



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

I Background Information:

A 510(k) Number

K240865

B Applicant

Immunodiagnostic Systems Limited

C Proprietary and Established Names

IDS-iSYS Free 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 (free)

C Type of Test:

Quantitative, Enzyme Immunoassay (EIA)

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The IDS-iSYS Free Testosterone assay is an in vitro diagnostic device intended for the quantitative determination of free testosterone in human serum or plasma on the IDS system. Measurement of free testosterone is used in the diagnosis and treatment of disorders involving the male sex hormones (androgens), including primary and secondary hypogonadism, impotence in male and in females; hirsutism (excessive hair) and virilization (masculinization) due to tumors, polycystic ovaries and androgenital syndromes.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

IDS-iSYS Multi-Discipline Automated Analyzer

IV Device/System Characteristics:

A Device Description:

The IDS-iSYS Free Testosterone assay consists of a reagent cartridge. The reagent cartridge contains:

- Magnetic particles coated with Streptavidin in a buffer containing preservative, one bottle, 2.5 mL.
- Monoclonal anti-testosterone labelled with biotin, in a buffer containing preservative, one bottle, 7.5 mL
- Testosterone labelled with an acridinium ester derivative, in a buffer containing protein and preservative, one bottle, 3.5 mL

B Principle of Operation:

The IDS-iSYS Free Testosterone test system uses a competitive enzyme immunoassay technology. 20 µL of patient sample or calibrators are incubated with the biotinylated monoclonal anti- testosterone 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; the resulting light emitted by the acridinium label is directly proportional to the concentration of analyte in the original sample.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Free Testosterone AccuBind ELISA Test System

B Predicate 510(k) Number(s):
K181017

C Comparison with Predicate(s):

Device & Predicate Device(s):	<u>K240865</u>	<u>K181017</u>
Device Trade Name	IDS-iSYS Free Testosterone	Free Testosterone AccuBind ELISA Test System
General Device Characteristic Similarities		
Intended Use	Quantitative determination of Free Testosterone	Same
Test Principle	Competitive immunoassay	Same
General Device Characteristic Differences		
Sample Type	Human serum or plasma	Human serum
Detection Method	Chemiluminescence	Microplate colorimetric reader
Measuring Range	0.40 – 60 pg/mL	0.11 – 60 pg/mL

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 EP06-2nd Edition-: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline

CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition

CLSI EP07-A3 Interference Testing in Clinical Chemistry.

CLSI EP37 1st Edition: Supplemental Tables for Interference Testing in Clinical Chemistry

CLSI EP09c 3rd Edition: Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Third Edition

CLSI C28-A3: Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Precision studies were conducted in accordance with the CLSI Guideline EP05-A3.

A precision study was conducted to estimate repeatability and within-laboratory precision. Ten 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. The results are provided in the table below:

Sample	Concentration (pg/mL)	Repeatability		Within Laboratory	
		SD	CV	SD	CV
S1	0.67	0.05	7.0%	0.07	9.9%
S2	1.15	0.05	4.3%	0.06	5.2%
S3	1.19	0.04	3.0%	0.06	5.0%
S4	3.75	0.06	1.7%	0.12	3.3%
S5	9.17	0.20	2.2%	0.27	2.9%
S6	10.78	0.15	1.4%	0.39	3.7%
S7	19.71	0.28	1.4%	0.44	2.3%
S8	32.18	0.77	2.4%	1.03	3.2%
S9	43.37	0.76	1.8%	1.45	3.3%
S10	56.04	1.24	2.2%	1.90	3.4%

A reproducibility study was conducted in which nine (9) 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	Concentration (pg/mL)	Reproducibility	
		SD	CV
S1	1.2	0.09	7.8%
S2	1.3	0.07	5.8%
S3	3.7	0.15	4.1%
S4	9.3	0.44	4.7%
S5	10.9	0.46	4.2%
S6	20.1	0.89	4.4%
S7	33.1	2.85	8.6%
S8	45.4	2.36	5.2%
S9	58.9	2.80	4.7%

A reproducibility study was conducted in which nine (9) 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	Concentration (pg/mL)	Reproducibility	
		SD	CV
S1	1.2	0.13	11.1%
S2	1.3	0.08	6.5%
S3	3.8	0.17	4.5%
S4	9.2	0.35	3.8%
S5	10.8	0.44	4.1%
S6	19.8	0.83	4.2%
S7	32.6	1.82	5.6%
S8	44.4	1.94	4.4%
S9	57.9	2.24	3.9%

2. Linearity:

A study was performed based on the CLSI Guideline EP06-Ed2. Three dilution series spanning the analytical measuring interval were prepared by mixing high and low serum samples pools. All dilution levels were assayed in replicates of four. Linearity was evaluated using linear regression analysis. The deviation from linearity did not exceed -6.6% for samples with free testosterone concentrations from the LoQ to 68.21 pg/mL.

The combined linear regression for all 3 dilution series was as follows:
 $Y = 1.00x - 0.04$; $R = 1.00$

The results from the linearity study support an analytical measuring interval of 0.40 - 60 pg/mL.

3. Analytical Specificity/Interference:

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

Interference:

Aliquots from pools of human serum with free testosterone concentrations of 1.0 pg/mL or 45 pg/mL were spiked with potentially interfering substances. The samples were assayed, and the free testosterone concentrations of the spiked samples were compared to control samples without interferent. No significant interference ($\leq \pm 10\%$ bias) was observed when the interfering substances were tested at the following concentrations:

Substance	Highest concentration at which no significant interference was observed
Acetylsalicylic Acid	1.67 mmol/L
Acetaminophen	1030 μ mol/L
Bilirubin (Conjugated)	40 mg/dL
Bilirubin (Unconjugated)	40 mg/dL
Human Anti Mouse Antibody (HAMA)	1000 ng/dL
Hemoglobin	300 mg/dL
Ibuprofen	1060 μ mol/L
Total Protein	10 g/dL
Salicylic Acid	2.07 mmol/L
Triglycerides	1500 mg/dL
Rheumatoid Factor (RhF)	1500 IU/mL

To evaluate the candidate device's susceptibility to biotin interference, biotin was spiked into serum samples containing different concentrations of free testosterone (approximately 1.5 pg/mL, 20 pg/mL, or 45 pg/mL). Samples were assayed in multiple replicates. The sponsor defined no significant interference as $\leq 10\%$ bias. The results are summarized below.

% Bias for Samples Containing Various Concentration of Biotin					
Testosterone Concentration	Biotin Concentration (ng/mL)				
	250	500	750	870	1750
1.5 pg/mL	5.4%	-10%	11%	-6%	61%
20 pg/mL	6.0%	-9%	8%	NT	NT
45 pg/mL	9.0%	-10%	16%	33%	64%

NT = Not Tested

The sponsor has included the following limitations in the labeling:

- Specimens that contain biotin at a concentration of 500 ng/mL demonstrate a less than or equal to $\pm 10\%$ 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 μ g/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.

- The lowest Total Protein level that does not significantly interfere ($\leq \pm 10\%$ bias) with IDS Free Testosterone assay is 10 g/dL.
- The lowest Hemoglobin level that does not significantly interfere ($\leq \pm 10\%$ bias) with the assay is 300 mg/dL. Visual hemolysis in the sample is typically already seen in samples with hemoglobin concentration of 50 mg/dL or greater. Visibly hemolyzed samples must not be used with IDS Free Testosterone assay.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed.

Cross-Reactivity:

A cross-reactivity study was performed to evaluate the following substances. Aliquots from pools of human serum with a free testosterone concentration of 1.0 pg/mL or 15 pg/mL were spiked with potentially cross-reactive substances. The samples were assayed and the resulting percent cross-reactivity was calculated using the following formula:

$$\% \text{ Cross Reactivity} = 100 \times (\text{Average "spike" concentration} - \text{Average "blank" concentration}) / \text{Spike concentration}$$

Results are shown in the table below:

Cross-reactant	Test Concentration (ng/mL)	% Cross Reactivity
11-Deoxycortisol	100,000	< 0.01%
DHEA	1000; 50000	< 0.01%
Aldosterone	3000	< 0.01%
Cortisol	1000	< 0.01%
Androstenedione	100	0.018%
17 α -Ethinilestradiol	500	< 0.01%
Androsterone	500	< 0.01%
Dihydrotestosterone	500	< 0.01%
Epitestosterone	500	< 0.01%
Norgestrel	1000	< 0.01%
Cortisone	2000	< 0.01%
Danazol	1000	< 0.01%
Estriol	100	< 0.01%
Testosterone propionate	100	< 0.01%
Prednisone	1000	< 0.01%
Oxymetholone	10,000	< 0.01%
Estradiol	1000	< 0.01%
5 α -Androstane-3 β ,17 β -diol	1000	< 0.01%
Ethisterone	1000	< 0.01%
11- β -Hydroxytestosterone	1000	< 0.01%
Prednisolone	1000	< 0.01%
Estrone	1000	< 0.01%

Cross-reactant	Test Concentration (ng/mL)	% Cross Reactivity
Pregnenolone	5000	< 0.01%
Progesterone	1000	< 0.01%
11-Ketotestosterone	100	0.016%
Cyproterone	2000	< 0.01%
Dexamethasone	2000	< 0.01%
17aOH-Progesterone	500	< 0.01%
Methyltestosterone	100	0.039%
17α-Estradiol	1000	< 0.01%
17-Hydroxypregnenolone	1000	< 0.01%
Estriol 3-glucuronide	1000	< 0.01%
3-EstriolSulfate	1000	< 0.01%
D-5-Androstene-3β,17β-diol	1000	< 0.01%
Amitriptyl HCl	1000	< 0.01%
Clomiphene Citrate	1000	< 0.01%
Corticosterone	1000	< 0.01%
Cyproterone acetate	1000	< 0.01%
DHEA-S	100000	< 0.01%
Desogestrel	100	< 0.01%
Ethinodiol	1000	< 0.01%
Ethinodiol diacetate	50	< 0.01%
Flunisolide	1000	< 0.01%
Fluoxymesterone	1000	< 0.01%
Lynestrenol	1000	< 0.01%
Medoxyprogesterone acetate	1000	< 0.01%
Mestranol	1000	< 0.01%
Norethindrone	50	< 0.01%
Norethinodrone acetate	50	< 0.01%
Norgestimate	1000	< 0.01%
Norethynodrel	50	< 0.01%
Salbutamol	1000	< 0.01%
Spirolactone	1000	< 0.01%
Stanozolol	1000	< 0.01%
Testosterone Cypionate	12	< 0.01%
Testosterone enanthate	100	< 0.01%
Testosterone SO4	1000	< 0.01%
Testosterone Undecanoate	12	< 0.01%
Triamcinolone	50	< 0.01%

4. Assay Reportable Range:

The sponsor claims a range of 0.40-60 pg/mL.

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

The IDS-iSYS Free Testosterone assay is traceable to an internal reference material which is traceable to another commercially available assay.

6. Detection Limit

Determination of the Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) were conducted in accordance with the CLSI guideline EP17-A2.

Limit of Blank (LoB)

For determination of LoB, four analyte-free samples were measured in replicates of 5 using 3 reagent lots over 5 days for a total of 60 replicates per reagent lot on one IDS-iSYS Multi-Discipline Automated Analyzer. LoB was calculated according to the parametric function as described in CLSI EP17-A2.

Limit of Detection (LoD)

For determination of LoD, seven serum samples with low-analyte concentrations were measured in replicates of 5 across 3 reagent lots over 3 days for a total of 105 replicates per reagent lot on one IDS-iSYS Multi-Discipline Automated Analyzer. LoD was calculated according to CLSI EP17-A2.

Limit of Quantitation (LoQ)

For the determination of LoQ, seven serum samples were measured in replicates of 5 across 3 reagent lots over 3 days for a total of 105 replicates per reagent lot on one IDS-iSYS Multi-Discipline Automated Analyzer. The LoQ was defined as the concentration of analyte which has imprecision less than 20% CV.

The summary results for LoB, LoD and LoQ are shown below.

Limits of Detection	Free Testosterone Concentration
LoB	0.08 pg/mL
LoD	0.17 pg/mL
LoQ	0.40 pg/mL

7. Assay Cut-Off:

Not applicable

B Comparison Studies:

1. Method Comparison with Predicate Device:

A method comparison study was performed comparing the IDS-iSYS Free Testosterone assay to the predicate device, using a protocol based on CLSI EP09c-A3. A total of 241 (220 native and 21 contrived) human serum samples with free testosterone concentrations ranging from 0.276 to 50.35 pg/mL (as measured by the predicate device) were evaluated with the candidate and predicate devices. 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 (pg/mL)*	Slope	Slope 95% CI	Intercept	Intercept 95% CI	Correlation Coefficient (r)
241	00.41-55.98	1.02	0.97 – 1.06	-0.02	-0.26 – 0.07	0.98

*As measured by the candidate device

2. Matrix Comparison:

A matrix comparison study was conducted using 40 matched serum (without additives), serum gel separator tubes (SST) and plasma (K₂ EDTA, Lithium Heparin, and Sodium Heparin) samples with concentrations ranging from 0.40 to 58.45 pg/mL. The samples were tested in duplicate using one reagent lot, but only the first replicate was used for data analysis. The Passing-Bablok regression analysis was performed. The summary results are shown below.

Tube type	N	Slope	Intercept	Correlation coefficient r
SST	40	0.96	0.08	1.00
K ₂ EDTA	40	0.97	0.08	0.99
Li Heparin	40	0.97	0.03	0.99
Na Heparin	40	0.97	0.00	0.99

The results demonstrate equivalency between serum, serum gel separator tubes (SST) and plasma (K₂ EDTA, Lithium Heparin, and Sodium Heparin) sample matrices.

C Clinical Studies:

1. Clinical Sensitivity:

Not applicable

2. Clinical Specificity:

Not applicable

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

Not applicable.

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

A reference interval study was performed for the IDS Free Testosterone assay in accordance with CLSI EP28-A3c guideline. A total of 563 adult serum samples were collected from apparently healthy individuals. The sample groups tested consisted of:

- 309 adult males between 21 and 77 years of age
- 254 adult females between 21 and 77 years of age

The data were analyzed to generate a nonparametric 95% reference interval using the 2.5th and 97.5th percentiles as reference limits. The resulting reference interval is summarized in the following table:

Males	21 to 39 years	40 to 59 years	≥ 60 years
N of subjects	129	138	42
Median (pg/mL)	12.36	8.70	7.78
Observed Range (pg/mL) (2.5th to 97.5th percentile)	4.91 to 21.64	3.73 to 14.96	2.25 to 11.37

Females	21 to 39 years	40 to 59 years	≥ 60 years
N of subjects	130	57	67
Median (pg/mL)	1.13	0.71	0.89
Observed Range (pg/mL) (2.5th to 97.5th percentile)	0.46 to 2.20	0.40 to 1.74	0.42 to 2.20

Other Supportive Information

The sponsor evaluated whether the conjugate binds to SHBG (sex hormone binding globulin). Charcoal-stripped human serum was spiked with SHBG at concentrations ranging from 6.25 to 200 µg/mL and then assayed. All concentrations tested exhibited less than or equal to 7% binding.

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.