercept	1.01
Correlation Coefficient	0.997
Number of Samples	151

SPECIFICITY

The following substances were tested for cross-reactivity. The cross-reactivity (mol%) is the percentage of the compound which will be identified as SHBG. If these compounds are present in the specimen at the same concentration as SHBG, the final result will be increased by these percentages.

Compound	Added concentration	Cross-reactivity (mol%)
Alpha-Fetoprotein (AFP)	48.4 µg/dL	< 0.1
Cortisol	100 µg/mL	< 0.1
11-Deoxycortisol	4 µg/mL	< 0.1
5alpha-Dihydroxytestosterone	20 µg/mL	< 0.1
Estradiol	3600 pg/mL	< 0.1
Testosterone	20 µg/mL	< 0.1
Thyroglobulin	300 µg/mL	1.1
Thyroxin-Binding Globulin	200 µg/mL	< 0.1
Transferrin	4 mg/mL	< 0.1
Fibrinogen	4.5 g/L	< 0.1
Plasminogen	250 mg/L	< 0.1
Human IgA	367 mg/dL	< 0.1
Human IgG	335 mg/dL	< 0.1
CBG	35 mg/dL	< 0.1
TSH	180 mIU/L	< 0.1

SENSITIVITY

Limit of Detection: The limit of detection for CL AIA-PACK SHBG TEST CUP was determined in accordance with CLSI guideline EP17-A2. The blank sample was measured in 60 replicates. The five low level samples were measured in 12 replicates each. As a result, the limit of detection for CL AIA-PACK SHBG TEST CUP was estimated to be 0.00024 nmol/L, which corresponds to 0.0048 nmol/L in original samples (dilution factor 20).

INTERFERENCE

Interference is defined, for the purposes of this study, with recovery outside of 10 % of the known concentration of the specimen after the following substances are added to human specimens.

1. Haemoglobin (up to 440 mg/dL), free bilirubin (up to 17 mg/dL) and conjugated bilirubin (up to 18 mg/dL) do not interfere with the assay.

2. Lipemia, as indicated by triglyceride concentration (up to 1,600 mg/dL), does not interfere with the assay.
3. Ascorbic acid (up to 20 mg/dL) does not interfere with the assay.
4. Protein, as indicated by human albumin concentration (up to 5.0 g/dL), does not interfere with the assay.
5. Heparin (up to 100 U/mL) does not interfere with the assay.

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