June 17, 2019

Immunodiagnostic Systems Ltd.
Mick Henderson
Regulatory Affairs Manager
10 Didcot Way
Boldon Business Park
Boldon
NE35 9PD
GB

Re: K190121
Trade/Device Name: IDS SHBG
Regulation Number: 21 CFR 862.1680
Regulation Name: Testosterone test system
Regulatory Class: Class I, reserved
Product Code: CDZ
Dated: May 15, 2019
Received: May 17, 2019

Dear Mick Henderson:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Kellie B. Kelm, Ph.D.
Acting Director
Division of Chemistry
and Toxicology Devices
OHT7: Office of In Vitro Diagnostics
and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
k190121

Device Name
IDS SHBG

Indications for Use *(Describe)*
The IDS SHBG assay is an in vitro diagnostic device intended for the quantitative determination of SHBG in human serum or plasma on the IDS System. Results are to be used as an aid in the diagnosis of androgen disorders

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) SUMMARY

510k Number k190121

Introduction According to the requirements of 21CFR807.92, the following information provides sufficient detail to understand the basis for a determination of substantial equivalence.

Submitter

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Date prepared: 11 June 2019

Device Name

Proprietary name: IDS SHBG

Common name: As above

Classification: 21CFR862.1680 Testosterone test system

Product Code: CDZ

Predicate Device The IDS SHBG is substantially equivalent to other products in commercial distribution intended for similar uses. We claim equivalency to the currently marketed Siemens ADVIA Centaur SHBG (k151986).

Device Description

Description for IDS-iSYS SHBG assay

The IDS SHBG assay is an *in vitro* diagnostic device intended for the quantitative determination of sex hormone binding globulin (SHBG) in human serum and plasma on the IDS-iSYS Multi-Discipline Automated System. Results are to be used in conjunction with other clinical and laboratory data to assist the clinician in the diagnosis of androgen disorders.

The assay is based on chemiluminescence technology. 5 µL of patient sample or calibrators are incubated with biotinylated monoclonal anti-SHBG antibody, an acridinium labelled monoclonal anti-SHBG conjugate and streptavidin labelled magnetic particles. The magnetic particles are captured using a magnet and a wash step performed to remove any unbound analyte. Trigger reagents are added; the resulting light emitted by the acridinium label is directly proportional to the concentration of analyte in the original sample.

The IDS SHBG assay is an *in vitro* diagnostic device consisting of ready to use reagents provided in individual compartments within the reagent cartridge.

The reagent cartridge contains:

- Magnetic particles – magnetic particles coated with streptavidin in a phosphate buffer containing preservatives
- Biotin antibody – monoclonal anti-SHBG labelled with biotin in a buffer containing proteins and preservatives
- Conjugate – monoclonal anti-SHBG labelled with an acridinium ester derivative in a buffer containing proteins and preservatives

The calibrators consist of:

- Calibrators A and B are included in the assay kit. The calibrators consist of a human serum matrix with defined concentrations of SHBG and preservatives. Together with a lot specific master

calibration curve, the calibrators will be used to perform the adjustment of the master calibration curve.

Indications for Use

IDS SHBG

The IDS SHBG assay is an *in vitro* diagnostic device intended for the quantitative determination of SHBG in human serum or plasma on the IDS System. Results are to be used as an aid in the diagnosis of androgen disorders

Rx Only

Conditions for use: For in vitro diagnostic use only.
Rx Only

Special instrument Requirements:

IDS-iSYS Multi-Discipline Automated System (k091849)

Comparison Tables

Similarities compared to the chosen (FDA cleared; marketed) predicate device (k151986)

Performance	**Predicate Device ADVIA Centaur SHBG (k151986)**	**Candidate Device IDS SHBG**
Intended use	for use as an aid in the diagnosis of androgen disorders	Same
Analyte	Sex hormone binding globulin (SHBG)	Same
Reagent storage	2-8 °C	Same
Range of assay	1.6-180 nmol/L	1.6 to 180 nmol/L
Sample preparation (pre-treatment)	Performed on-board the analyzer	Same
Method of detection (Test methodology)	Chemiluminescent Magnetic Latex Particle Immunoassay	Same
Automation	Fully automated assay	Same
Quality Control	Requires three controls to validate the calibration	Same
Calibration procedure	2 point calibration	Same
Traceability/ Standardization	Standardised to WHO 2nd International Standard (08/266)	Same
Specificity, Interfering substances & Cross Reactivity	<u>Interference</u> Haemoglobin No Interference up to 500 mg/dL <u>Cross Reactivity</u> Cortisol Not detectable 5α-dihydroxytestosterone Not detectable Testosterone Not detectable Fibrinogen Not detectable Plasminogen Not detectable Human IgA Not detectable	<u>Interference</u> Haemoglobin Same <u>Cross Reactivity</u> Cortisol Same 5α-dihydroxytestosterone Same Testosterone Same Fibrinogen Same Plasminogen Same Human IgA Same

	Human IgG Not detectable Corticosteroid binding globulin Not detectable Thyrotropin (TSH) Not detectable	Human IgG Same Corticosteroid binding globulin Same Thyrotropin (TSH) Same

Differences compared to the chosen predicate device (k151986)

Performance	**Predicate Device ADVIA Centaur SHBG (K151986)**	**Candidate Device IDS SHBG**
Indications for Use	The ADVIA Centaur SHBG assay is an *in vitro* diagnostic immunoassay for the quantitative determination of sex hormone-binding globulin (SHBG) in human serum and plasma using the ADVIA Centaur XP system.	The IDS SHBG assay is an *in vitro* diagnostic device intended for the quantitative determination of SHBG in human serum or plasma on the IDS System. Results are to be used as an aid in the diagnosis of androgen disorders.
Calibrator matrix	Equine serum	Human serum
Sample matrix (primary tube type)	Human Serum, plasma (lithium heparin)	Human serum, Plasma (K_2 EDTA)
Sample volume	10µL	5µL
Kit reagent components	Each SHBG Ready Pack contains: 1) Solid Phase (11.0 mL) streptavidin coupled magnetic latex particles (~150 µg/mL), 2) Lite Reagent (3.0 mL) containing mouse monoclonal anti-SHBG antibody (~130 µg/mL) labeled with acridinium ester, and 3) Ancillary Well Reagent (3.0 mL) containing a biotinylated monoclonal mouse anti-SHBG antibody (~6 µg/mL).	Reagent cartridge (1 vial each of MPT1, CONJ, Ab-BIOT & DIL), two concentration levels of calibrators (A&B) (1 vial of each) & a mini CD

	Each Ready Pack contains reagents for 50 tests.	
Kit reagent component volumes	Reagent cartridge (1 vial each): MPV1 (2.0mL), CONJ (10.1mL), NaOH (13mL) & BUF (26.0mL)	Reagent cartridge (1 vial each): MPT1 (2.5mL), CONJ (7.5mL), Ab- BIOT (7.55mL) & DIL (18.0mL) Calibrators(1.0mL)
Calibration interval	35 days	10 days
On board the analyzer reagent stability	60 days	14 days
Sensitivity	LoB: 1.2 nmol/L LoD: 1.6 nmol/L LoQ: 1.8 nmol/L	LoB: 0.01 nmol/L LoD: 0.15 nmol/L LoQ: 0.30 nmol/L
Expected values	Males 21 to 49 years 14.55 to 94.64 nmol/L Males > 50 years 21.63 to 113.13 nmol/L Premenopausal Females 10.84 to >180.00 nmol/L Postmenopausal Females 23.15 to 159.07 nmol/L	Males 21 to 49 years 11.47 to 58.07 nmol/L Males > 50 years 14.85 to 65.21 nmol/L Premenopausal Females 20.30 to 140.18 nmol/L Postmenopausal Females 11.30 to 127.31 nmol/L
Precision	Within Run Precision n = 40 2.5% to 3.8% in the concentration range 9.04 to 142.87 nmol/L Total Precision n = 40 3.1% to 6.5% in the concentration range 9.04 to 142.87 nmol/L	Within Run Precision n = 84 1.7% to 3.7% in the concentration range 5.57 to 201.67 nmol/L Total Precision n = 84 3.2% to 5.0% in the concentration range 5.57 to 201.67 nmol/L
Specificity, Interfering substances & Cross Reactivity	Interference Bilirubin, conjugated No Interference up to 20 mg/dL Bilirubin, unconjugated No Interference up to 20 mg/dL Biotin No Interference up to 100 ng/mL Cholesterol, total No Claim Human Anti Mouse Antibody (HAMA) No Claim	Interference Bilirubin, conjugated No Interference up to 40 mg/dL Bilirubin, unconjugated No Interference up to 40 mg/dL Biotin No Interference up to 1200 ng/mL Cholesterol, total No Interference up to 456 mg/dL Human Anti Mouse Antibody (HAMA) No Interference up to 3000 ng/mL

	Rheumatoid Factor No Claim	Rheumatoid Factor No Interference up to 7000 IU/mL
	Total Protein No Claim	Total Protein No Interference up to 12 g/dL
	Triglyceride No Interference up to 1000 mg/dL	Triglyceride No Interference up to 3000 mg/dL
	Acetaminophen No Claim	Acetaminophen No Interference up to 1324 µmol/L
	Ibuprofen No Claim	Ibuprofen No Interference up to 2425 µmol/L
	Ascorbic acid No Claim	Ascorbic acid No Interference up to 1700 µmol/L
	Acetylsalicylic acid No Claim	Acetylsalicylic acid No Interference up to 3.62 nmol/L
	Salicylic acid No Claim	Salicylic acid No Interference up to 4.34 mmol/L
	Creatinine No Claim	Creatinine No Interference up to 2.65 mmol/L
	Dopamine No Claim	Dopamine No Interference up to 850 µmol/L
	Tetracycline No Claim	Tetracycline No Interference up to 90 µmol/L
	Tolbutamine No Claim	Tolbutamine No Interference up to 3.7 mmol/L
	Tolazamide No Claim	Tolazamide No Interference up to 3.21 mmol/L
	Uric acid No Claim	Uric acid No Interference up to 1.4 mmol/L

Specificity, Interfering substances & Cross Reactivity	<u>Cross Reactivity</u> AFP Not detectable Thyroglobulin Not detectable Thyroxin binding globulin Not detectable Transferrin 0.04 % 11-deoxycortisol 0.24 % Estradiol Not detectable	<u>Cross Reactivity</u> AFP 0.5 % Thyroglobulin 90.2 % Thyroxin binding globulin -0.1 % Transferrin 0.0 % 11-deoxycortisol -0.1 % Estradiol -7.3 %
Method comparison	Against the Elecsys SHBG assay n = 194 Linear Regression ADVIA Centaur SHBG = 0.99(Elecsys) – 0.11 nmol/L Correlation coefficient (r) = 0.99	Against the ADVIA Centaur SHBG assay. N = 136 Passing Bablok regression IDS SHBG =0.9112 (ADVIA Centaur SHBG) 0.1556 nmol/L Correlation coefficient (r) = 0.989
Linearity	Weighted Linear regression of the observed concentrations versus the expected concentrations: Observed = 0.984 x (Expected) + 0.245ng/dL	Linear regression of the observed concentrations versus the expected concentrations: Serum Observed = 1.00 x (Expected) - 0.54 nmol/L Regression of coefficient (R2) = 0.999 K_2 EDTA Observed = 0.97 x (Expected) - 0.46 nmol/L Regression of coefficient (R2) = 0.998

Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision was determined in accordance with CLSI EP5-A3, "Evaluation of Precision Performance of Quantitative Measurement Methods". Assessment was made for the following variables: within run precision, total precision.

Fourteen serum-based samples were used, at different SHBG concentration levels ranging from 1.6 nmol/L to approximately 180.00 nmol/L. This was to ensure that the assay measuring range of the IDS SHBG was covered.

Final claims data reported is representative of one kit lot (MB2) where 21 days were available for analysis.

Results from one representative lot:

ID	Mean	Within Run SD	Within Run CV	Between Run SD	Between Run CV	Within Day SD	Within Day CV	Between Day SD	Between Day CV	Total SD	Total CV
CALB MB2	124.95	3.93	3.1%	1.64	1.3%	4.26	3.4%	2.02	1.6%	4.71	3.8%
CTL1 MB2	9.08	0.20	2.2%	0.12	1.3%	0.23	2.5%	0.33	3.6%	0.40	4.4%
CTL2 MB2	36.54	1.03	2.8%	0.00	0.0%	1.03	2.8%	1.32	3.6%	1.67	4.6%
CTL3 MB2	93.43	2.93	3.1%	3.61	3.9%	4.65	5.0%	0.00	0.0%	4.65	5.0%
CV 1 MB2	8.96	0.20	2.2%	0.12	1.4%	0.23	2.6%	0.29	3.3%	0.37	4.2%
CV 2 MB2	90.58	2.68	3.0%	2.86	3.2%	3.92	4.3%	0.37	0.4%	3.94	4.3%
CV 3 MB2	201.67	7.49	3.7%	4.90	2.4%	8.95	4.4%	2.56	1.3%	9.31	4.6%
IQC 1	5.57	0.09	1.7%	0.06	1.1%	0.11	2.0%	0.20	3.5%	0.23	4.1%
IQC 2	52.45	1.24	2.4%	0.92	1.8%	1.55	2.9%	0.87	1.7%	1.77	3.4%
IQC 3	96.82	2.76	2.8%	0.43	0.4%	2.79	2.9%	1.26	1.3%	3.06	3.2%
Sample 1	16.56	0.28	1.7%	0.14	0.9%	0.32	1.9%	0.51	3.1%	0.60	3.6%
Sample 2	85.46	2.15	2.5%	0.00	0.0%	2.15	2.5%	1.63	1.9%	2.70	3.2%
Sample 3	38.81	0.69	1.8%	0.40	1.0%	0.80	2.1%	0.93	2.4%	1.22	3.2%

b. Linearity/assay reportable range:

For the measuring range, a linearity study was conducted based on guidance from the CLSI EP6-A. A high human serum sample and a low human serum sample (low serum sample diluted in zero matrix) were analysed in addition to 14 evenly spaced dilutions which were created by mixing the high and low sample as indicated below:

Sample	Dilution	Dilution Factor (%)
1:	Low (L)	0
2:	0.995L + 0.005H	0.5
3:	0.99L + 0.01H	1
4:	0.9875L + 0.0125H	1.25
5:	0.975L + 0.025H	2.5
6:	0.95L + 0.05H	5
7:	0.90L + 0.10H	10
8:	0.80L + 0.20H	20
9:	0.70L + 0.30H	30
10:	0.60L + 0.40H	40
11:	0.50L + 0.50H	50
12:	0.40L + 0.60H	60
13:	0.30L + 0.70H	70
14:	0.20L + 0.80H	80
15:	0.10L + 0.90H	90
16:	High (H)	100

Serum:

Observed = 1.00 x (expected) -0.54 nmol/L
Regression coefficient R^2: 0.999

K_2 EDTA plasma:

Observed = 0.97 x (expected) -0.46 nmol/L
Regression coefficient R^2: 0.998

The measuring range is defined as the range of values the instrument can report directly without sample dilution. The reportable range is the range of values the instrument can report as a quantitative result with sample dilution.
The IDS SHBG assay is linear over the measuring range from 1.60 to 180.00 nmol/L. The IDS SHBG assay is also linear across the reportable range of the assay which is 0.30 to 720.00 nmol/L when any sample with SHBG concentrations above 180nmol/L is automatically diluted 1:4 by the iSYS System

To support the extended measuring range up to 720 nmol/L through the automated 1:4 dilution by the analyzer, the guideline CLSI EP34 1st ed. was followed.

Nine native samples with known SHBG concentration (obtained on the predicate device Siemens Advia Centaur SHBG assay) were tested on the iSYS using the automated post-dilution. Samples were tested in a single replicate on one kit lot.
When testing on the predicate device, samples above 180nmol/L were automatically 1:2 by the automate. If samples were still above the measurement range, the samples were manually diluted 1:5.
The accuracy of the IDS-iSYS system to read samples above 180 nmol/L using the automatic dilution is evaluated by calculating the recovery between IDS and Siemens Advia Centaur assays as below:

% Recovery = (SHBG estimate on iSYS / SHBG concentration on predicate) x 100

Table 1: Comparison of SHBG concentration of diluted samples

between IDS and Siemens Advia centaur (predicate) assays

sample ID	**IDS run 1**	**Predicate**	**% recovery**
sample 1	288.19	310.24	**93%**
sample 2	327.17	352.32	**93%**
sample 3	274.71	291.04	**94%**
sample 4	294.01	294.34	**100%**
sample 5	507.35	573.23	**89%**
sample 6	484.94	555.05	**87%**
sample 7	569.31	610.95	**93%**
sample 8	287.53	317.10	**91%**
sample 9	686.73	726.84	**94%**
		Mean recovery	**93%**

Conclusion

A recovery of between 87% to 100% was achieved.
IDS SHBG assay shows good accuracy when measuring samples in the range 180 – 720 nmol/L using the automatic dilution.

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

Traceability of calibrator to a reference material

The standardization of the IDS SHBG assay against the WHO 2nd international standard 08/266 was carried out in two phases, value assignment and verification, as described below:

Value assignment of kit calibrators
This phase includes the value assignment of the secondary standards (Internal reference calibrators, IRs). The candidate secondary standards (IRs) are value assigned directly to the WHO NIBSC 2nd international standard for SHBG (IS 08/266) through an internal QC procedure: at least 24 runs are performed using three iSYS instruments, 2 kit lots, 3 replicates for each run. Serial dilutions of the IS 08/266 in SHBG-depleted human serum are used as the reference to assign IR values.

The IDS SHBG kit calibrators A and B and master curve are value assigned off the secondary standards and the IS-08/266 through an internal QC procedure. At least 20 runs (14 runs using the previous IR lot – 6 runs using the international standard as the reference) are performed using one iSYS instrument, 2 replicates for each run.

Value assignment verification
The kit calibrator A and B values are then verified on three different iSYS instruments following an approved QC procedure by testing internal quality controls in five replicates in one run and on each instrument.
Internal quality controls are at 3 SHBG levels:

- IQC 1 between 5.00 and 8.00 nmol/L
- IQC 2 between 45.00 and 65.00 nmol/L
- IQC 3 between 80.00 and 120.00 nmol/L

Acceptance Criteria
The values of the calibrators must fall within specified acceptable ranges:

- Calibrator A between 0.10 to 0.30 nmol/L – precision CV ≤ 11%
- Calibrator B between 110.00 to 130.00 nmol/L – precision CV ≤ 8%

Internal quality controls must be within their respective ranges with a precision CV ≤ 11% for IQC1 and ≤ 8% for IQC2 and IQC3.

Traceability to the NIBSC 2nd IS 08/266
A correlation study is also performed between the results obtained when using the 2-point calibration and the results derived from the international standard IS 08/266 curve.

189 samples (139 serum – 50 K2 EDTA plasmas) were tested in two replicates using three lots (MB1, MB2 and MB3) on one iSYS instrument. Their concentrations were derived from the 2-point calibration and compared to the concentration calculated by using a serial dilution of the NIBSC 2nd international standard 08/266 (from 180 nmol/L to 0 nmol/L using SHBG depleted human serum). Results were analysed using Passing Bablok analysis on Analyse-it.

d. Detection limit:

The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were determined based on guidance from CLSI EP17-A2 "Protocols for the determination of limits of detection and limits of quantitation".
The statistical approach used when calculating Detection limits are:-

The equations used in Analyse-It are:

- $LoB - L_C = z_{(1-\alpha)}\sigma$ is the same as $M_B + c_pSD_B$
- $LoD - L_D = L_C + z_{(1-b)}\sigma$ is the same as $LoB + c_pSD_L$

EP17-A2 formula:

- Formula (2), page 25: $LoB = M_B + c_pSD_B$
- Formula (5), page 27: $LoD = LoB + c_\beta SD_L$

For the measurement of LoB, LoD and LoQ three manufacture batches (MB1, MB2B and MB3) were tested.

The LoB sample was run in 12 replicates for each of 5 runs over 3 days, by one operator and on one different instrument for each kit lot for a total of 60 replicates per lot.
Where two assays were performed in one day the set-up time between assays start time was at least 2 hours.

Each of the 7 LoD samples was measured in duplicate. For each kit lot, a total of 5 assays were run over 3 days by one operator and on a different instrument, for a total of 70 replicates. Where two assays were performed in one day the set- up time between assays start time was at least 2 hours.

For calculation of The LoQ panel of 9 samples were measured in singlicate two times per day. For each kit lot, a total of 10 assays were run over 5 days by one operator and on a different instrument, for a total of 90 replicates. Where two assays were performed in one day the set- up time between assays start time was at least 2 hours.

Sensitivity	**Concentration**
LoB	0.01 nmol/L
LoD	0.15 nmol/L
LoQ	0.30 nmol/L

e. Analytical specificity:

Interference and cross-reactivity studies were performed in accordance with the CLSI guidance EP7-A2"Interference testing in clinical chemistry".

To determine potential interference in the specific detection of SHBG, two serum samples at two different concentrations of SHBG were spiked with the potential interferent. Control samples (blank) for each of the two SHBG samples were spiked with a volume of relevant diluent equal to that of the spiked interferent. The mean concentration of the 26 replicate assays, determined for both the spiked and control samples, were then compared. The differences observed between the mean spiked and control sample values were examined and assessed according to acceptance criteria.

For total cholesterol the interference was tested by dilution of a high cholesterol native serum sample in zero matrix (SHBG depleted normal human serum) and calculation of the observed vs expected SHBG concentration.

% Interference was calculated using the formula below:

$$\% \text{ Interference} = \frac{(\text{mean spiked concentration} - \text{mean un-spiked concentration})}{\text{mean un-spiked concentration}} \times 100$$

% Observed/Expected (%O/E) was calculated using the formula below:

$$\% \text{ O/E} = \frac{(\text{observed mean concentration} - \text{expected concentration})}{\text{expected concentration}} \times 100$$

The following table provides the levels of interferents tested.

Potential interferents	**Highest concentration tested that demonstrated no significant interference**
Triglycerides	3000 mg/dL
Haemoglobin	500 mg/dL
Bilirubin, conjugated	40 mg/dL
Bilirubin, unconjugated	40 mg/dL
Total Protein	12 g/dL
Biotin	6000 ng/mL
Biotin	1500 ng/mL
Rheumatoid Factor	7000 IU/mL
HAMA	3000 ng/mL
Cholesterol	456 mg/dL
Acetaminophen	1324 µmol/L
Acetylsalicylic acid	3.62 nmol/L
Salicylic acid	4.34 mmol/L
Ibuprofen	2425 µmol/L

Ascorbic acid (Vit C)	1700 µmol/L
Creatinine	2.65 mmol/L
Dopamine	850 µmol/L
Tetracycline	90 µmol/L
Tolbutamide	3.7 mmol/L
Tolazamide	3.21 mmol/L
Uric Acid	1.4 mmol/L

Cross-reactivity testing was performed for alpha fetoprotein, cortisol, 11-deoxycortisol, estradiol, testosterone, 5-dihydrotestosterone, thyroglobulin, thyroxin binding globulin, transferrin, TSH, Human IgA, human IgG, plasminogen, fibrinogen and corticosteroid binding globulin.

Cross-reactants were prepared by manufacturing a 'top dose' of the relevant analyte which was then diluted down to a range of stock concentrations in undetectable SHBG matrix (serum based zero matrix). The stock cross reactant or zero matrix was spiked directly into a low sample and a high SHBG sample. The cross reactivity was then determined using the below equation

$$\% \text{ cross reactivity} = \frac{(\text{Mean conc. of spiked sample} - \text{mean conc. of un-spiked sample})}{\text{Spike concentration}} \times 100\%$$

The following table provides the levels of cross-reactants tested.

Cross Reactant	Spike Concentration	% Cross Reactivity in samples less than 30	% Cross Reactivity in samples between 55 and 130
AFP	5000 ng/mL	-0.3%	0.5%
Thyroglobulin	3000 ng/mL	5.1%	90.2%
Thyroxin binding globulin	200 µg/mL	0.0%	-0.1%
Transferrin	4 mg/mL	0.0%	0.0%
Cortisol	100000 ng/mL	0.0%	0.0%
11-deoxycortisol	4000 ng/mL	0.0%	-0.1%
5α-dihydroxytestosterone	20000 ng/mL	0.0%	0.0%
Estradiol	3600 pg/mL	-7.3%	-3.2%
Testosterone	20000 ng/mL	0.0%	0.0%
Fibrinogen	4.5 g/L	0.0%	0.0%
Corticosteroid binding globulin	35 mg/dL	0.0%	0.0%
Thyrotropin (TSH)	180 mIU/mL	0.0%	0.0%
Plasminogen	250 mg/L	0.0%	0.0%
Human IgA	367 mg/dL	0.0%	0.0%

Human IgG	335 mg/dL	0.0%	0.0%

f. Assay cut-off:

Not applicable

2. Comparison studies:

Method comparison:

The IDS SHBG assay was compared against a commercially available quantitative automated assay, following CLSI EP-9A3, "Method Comparison and Bias Estimation Using Patient Samples". A total of 136 samples, selected to represent a wide range of SHBG concentrations [sample concentration range: 2.54 – 172.12 nmol/L (0.24 – 16.35 µg/mL)], was assayed by each method. Passing-Bablok regression analysis was performed on the comparative data:

N	**Slope**	**95% CI**	**Intercept**	**95% CI**	**Correlation Coefficient (r)**
136	0.9112	0.88 to 0.94	0.1556 nmol/L (-0.0148 µg/mL)	-0.35 to 0.9 nmol/L (-0.03 to 0.85 µg/mL)	0.989

Matrix comparison:

The IDS SHBG matrix comparison study was performed to evaluate the difference across tube types (serum (without additives), serum gel separator tubes (TG), and K_2 EDTA plasma.) following the CLSI EP9-A3 guideline.

A total of 69 samples (68 native, 1 diluted) to cover the range of 0.51 to 238.45 mol/L. Passing-Bablok regression analysis was performed on the comparative data:

Sample matrix	Gel tube	K2 EDTA
n	69	69
Slope	1.019	0.9854
Intercept	0.4897	0.0308
r	0.999	0.998
Mean bias v Serum	0.7%	-1.3%

3. Expected values/Reference range:

The expected values were calculated using Analyse-it. Results were analysed to generate a nonparametric 95 % reference interval using the 2.5th and 97.5th percentiles as reference limits.

The package insert recommends considering "the above ranges as guidelines only; it is recommended that each laboratory establish its own expected range based upon its own patient population.

The expected values were assessed by using 671 serum samples from the United States in which the subjects were apparently healthy adults and aged between 21 and 77 years old.

The observed ranges were established, according to CLSI C28-A3c "Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory" is summarised in the table below:

	Males 21 – 49 years	Males > 50 years	Females premenopausal	Females postmenopausal
Number of subjects	165	180	206	120
Mean nmol/L	29.74	35.32	54.37	52.11
Median nmol/L	28.39	33.92	46.35	48.48
Observed 2.5th to 97.5th percentile nmol/L	11.47 – 58.07	14.85 – 65.21	20.30 – 140.18	11.30 – 127.31

	Males 21 – 49 years	Males > 50 years	Females premenopausal	Females postmenopausal
Number of subjects	165	180	206	120
Mean µg/mL	2.83	3.36	5.17	4.95
Median µg/mL	2.70	3.22	4.40	4.61
Observed 2.5th to 97.5th percentile µg/mL	1.09 – 5.52	1.41 – 6.19	1.93 – 13.32	1.07 – 12.09

The above ranges should be considered as guidelines only; it is recommended that each laboratory establish its own expected range based upon its own patient population.

Conclusion:

The IDS SHBG data presented and provided is complete and supports the basis for substantial equivalence to the predicate device.