510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K252728

B Applicant

Immunodiagnostic Systems Limited

C Proprietary and Established Names

IDS-iSYS Total Testosterone

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
CDZ	Class I, reserved	21 CFR 862.1680 - Testosterone Test System	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

New device

B Measurand:

Testosterone

C Type of Test:

Quantitative Immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The IDS-iSYS Total Testosterone assay is an in vitro diagnostic device intended for the quantitative determination of testosterone in human serum or plasma on the IDS system.

Measurement of testosterone are used in the diagnosis and treatment of disorders involving the male sex hormones (androgens), including primary and secondary hypogonadism, delayed or precocious puberty, impotence in males and, in females hirsutism (excessive hair) and virilization (masculinization) due to tumors, polycystic ovaries, and adrenogenital syndromes.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

IDS-iSYS Multi-Discipline Automated Analyzer (K091849)

IV Device/System Characteristics:

A Device Description:

The IDS-iSYS Total Testosterone reagent cartridge contains the following reagents:

MP3: Magnetic particles coated with Streptavidin in a PBS Pluronic buffer with sodium azide as preservative (<0.1 %), 1 bottle, 2.5 mL

CONJ: Testosterone linked to mouse protein labelled with an acridinium ester derivative, in a phosphate buffer with ProClin® 300 as preservative (<0.0015%), 1 bottle, 3.5 mL

Ab-BIOT: Anti-Testosterone sheep monoclonal antibody labelled with biotin, in a phosphate buffer containing bovine and sheep protein with ProClin® 300 as preservative (<0.0015%), 1 bottle, 11.5 mL

BUF: MES buffer containing BSA (0.1%), 4-Cloro-m-cresolo (0.1 mg/mL), Tween 20 (0.055%) and ProClin® 300 (0.0025%), 1 bottle, 11.5 mL

B Principle of Operation:

The IDS-iSYS Total Testosterone test system uses a competitive chemiluminescent immunoassay format. Patient samples or calibrators (30 µL) are first incubated with biotinylated anti-testosterone monoclonal antibody, an acridinium-labeled testosterone conjugate and streptavidin labeled magnetic particles. The magnetic particles are captured using a magnet and a wash step is performed to remove any unbound analyte. Trigger reagents are added and the chemiluminescent signal produced by the acridinium label is inversely proportional to the testosterone concentration in the sample.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Elecsys Testosterone II

B Predicate 510(k) Number(s):

K211685

C Comparison with Predicate(s):

Device & Predicate Device(s):	**K252728**	**K211685**
Device Trade Name	IDS-iSYS Total Testosterone	Elecsys Testosterone II
General Device Characteristic Similarities		
Intended Use/Indications For Use	Quantitative determination of Testosterone	Same
Test Principle	Competitive immunoassay	Same
General Device Characteristic Differences		
Detection Method	Chemiluminescence	Electrochemiluminescence
Measuring Range	14 – 1500 ng/dL	2.50 – 1500 ng/dL
Expected Range of Values (ng/dL)	Females, 21 - 49 years <14 – 51 Females, ≥ 50 years <14 – 48 Males, 21 - 49 years 213 – 818 Males, ≥ 50 years 180 - 711	Females, 20 – 49 8.4 – 48.1 Females, ≥ 50 years 2.9 – 40.8 Males, 20 – 49 249 – 836 Males, ≥ 50 years 193 - 740

VI Standards/Guidance Documents Referenced:

Clinical & Laboratory Standards Institute (CLSI) EP05-A3: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Third Edition

CLSI EP07-A3; Interference testing in Clinical Chemistry; Approved Guideline – 3rd Edition

CLSI-EP06; Evaluation of the Linearity of Quantitative Measurement Procedures, 2nd Edition

CLSI-EP09c; Measurement Procedure Comparison and Bias Estimation Using Patient Samples, 3rd Edition

CLSI-EP17-A2; Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline, 2nd Edition

CLSI-E28-A3c; Defining Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline, 3rd Edition

CLSI-EP37; Supplemental Tables for Interference Testing in Clinical Chemistry, 1st Edition

CLSI-EP25; Evaluation of Stability of In Vitro Medical Laboratory Test Reagents, 2nd Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Precision and repeatability were evaluated according to CLSI guideline EP05-A3.

Repeatability
A precision study was conducted to estimate repeatability and within-laboratory precision. Eight human serum samples with testosterone concentrations spanning the analytical measuring interval were assayed in duplicate in two runs per day over 20 days using one reagent lot on one IDS-iSYS Multi-Discipline Automated Analyzer. A total of 80 replicates per sample were measured. Repeatability and within laboratory precision were calculated using a two-way nested ANOVA according to CLSI EP05-A3. The results are provided in the table below:

Sample ID	N	Mean Conc. (ng/dL)	Repeatability		Within Laboratory	
			SD	CV	SD	CV
S1	80	42.5	2.2	5.2%	4.3	10.2%
S2	80	73.1	3.5	4.8%	5.5	7.5%
S3	80	125	3.9	3.1%	7.3	5.9%
S4	80	180	5.2	2.9%	9.6	5.3%
S5	80	402	7.6	1.9%	16.4	4.1%
S6	80	588	16.5	2.8%	28.2	4.8%

Sample ID	N	Mean Conc. (ng/dL)	Repeatability		Within Laboratory	
			SD	CV	SD	CV
S7	80	1146	24.2	2.1%	52.7	4.6%
S8	80	1228	27.1	2.2%	45.9	3.7%

Reproducibility – Site to Site

A reproducibility study was conducted in which eight (8) human serum samples with testosterone concentrations spanning the analytical measuring interval were tested using one reagent lot on three instruments (IDS-iSYS Multi-Discipline Automated Analyzer) at 3 sites by 3 operators (one operator per instrument/site). Each sample was tested in replicates of 5 per run, 1 run per day for 5 days for a total of 75 replicates per sample. The results are provided in the table below:

Sample ID	N	Mean Conc. (ng/dL)	Repeatability		Between Site		Reproducibility	
			SD	CV	SD	CV	SD	CV
S1	75	43.8	3.2	7.3%	0.0	0.0%	4.0	9.2%
S2	75	79.3	4.4	5.5%	4.6	5.8%	8.8	11.1%
S3	75	140.5	5.4	3.8%	6.7	4.7%	14.2	10.1%
S4	75	198.3	8.0	4.1%	9.5	4.8%	16.6	8.4%
S5	75	442.9	9.7	2.2%	22.7	5.1%	33.1	7.5%
S6	75	614.4	23.4	3.8%	36.3	5.9%	52.3	8.5%
S7	75	1167.6	53.6	4.6%	28.8	2.5%	77.5	6.6%
S8	75	1258.7	37.8	3.0%	52.4	4.2%	85.4	6.8%

Reproducibility – Lot to Lot

A reproducibility study was conducted in which eight (8) human serum samples with testosterone concentrations spanning the analytical measuring interval were tested by one operator using three reagent lots on one IDS-iSYS Multi-Discipline Automated Analyzer. Each sample was tested in replicates of 5 per run, 1 run per day for 5 days for a total of 75 replicates per sample. The results are provided in the table below:

Sample ID	N	Mean Conc. (ng/dL)	Repeatability		Between Lot		Reproducibility	
			SD	CV	SD	CV	SD	CV
S1	75	43.4	2.8	6.4%	2.3	5.4%	4.3	10.0%
S2	75	73.6	4.0	5.4%	3.9	5.3%	7.5	10.2%
S3	75	136.8	6.4	4.7%	6.3	4.6%	12.8	9.3%
S4	75	191.4	5.5	2.9%	7.5	3.9%	12.4	6.5%
S5	75	438.2	13.2	3.0%	23.4	5.3%	31.1	7.1%
S6	75	576.8	14.3	2.5%	12.7	2.2%	27.1	4.7%
S7	75	1137.8	40.0	3.5%	29.5	2.6%	57.1	5.0%
S8	75	1206.5	44.0	3.7%	31.4	2.6%	67.1	5.6%

2. Linearity:

A study was performed based on the CLSI guideline EP06-Ed2. Two dilution series spanning a concentration range from 8-1,500 ng/dL were prepared by mixing high and low serum samples pools. Samples were assayed in replicates of seven or nine. Linearity was evaluated using linear regression analysis. The deviation from linearity did not exceed -6.5% for samples with total testosterone concentrations within the claimed measuring range.

The combined linear regression for two dilution series was as follows:
Y= 0.97x-1.44

3. Analytical Specificity/Interference:

Interference and cross-reactivity studies were conducted following the CLSI EP7-Ed3 guideline.

Endogenous Interference

Serum samples with testosterone concentrations of 30, 75, 300 and 1200 ng/dL were spiked with potentially interfering substances. The samples were assayed, and the total testosterone concentrations of the spiked samples were compared to control samples without interferent. No significant interference (≤±10% bias) was observed when the interfering substances were tested at the following concentrations:

Interferent	Highest Concentration tested without interference
Hemoglobin	1000 mg/dL
Triglycerides	1500 mg/dL
Bilirubin, conjugated	40 mg/dL
Bilirubin, unconjugated	40 mg/dL

The sponsor includes the following limitation in the labeling:

- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays [4]. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed.
  4. *Boscato, LM. and Stuart, MC., 'Heterophilic antibodies: a problem for all immunoassays'. Clin Chem, 34, 1988, pp 27–33*

Biotin

To evaluate the candidate device's susceptibility to biotin interference, biotin was spiked into serum samples containing different concentrations of testosterone (approximately 40, 70, 300, and 1100 ng/dL). Samples were assayed in multiple replicates. The sponsor defined no significant interference as ≤10% bias. The results are summarized below.

% Bias for Samples Containing Various Concentration of Biotin							
Testosterone Concentration (ng/dL)	Biotin Concentration (ng/mL)						
	250	350	450	500	750	1000	3500
40	-4%	5%	86%	>100%	>100%	>100%	>100%
70	-1%	4%	52%	NT	NT	NT	>100%
300	2%	10%	50%	NT	NT	NT	NT
1100	-3%	-1%	14%	>100%	>100%	>100%	>100%

NT = Not Tested

The sponsor includes the following limitation in the labeling:

- Specimens that contain biotin at a concentration of 350 ng/mL demonstrate a less than or equal to ±10% change in results. Biotin concentrations greater than this may lead to falsely elevated results for patient samples. The recommended adult daily dietary intake for biotin is 30 µg/day. Over the counter dietary supplements promoted for use in hair, skin and nail health may contain 5-10 mg of biotin. Pharmacokinetic studies in healthy adults have shown that ingesting 5 mg of biotin can result in serum levels as high as 73 ng/mL. In rare cases, subjects are prescribed up to 300 mg of biotin per day for therapeutic applications, resulting in serum biotin levels as high as 1,160 ng/mL.

Exogenous Interferences

The effect on quantitation of analyte in the presence of drugs was determined by comparing values obtained from samples spiked with 17 common pharmaceutical compounds with the reference sample (unspiked). All samples used were native human serum pools. Samples (with testosterone concentrations near 60 ng/dL and 500 ng/dL) were divided into aliquots and spiked with the common drug interferents. The reference sample without drug was spiked with the respective amount of solvent. The definition of significant interference was set to ≤10% bias compared to the reference sample.

Drug	Highest Drug Concentration tested without Interference
Acetaminophen	15.6 mg/dL
Acetylcysteine	15.0 mg/dL
Acetylsalicylic Acid	3.0 mg/dL
Ampicillin	7.5 mg/dL
Ascorbic acid	5.25 mg/dL
Cefoxitin	75.0 mg/dL
Cyclosporine	0.18 mg/dL
Doxycycline	1.8 mg/dL
Heparin	330.0 IU/dL
Ibuprofen	21.9 mg/dL
Itraconazole	3.0 mg/dL
Levodopa	0.75 mg/dL
Methyldopa	2.25 mg/dL
Metronidazole	12.3 mg/dL
Phenylbutazone	10.7 mg/dL

Drug	Highest Drug Concentration tested without Interference
Rifampicin	4.8 mg/dL
Theophylline	6.0 mg/dL
Testosterone undecanoate	3.2 mg/dL
Nandrolone	11.4 mg/L

Significant interference was observed for Testosterone undecanoate and Nandrolone at the concentrations tested above. The sponsor includes the following limitation in the labeling:

- A significant interference with Nandrolone and Testosterone undecanoate was identified. The IDS Total Testosterone assay does not distinguish between endogenous testosterone and testosterone derived from supplementation therapy. Do not test samples from individuals undergoing treatment with Nandrolone or Testosterone undecanoate using the IDS Total Testosterone method."

Cross Reactivity

A cross-reactivity study was performed to evaluate the following substances. Aliquots from pools of human serum with testosterone concentrations of 60 ng/dL and 500 ng/dL were spiked with potentially cross-reactive substances and measured in the presence or absence of the potential cross-reactants and cross reactivity was calculated using the following equation:

% Cross Reactivity = 100 x (Average "spike" concentration – Average "blank concentration) / Spike concentration of cross reactant

Cross Reactant	Cross Reactant Concentration	% Cross Reactivity
*11-Ketotestosterone	200 ng/mL	4.9%
11-Ketotestosterone	100 ng/mL	6.1%
*11-β-Hydroxy testosterone	50 ng/mL	24.3%
11-β-Hydroxy testosterone	25 ng/mL	27.4%
19-Norethisterone	40 ng/mL	0.80%
5α-Androstane-3β,17β-diol	1000 ng/mL	0.10%
5-α-Androstene-3β,17β-diol	1000 ng/mL	0.10%
Androstenedione	100 ng/mL	2.30%
Cortisol	5000 ng/mL	n.d
Cortisone	5000 ng/mL	n.d
Danazol	1000 ng/mL	n.d
Dexamethasone	2000 ng/mL	n.d
DHEA	5000 ng/mL	n.d
DHEA-S	50000 ng/mL	n.d
Dihydrotestosterone	500 ng/mL	0.40%
Estradiol	5000 ng/mL	0.10%
Estrone	5000 ng/mL	n.d
Ethisterone	1000 ng/mL	0.20%
Norgestrel	1000 ng/mL	0.10%

Cross Reactant	Cross Reactant Concentration	% Cross Reactivity
Prednisolone	5000 ng/mL	n.d
Prednisone	5000 ng/mL	n.d
Progesterone	5000 ng/mL	n.d
Testosterone propionate	100 ng/mL	n.d

**Serum with testosterone level ~20 ng/dL*

Interference was observed for 11-Ketotestosterone and 11-β-Hydroxy testosterone at the concentrations tested above. The sponsor includes the following limitation in the labeling:

- A strong interaction with 11-Ketotestosterone and 11β-Hydroxy testosterone was found using the IDS Total Testosterone method. n.d. = not detectable; cross-reactivity is < 0. 1%.

4. Detection Limit and Assay Reportable Range:

Limit of Blank (LoB)

For determination of LoB four analyte free samples were measured in 5 replicates, once per day for 3 days, with 2 different reagent lots resulting in 15 replicates per sample for a total of 60 replicates per reagent lot on one IDS-iSYS analyzer. LoB was calculated according to the parametric function as described in CLSI EP17-A2.

The LoB claim in the labeling is 4 ng/dL.

Limit of Detection (LoD)

For determination of LoD, seven serum samples with low-analyte concentrations were measured in replicates of 5, with one reagent lot over 5 days, resulting in 25 replicates per sample for a total of 175 replicates per reagent lot on one IDS-iSYS Analyzer. LoD was calculated according to CLSI EP17-A2.

The LoD claim in the labeling is 8 ng/dL.

Limit of Quantitation (LoQ)

For the determination of LoQ, seven serum samples with low-analyte concentrations were measured in replicates of 5, with one reagent lot over 5 days, resulting in 25 replicates per sample for a total of 175 replicates per reagent lot on one IDS-iSYS Analyzer. The LoQ was defined as the concentration of analyte which has imprecision less than 20% CV.

The LoQ claim in the labeling is 14.0 ng/dL.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

The device is traceable to the certified reference testosterone material from the National Measurement Institute of Australia (NMIA), product code M914c, which is a certified reference material reviewed for compliance with ISO 15194.

Sample stability

The sponsor provided information to support the following sample stability labeling claims using serum and K_2EDTA plasma samples:

Storage Condition	Duration
Room temperature (20±3°C)	72 hours (3 days)
5±3°C (2 - 8°C)	22 days
-20°C or below	35 days (5 weeks)
Freeze/Thaw cycle	3
On-board the system*	6 hours

*Continuous on-board stability

6. Assay Cut-Off:

Not applicable

B. Comparison Studies:

1. Method Comparison with Predicate Device:

A method comparison study was performed comparing the IDS-iSYS Testosterone assay to the predicate device, using a protocol based on CLSI EP09c-A3. A total of 125 native human serum samples with testosterone concentrations ranging from 14 to 1490 ng/dL (as measured by the predicate device) were evaluated with the candidate and predicate devices in singlicate. The Passing-Bablok regression analysis results between the candidate device (dependent variable, y) and the comparator device (x, comparator), are shown below:

N	Concentration Range (ng/dL)*	Slope	Slope 95% CI	Intercept	Intercept 95% CI	Correlation Coefficient (r)
125	14-1449	0.96	0.93 – 0.98	1.06	-2.01 – 4.29	1.00

*As measured by the candidate device

2. Matrix Comparison:

A matrix comparison study was conducted to assess the equivalence between serum (serum without additives, serum gel separator tubes (SST)) and plasma (K_2 EDTA, K_3 EDTA, Sodium Heparin and Lithium Heparin) sample matrices when using the IDS Total Testosterone assay. A total of 50 matched serum, serum gel separator tubes (SST) and plasma (K_2EDTA, Lithium Heparin, and Sodium Heparin) and 39 matched serum and plasma (K_3EDTA) samples with concentrations ranging from 14 to 1500 ng/dL were used. The samples were tested in singlicate by one operator on one instrument using one reagent lot. Data was assessed by Passing-Bablok regression analysis. The results are summarized below.

Tube type	N	Slope	Intercept	Correlation coefficient r
SST	50	0.99	0.42	1.00
K_2 EDTA plasma	50	0.99	-1.25	1.00
K_3 EDTA plasma	39	0.99	-0.49	1.00
Li Heparin plasma	50	0.98	2.36	1.00
Na Heparin plasma	50	0.99	1.94	1.00

The results demonstrate equivalency between serum, serum gel separator tubes (SST) and plasma (K_2 EDTA, K_3 EDTA, Lithium Heparin, and Sodium Heparin) sample matrices.

B Clinical Studies:

1. Clinical Sensitivity:

   Not Applicable

2. Clinical Specificity:

   Not Applicable

3. Clinical Cut-Off:

   Not Applicable

4. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

   Not Applicable

C Expected Values/Reference Range:

A reference interval study was performed for the total Testosterone assay in accordance with the CLSI EP28-A3c guideline. A total of 776 adult serum samples were collected in the USA from apparently healthy individuals. The sample groups tested consisted of:

- 497 males between 21 and 78 years of age
- 279 females between 21 and 78 years of age

The samples were categorized into two age groups: 21 to 49, and ≥ 50 years. The data were analyzed applying a nonparametric method with 90% confidence interval using the 5th and 95th percentiles of measured data. The resulting reference interval is summarized in the following table:

Males	21 to 49 years	≥ 50 years
N of subjects	266	231
Median (ng/dL)	459	402
Observed Range (ng/dL) (5th to 95th percentile)	213-818	180-711

Females	21 to 49 years	≥ 50 years
N of subjects	119	108
Median (ng/dL)	23	22
Observed Range (ng/dL) (5th to 95th percentile)	<14-51	<14-48

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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