510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k143075

B. Purpose for Submission:

New Device

C. Measurand:

Sex Hormone Binding Globulin (SHBG)

D. Type of Test:

Quantitative immunoassay

E. Applicant:

Tosoh Bioscience

F. Proprietary and Established Names:

ST AIA-PACK SHBG
ST AIA-PACK SHBG Calibrator Set

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
CDZ	Class I, reserved	21 CFR 862.1680	Clinical Chemistry (75)
JIT	Class II	21 CFR 862.1150	Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):
   See Indication(s) for use below

2. Indication(s) for use:

   ST AIA-PACK SHBG is designed for In Vitro Diagnostic Use Only for the quantitative measurement of sex hormone binding globulin (SHBG) in human serum or Na heparinized plasma on Tosoh AIA System Analyzers. The ST AIA-PACK SHBG assay is intended for use as an aid in the diagnosis of androgen disorders.

   The ST AIA-PACK SHBG Calibrator Set is intended for In Vitro Diagnostic Use Only for the calibration of the ST AIA-PACK SHBG assay

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Tosoh AIA-2000 System Analyzer

I. Device Description:

The ST AIA-PACK SHBG consists of 5 trays of 20 test cups which contain twelve lyophilized magnetic beads coated with anti-SHBG mouse monoclonal antibody and 100 µL of anti-SHBG mouse monoclonal antibody conjugated to bovine alkaline phosphatase with sodium azide as a preservative.

ST AIA-PACK Sample Diluting Solution (sold separately) consists of a protein matrix with no detectable concentration of SHBG with sodium azide as a preservative.

The ST AIA-PACK SHBG Calibrator Set contains a protein matrix with assigned levels of sex hormone binding globulin (SHBG). The calibrator set contains six levels of calibrators with the following SHBG concentrations: 0, 0.10, 0.30, 1.25, 6.25, and 15.0 nmol/L. The calibrators are supplied at a 1:20 dilution; therefore, the measured concentrations would be equivalent to 0, 2.0, 6.0, 25.0, 125.0, and 300 nmol/L after the analyzer performs an automatic multiplication by a factor of 20.

Each serum/plasma donor unit used in the preparation of these products has been tested by an U.S. FDA approved method and found to be non-reactive for the presence of the antibody to Human Immunodeficiency Virus 1 and 2, the Hepatitis B surface antigen, and the antibody to Hepatitis C.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Architect SHBG Reagent Kit
   Architect SHBG Calibrator Kit

2. Predicate 510(k) number(s):

   k060818

3. Comparison with predicate:

SHBG assay

Similarities		
Item	Candidate Device ST AIA-PACK SHBG	Predicate Device Architect SHBG Reagent Kit (k060818)
Intended Use	For the quantitative measurement of sex hormone binding globulin (SHBG) in human serum and plasma for use as an aid in the diagnosis of androgen disorders.	Same
Antibody	Anti-SHBG mouse monoclonal antibody	Same

SHBG assay

Differences		
Item	Candidate Device ST AIA-PACK SHBG	Predicate Device Architect SHBG Reagent Kit (k060818)
Reference range	Premenopausal women: 18-260 nmol/L Postmenopausal women: 15-185 nmol/L Males (21-49 years old): 10-68 nmol/L Males (≥50 years old): 16-125nmol/L	Females: 11.7-137.2 nmol/L Males: 11.2-78.1 nmol/L
Test Methodology	Fluorescence Immunoassay	Chemiluminscent Microparticle Immunoassay (CMIA)
Specimen type	Serum or sodium heparinized plasma	Serum or lithium heparin, sodium heparin, ammonium heparin, potassium EDTA plasma.
Measuring Range	0.2 to 250 nmol/L	0.1- 250 nmol/L

SHBG Calibrators

Similarities and Differences		
Item	Candidate Device ST AIA-PACK SHBG Calibrator Set	Predicate Device Architect SHBG Calibrator Kit (k060818)
Intended Use	Intended for the calibration of the SHBG assay.	Same
Number of Levels	6	Same
Matrix	Bovine serum albumin	Human serum in buffer
Traceability	Traceable to the WHO 2nd International Standard for SHBG from the National Institute for Biological Standards and Control (NIBSC) code 08/266.	Traceable to the WHO standard Material NIBSC code: 95/560

K. Standard/Guidance Document Referenced (if applicable):

CLSI EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition

CLSI EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures: a Statistical Approach; Approved Guideline

CLSI EP28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline

CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline-Second Edition

CLSI EP9-A2, Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline-Second Edition

L. Test Principle:

The ST AIA-PACK SHBG is a two-site immunoenzymometric assay which is performed entirely in the ST AIA-PACK SHBG test cups. SHBG present in the test sample is bound with monoclonal antibody immobilized on a magnetic solid phase and enzyme-labeled monoclonal antibody in the test cups. The magnetic beads are washed to remove unbound

enzyme-labeled antibody and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled monoclonal antibody that binds to the beads is directly proportional to the SHBG concentration in the test sample. A standard curve is constructed, and unknown sample concentrations are calculated using this curve. The concentration of range of the calibration curve is displayed in $1/20^{th}$ of the assay range in patient specimens. The concentrations of patient specimens and control material are calculated by multiplying the concentrations obtain on the calibration curve with the dilution factor. The AIA-2000 analyzer will automatically calculate the concentrations of patient samples and controls using the dilution factor and report the results.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Precision was assessed by assaying three levels of serum and Na-heparin plasma samples. The precision for the ST AIA-PACK SHBG assay was evaluated using three AIA-2000 analyzers and three different lots of reagent for both serum and Na-heparin plasma samples. Total and within run precision were obtained from measurements of 2 replicates in a single run, 2 times a day for 20 non-consecutive days (n=80).

   The precision results for each of the three lots assayed were similar. The study results of one representative lot are provided in the table below:

   Precision data from one representative lot:

			Within-Run		Between-Run		Total	
Sample	n	Mean SHBG (nmol/L)	SD	%CV	SD	%CV	SD	%CV
Serum 1	80	18.0	0.39	2.1	0.37	2.0	0.53	3.0
Serum 2	80	54.8	1.29	2.3	1.32	2.4	1.8	3.3
Serum 3	80	158.9	4.07	2.6	3.39	2.1	4.93	3.1
Plasma 1	80	16.1	0.40	2.4	0.4	2.5	0.6	3.4
Plasma 2	80	65.2	1.0	1.6	1.0	1.5	1.5	2.3
Plasma 3	80	142.2	3.7	2.6	2.9	2.0	2.0	3.1

   All the data were combined to assess the within-run, between-run, between-day, between-lot and total precision. The results are provided in the table below:

Combined precision data:

		Within-Run		Between-Run		Between-Lot		Total	
Sample n=240	Mean SHBG (nmol/L)	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Serum 1	18.1	0.47	2.6	0.38	2.1	0.77	4.3	0.84	4.6
Serum 2	55.3	1.39	2.5	1.21	2.2	1.54	2.8	2.88	5.2
Serum 3	163.1	4.79	2.9	4.10	2.5	2.77	1.7	9.42	5.8
Plasma 1	16.3	0.43	2.7	0.42	2.6	0.84	5.1	0.85	5.3
Plasma 2	66.3	1.47	2.2	1.36	1.0	1.59	2.4	3.75	5.7
Plasma 3	147.0	4.21	2.9	3.83	2.6	1.79	1.2	10.4	6.8

b. *Linearity/assay reportable range:*

A linearity study was performed in accordance to CLSI EP6-A for both serum and Na-heparinized plasma samples. For both serum and Na-heparin plasma, a total of eleven samples ranging from 0.07 to 257 nmol/L SHBG were prepared from performing intermediate dilutions of a low-level and a high-level SHBG sample. The linearity of the ST AIA-PACK SHBG assay was evaluated in a single study, performed on one day, using one AIA-2000 analyzer. Each of the eleven linearity samples was run in replicates of four. The mean, SD and CV% were calculated for each replicate. The linear regression analysis results are summarized below:

Serum: $y = 1.038x - 1.4013$, $R^2 = 0.9924$

Na-Heparin Plasma: $y = 1.001x - 0.6649$, $R^2 = 0.9997$

The linearity results for both serum and Na-heparin plasma support the claimed measuring range of this assay (0.2- 250 nmol/L).

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Traceability:

The ST AIA-PACK SHBG calibrators have been standardized against WHO (Sex Hormone Binding Globulin 2nd International Standard NIBSC Code 08/266 (version

2.0, dated 03/28/2013). The calibrators are a gravimetrically prepared dilution of human serum SHBG antigen with buffered bovine serum base.

Value assignment:

The values of the product calibrator were assigned using two AIA 2000 analyzers with three lots of reagents with 5 replicate runs per calibrator (n=30). The values are verified by an internal procedure and must meet specifications. The target values for the calibrators are as follows:

ST AIA-PACK SHBG Calibrator 1: 0.10 nmol/L
ST AIA-PACK SHBG Calibrator 2: 0.30 nmol/L
ST AIA-PACK SHBG Calibrator 3: 1.25 nmol/L
ST AIA-PACK SHBG Calibrator 4: 6.25 nmol/L
ST AIA-PACK SHBG Calibrator 5: 15.0 nmol/L

Stability:

Real time stability studies were performed to determine the shelf-life stability and the open-vial stability of the ST AIA-PACK SHBG Calibrators. The stability study protocols and acceptance criteria were reviewed and were found to be adequate to support the sponsor's following stability claims:

The ST AIA-PACK SHBG Calibrator Set is stable for 12 months when stored at 2-8^{o} C (shelf life) and for 24 hours after reconstitution when stored at 2-8^{o} C (open-vial).

d. Detection limit:

Limit of Blank (LoB):
Four lots of calibrator 1(blank samples) were measured in 60 replicates. The following equation was used to determine the LoB:

LoB= Result at Position [NB(p/100)=0.5] where p represents the 95th percentile and NB represent the number of replicates.

The LoB was determined to be 0.017 nmol/L

Limit of Detection (LoD):
To determine the LoD, the standard deviation of the sample measurements was obtained from 12 measurements of 10 low-level human serum samples by running four replicates over a period of three days. The following equation was used to calculate the LoD: LoD = LoB + CB x SDs where CB represents the value derived from 95th percentile of the standard distribution.

The LoD was determined to be 0.063 nmol/L

Limit of Quantitation (LoQ):
To determine the LoQ, sample measurements was obtained from 12 measurements of 5 low level human serum samples by running four replicates over a period of three days using 2 lots of reagent. The LoQ of 0.2 nmol/L was based on a percent total error of 12%. The LoQ was determined to be 0.20 nmol/L

Detection Limits:

LoB	LoD	LoQ
0.017 nmol/L	0.063 nmol/L	0.20 nmol/L

The claimed measuring range of the assay is 0.2 to 250 nmol/L

e. *Analytical specificity:*

Cross reactivity to structurally similar endogenous steroids with the ST AIA-PACK SHBG assay was determined by spiking human serum samples with each compound and comparing the SHBG results to a control sample spiked with an equivalent volume of sample diluting solution. The cross reactivity study was performed at one site using one AIA-2000 analyzer and one lot of reagent. The sponsor's defined significant cross reactivity as ≥ ±10% of the control value. The equation that was used to calculate % cross reactivity is as follows:

$$\text{Cross-reactivity (\%)} = \frac{(\text{Conc. of spiked sample} - \text{Conc. of non-spiked sample})}{\text{Conc. of cross-reactant}} \times 100$$

The cross reactivity results are summarized in the table below:

Compound	Cross-Reactant Concentration	Cross- reactivity (%)
AFP	484 µg/L	N.D.*
Cortisol	100,000 ng/mL	0.003
11-Deoxycortisol	4,000 ng/mL	0.114
Estradiol	3,600 pg/mL	N.D.
Testosterone	20,000 ng/mL	0.019
5(alpha)-dihydrotestosterone	20,000 ng/mL	0.007
Thyroglobulin	300 µg/mL	2.544
Thyroxine binding globulin	200 µg/mL	N.D.
Transferrin	4.0 mg/mL	N.D.
TSH	180 mIU/L	N.D.
Human IgA	367 mg/dL	0.072

Human IgG	335 mg/dL	0.218
Plasminogen	250 mg/L	N.D.
Fibrinogen	4.5 g/L	0.120
Corticosteroid binding globulin	35 mg/dL	0.012

*N.D.= not detectable

An interference study was performed to determine the level of endogenous interference in a specimen using the ST AIA-PACK SHBG assay on the AIA-2000 analyzer. Three levels of serum and Na-heparin plasma specimens were spiked with increasing concentrations of each interfering substance and assayed in triplicate at one site using one lot of reagent. The sponsor defines significant interference as ≥ ±10% of the expected value. The interference study results are summarized in the table below:

Substance	Highest concentration tested which showed no significant interference.
Hemoglobin	446 mg/dL
Free Bilirubin	17.6 mg/dL
Conjugated Bilirubin	18.5 mg/dL
Triglycerides	1667 mg/dL
Ascorbic Acid	20 mg/dL
Rheumatoid factor	550 IU/L
Na-Heparin	100.0 U/mL
Albumin	5.0 g/dL*

*the sponsor states in the labeling that: "Protein, as indicated by human albumin concentration (up to 5.00 g/dL added to samples from apparently healthy subjects), does not interfere with the assay.

HAMA Interference Study:

A study was performed to determine the interfering effects from HAMA. A serum sample with a SHBG concentration of 38.1 nmol/L was spiked with 24,269 ng/mL of HAMA. The sponsor defines significant interference as ≥ ±10% of the expected value. The study demonstrated that HAMA did not interfere with the SHBG assay up to 24,269 ng/mL of HAMA concentration.

The sponsor states the following in the labeling regarding potentially interfering substances:

Grossly hemolyzed and lipemic samples should not be tested.

Specimens 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 falsely elevated or decreased SHBG values.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

The sponsor performed a method comparison study between the ST AIA-PACK SHBG assay and the predicate device, the Abbott Architect SHBG assay using a total of 126 serum samples (6 were diluted to cover the low end of the measuring range) which were assayed in singleton at one site, using one AIA-2000 analyzer and one reagent lot. The result range for the samples tested was 0.6 to 241 nmol/L by the candidate device. The Deming regression method comparison results are summarized below:

ST AIA-PACK SHBG and Abbott Architect SHBG assay comparison

n	Slope	95% CI	Intercept	95% CI	R
126	0.949	0.926 to 0.972	-0.64	-2.61 to 1.34	0.991

b. Matrix comparison:

A matrix comparison study was performed by the sponsor to validate that equivalent SHBG results are obtained when serum and Na heparin plasma samples are assayed using the ST AIA-PACK SHBG assay on the AIA-2000 analyzer. A total of 116 serum and Na heparin plasma samples were assayed at two sites using two analyzers and one lot of reagent. The serum results ranged from 0.2 to 219 nmol/L. Five samples each of serum and Na-heparin plasma were diluted in order to cover the entire claimed measuring range. The results of the matrix comparison study are summarized in the table below:

Serum vs Na-heparin plasma

n	Slope	95% CI	Intercept	95% CI	R
116	0.977	0.964 to 0.991	0.269	-0.629 to 1.168	0.997

The study data support the package insert claim that human serum and Na-Heparin plasma are acceptable sample types for use with ST AIA-PACK SHBG assay. The sponsor indicates in the package insert labeling that EDTA plasma or citrated plasma should not be used.

3. Clinical studies:

   a. Clinical Sensitivity:

   Not applicable

   b. Clinical specificity:

   Not applicable

   c. Other clinical supportive data (when a. and b. are not applicable):

   Not applicable

4. Clinical cut-off:

   Not applicable

5. Expected values/Reference range:

   The reference range study was conducted with reference to the CLSI C28-A3 guideline. A total of 122 pre-menopausal (21 years and older) and 122 post-menopausal female serum specimens were assayed in singleton utilizing the Tosoh ST AIA-PACK SHBG assay. In addition, all samples were assayed in singleton utilizing the Tosoh ST AIA-PACK Testosterone. A total of 121 males between the ages of 21 and 49 years of age and 123 males over the age of 50 years of age were assayed in singleton utilizing the Tosoh ST AIA-PACK SHBG assay. In addition, all samples were assayed in singleton utilizing the Tosoh ST AIAPACK Testosterone. Testing was done at one site utilizing AIA-2000 analyzer and three lot numbers of reagents.

   The results from both assays were used to calculate the Free Androgen Index (%FAI).

   Reference Range for SHBG

Subject Group	N	SHBG range (nmol/L)
Premenopausal women	122	18-260
Postmenopausal women	122	15-185
Men 21-49 years old	121	10-68
Men ≥50 years old	123	16-125

Reference Range for Free Androgen Index

Subject Group	N	Free Androgen Index 2.5 to 97.5th percentile
Premenopausal women	122	0.2-9.7%
Postmenopausal women	122	0.1-7.1%
Men 21-49 years old	121	14.7-130.4%
Men ≥50 years old	123	1.0-72.4%

The formula for calculating the Free Androgen Index is:

$$\text{FAI (\%)} = \frac{\text{testosterone value (nmol/L)}}{\text{SHBG value (nmol/L)}} \times 100$$

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.