

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY  
DEVICE ONLY TEMPLATE**

**A. 510(k) Number:**

k041866

**B. Purpose for Submission:**

New Device

**C. Analyte:**

Testosterone

**D. Type of Test:**

Quantitative Immunoassay

**E. Applicant:**

Axis-Shield Diagnostics Limited

**F. Proprietary and Established Names**

Abbott AxSYM Testosterone Microparticle Enzyme Immunoassay (MEIA) test

**G. Regulatory Information:**

1. Regulation Section:  
21 CFR § 862.1150 (Calibrator)  
21 CFR § 862.1680 (Testosterone Test System)
2. Classification:  
Class II  
Class I (reserved)
3. Product Code:  
JIS  
CDZ
4. Panel:  
Clinical Chemistry

**H. Intended Use:**

1. Intended Use / Indications for Use:

AxSYM Testosterone is a Microparticle Enzyme Immunoassay (MEIA) for the quantitative determination of testosterone in human serum and plasma on the AxSYM System. Testosterone monitoring is used clinically to diagnose and differentiate endocrine disorders. In males, these include hypogonadism, testicular

failure, infertility, hypopituitarism, and hyperprolactinemia. In females, polycystic ovary syndrome, adrenal hyperplasia, infertility, hirsutism, amenorrhea, obesity, and virilization can cause changes in serum testosterone levels.

The AxSYM Testosterone assay is used as an aid in the investigation of infertility in males and of hirsutism and virilization in females.

The AxSYM Testosterone Standard Calibrators are for the standard calibration of the AxSYM system when used for the quantitative determination of testosterone in human serum and plasma.

2. Special Conditions for Use Statement:

Prescription Use Only

3. Special Instrument Requirements:

The Abbott AxSYM analyzer is required.

**I. Device Description:**

This device consists of an AxSYM Testosterone Reagent Pack and a set of an AxSYM Testosterone Standard Calibrators. The bar coded Reagent Pack contains three reagents: Anti-testosterone Coated Microparticles, Testosterone:Alkaline Phosphatase Conjugate, and Wash Buffer. The Standard Calibrators consist of six calibrators A through F with testosterone concentrations of 0, 0.2, 1.0, 2.5, 7.0, and 15.0 ng/mL.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):  
Roche Elecsys Testosterone
2. Predicate K number(s):  
K964889
3. Comparison with predicate:

<b>Similarities</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Matrix	Same	Serum, plasma
Calibrator Range	Same	0-15 ng/mL

<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Methodology	Microparticle Enzyme Immunoassay	Electrochemiluminescence Immunoassay
Assay End-Point	Fluorescence	Electrochemiluminescence
On-board Reagent Stability	2 weeks	4 weeks (Elecsys 1010)

<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
		8 weeks (Elecsys 2010/E170)
Calibration Curve	Results are determined from a six-point standard calibration curve generated and stored on the instrument	Results are determined from a calibration curve generated on the instrument via a two-point calibration and a master curve provided by the reagent bar code
Analytical Sensitivity	$\leq 0.1$ ng/mL	0.02 ng/mL
Functional Sensitivity	$\leq 0.2$ ng/mL	0.12 ng/mL

**K. Standard/Guidance Document Referenced (if applicable):**  
NCCLS EP5-A

**L. Test Principle:**

The sample, Displacement Agent, and the antibody-coated microparticles are added together, forming the reaction mixture. As the mixture incubates, testosterone in the sample binds to the anti-testosterone on the microparticles, forming an antigen-antibody complex. Next the Testosterone:Alkaline Phosphatase Conjugate is added and incubated with the reaction mixture, during which time it competes for available anti-testosterone binding sites on the microparticles. After incubation, the mixture is transferred to the matrix cell, where the microparticles bind irreversibly to the glass fiber matrix. After washing to remove unbound substances, the 4-Methylumbelliferyl Phosphate substrate is added to the matrix cell. The alkaline-phosphatase labelled conjugate catalyzes the removal of a phosphate group from the substrate, yielding the fluorescent product 4-Methylumbelliferone. The fluorescence is measured by the AxSYM optical assembly and converted to quantitative units.

**M. Performance Characteristics (if/when applicable):**

1. Analytical Performance:

*a. Precision/Reproducibility:*

The precision performance of this device was evaluated using NCCLS protocol EP5-A. Five plasma-based panels were tested, in replicates of two, at two separate times per day, for twenty days. Two AxSYM analyzers were used. A single lot of reagent was used and each analyzer was calibrated only once. Results were as follows:

PANEL 1

			Within Run		Total	
Analyzer	n	Mean (ng/mL)	SD	%CV	SD	%CV
1	80	0.84	0.07	7.9	0.11	13.7
2	80	0.82	0.07	8.3	0.10	12.7

PANEL 2

			Within Run		Total	
Analyzer	n	Mean (ng/mL)	SD	%CV	SD	%CV
1	80	2.15	0.13	5.9	0.19	8.9
2	80	2.09	0.12	5.5	0.17	8.1

PANEL 3

			Within Run		Total	
Analyzer	n	Mean (ng/mL)	SD	%CV	SD	%CV
1	80	3.82	0.20	5.3	0.26	6.8
2	80	3.76	0.17	4.6	0.31	8.4

PANEL 4

			Within Run		Total	
Analyzer	n	Mean (ng/mL)	SD	%CV	SD	%CV
1	80	4.28	0.24	5.5	0.40	9.3
2	80	4.15	0.21	5.2	0.37	8.9

PANEL 5

			Within Run		Total	
Analyzer	n	Mean (ng/mL)	SD	%CV	SD	%CV
1	80	9.68	0.38	3.9	0.58	6.0
2	80	9.89	0.39	3.9	0.71	7.2

**b. Linearity/assay reportable range:**

To assess linearity, five serum samples were selected ranging in testosterone concentration from 5.38 to 14.4 ng/mL. Each sample was analyzed undiluted and at a dilution factor of X2, X4, X8, and X16, in triplicate. The % recovery was calculated by:

$$\% \text{ recovery} = \text{observed value (ng/mL)} / \text{expected value (ng/mL)} \times 100$$

The observed value was the mean of the three replicate measurements.

Results were as follows:

	Dilution Factor	Observed Value	% Recovery
1	Undiluted	7.91	-----
	X2	4.14	104.6
	X4	2.18	110.0
	X8	0.98	99.3
	X16	0.54	108.8
2	Undiluted	5.38	-----
	X2	3.01	112.0
	X4	1.55	114.9
	X8	0.86	128.5
	X16	0.36	108.3
3	Undiluted	6.59	-----
	X2	3.62	110.0
	X4	1.83	111.0
	X8	0.85	103.2
	X16	0.48	116.6
4	Undiluted	14.40	-----
	X2	7.30	101.3
	X4	3.79	105.4
	X8	1.88	104.3
	X16	1.05	116.4
5	Undiluted	7.87	-----
	X2	4.46	113.3
	X4	2.39	121.4
	X8	1.22	124.2
	X16	0.66	134.6

**c. Traceability, Stability, Expected Values (controls, calibrators, or method):**

The calibrators for this assay are traceable to USP grade testosterone. A testosterone stock solution is first prepared gravimetrically by spiking in USP grade testosterone into testosterone free defibrinated plasma. This stock solution is assigned a value by assaying against calibrators which had been verified by GC-MS. Reference calibrators are prepared gravimetrically from the stock solution, and the standard kit calibrators are rate-matched to the reference calibrators. The overall acceptable rate mean ratio is 0.98 – 1.02.

In addition to real-time stability studies, the calibrators are subjected to freeze/thaw and heat stress conditions. After stressing calibrator performance is compared to the sponsor's acceptance criteria to determine the failure point. Both real-time and accelerated testing is used to establish claimed shelf-life stability.

**d. Detection limit:**

The sponsor calculated both an analytical and functional sensitivity.

Analytical sensitivity was defined as the concentration at two standard deviations from the mean of Calibrator A rates, and represents the lowest measurable concentration of analyte that can be distinguished from zero. Calibrator A was run in replicates of 10, on two AxSYM analyzers, using two lots of reagent. A total of 12 runs were performed, and the sponsor reports an analytical sensitivity of <0.1 ng/mL.

Functional sensitivity was defined as the lowest measurable concentration of analyte that can be measured with an inter-assay coefficient of variation less than or equal to 20%. To assess functional sensitivity, 7 human serum samples, ranging in concentration from 0.11 to 0.46 ng/mL, were tested once per day, in replicates of 5, on one analyzer, for 5 days. Results were as follows:

Sample ID	1	2	3	4	5	6	7
Mean	0.10492	0.13996	0.17036	0.22376	0.31324	0.3794	0.4585
SD	0.01842	0.02868	0.04127	0.04049	0.05702	0.05055	0.06091
CV%	18	20	24	18	18	13	13

The calculated % CV was plotted against the mean concentration of each sample. The functional sensitivity was determined to be <0.2 ng/L, as the concentration corresponding to a CV of 20% on a fitted curve.

**e. Analytical specificity:**

The sponsor evaluated the potential for interference from similar compounds by spiking calibrator A (0.0 ng/mL testosterone) and calibrator D (2.5 ng/mL testosterone) with the following potential cross-reactants:

Potential Cross-reactant	Calibrator A	
	Test Concentration (ng/mL)	% Cross-reactivity
Androstenedione	200	<± 1%
5 $\alpha$ -dihydrotestosterone	100	<± 1%
5 $\alpha$ -androstane-3,17-dione	1000	<± 1%
Oxymetholone	100	<± 1%
Methyltestosterone	100	<± 1%
17B-Estradiol	1000	<± 1%
Androsterone	1000	<± 1%
Cortisol	1000	<± 1%
Cyproterone acetate	1000	<± 1%
Danazol	1000	<± 1%
DHEA-sulfate	1000	<± 1%
11-deoxycortisol	1000	<± 1%
Dexamethasone	1000	<± 1%
Estrone	1000	<± 1%
Progesterone	1000	<± 1%
17 $\alpha$ -Ethinylestradiol 3 methyl ether	1000	<± 1%
17 $\alpha$ -Ethinyl estradiol	1000	<± 1%
Cortisone	1000	<± 1%
Deoxycorticosterone	1000	<± 1%
Estriol	1000	<± 1%
Ethinodiol diacetate	50	<± 1%
Norethindrone acetate	50	<± 1%
D(-) Norgestrel	50	<± 1%
Spirolactone	1000	<± 1%
Corticosterone	1000	<± 1%

Potential Cross-reactant	Calibrator D	
	Test Concentration (ng/mL)	% Cross-reactivity
5 $\alpha$ -dihydrotestosterone	100	< $\pm$ 1%
Oxymetholone	100	< $\pm$ 1%
Methyltestosterone	100	< $\pm$ 1%
17B-Estradiol	1000	< $\pm$ 1%
Androsterone	1000	< $\pm$ 1%
Cortisol	1000	< $\pm$ 1%
Cyproterone acetate	1000	< $\pm$ 1%
Danazol	1000	< $\pm$ 1%
DHEA-sulfate	1000	< $\pm$ 1%
11-deoxycortisol	1000	< $\pm$ 1%
Dexamethasone	1000	< $\pm$ 1%
Estrone	1000	< $\pm$ 1%
Progesterone	1000	< $\pm$ 1%
17 $\alpha$ -Ethinylestradiol 3 methyl ether	1000	< $\pm$ 1%
17 $\alpha$ -Ethinyl estradiol	1000	< $\pm$ 1%
5 $\alpha$ -androstane-3,17-dione	1000	< $\pm$ 1%
Cortisone	1000	< $\pm$ 1%
Deoxycorticosterone	1000	< $\pm$ 1%
Estriol	1000	< $\pm$ 1%
Ethinodiol diacetate	50	< $\pm$ 1%
Norethindrone acetate	50	< $\pm$ 1%
D(-) Norgestrel	50	< $\pm$ 1%
Spirolactone	1000	< $\pm$ 1%
Corticosterone	1000	< $\pm$ 1%
Androstenedione	200	< $\pm$ 1%

The sponsor also evaluated the potential for positive or negative interference from endogenous compounds by spiking in potential interferents to human serum samples and observing the change from the unspiked samples. Results were as follows:

Unspiked conc (ng/mL)	Spiked with 750 mg/dL Hemoglobin (ng/mL)	% Interference
0.86	0.88	2.3
0.64	0.67	4.7
2.98	3.28	10.1
5.12	5.24	2.3
6.52	6.91	6.0
4.89	5.09	4.1

Unspiked conc (ng/mL)	Spiked with 20 mg/dL Bilirubin (ng/mL)	% Interference
12.16	13.02	7.0
11.77	11.40	-3.1
12.30	12.78	3.9
10.25	11.30	10.2
0.15	0.17	10.4
0.96	1.10	15.0

Unspiked conc (ng/mL)	Spiked with 500 mg/dL Triglyceride (ng/mL)	% Interference
14.88	14.25	-4.3
6.71	6.00	-10.5
10.73	10.36	-3.5
6.21	5.95	-4.2
0.58	0.51	-13.1
2.66	2.66	0.1
12.54	12.87	2.6
4.89	4.75	-2.9

Unspiked conc (ng/mL)	Spiked with 90 nmol/L SHBG (ng/mL)	% Interference
2.65	2.79	5.2
3.10	2.92	-5.7
4.69	4.61	-1.6
7.89	7.28	-7.8
8.38	8.29	-1.1
10.39	8.87	-14.7

Unspiked conc (ng/mL)	Spiked with 10 g/dL Protein (ng/mL)	% Interference
0.193	0.174	-9.8
3.061	3.068	0.3
5.320	5.291	-0.5
12.092	10.836	-10.4

Unspiked conc (ng/mL)	Spiked with 0.4% v/v RBCs (ng/mL)	% Interference
9.05	8.69	-3.9
6.51	6.06	-6.9
10.1	9.77	-3.3
7.76	7.33	-5.5
0.50	0.56	12.0
0.63	0.64	1.1

*f. Assay cut-off:*  
Not applicable.

2. Comparison studies:

*a. Method comparison with predicate device:*

The sponsor did a method comparison study versus both the predicate device and gas chromatography – mass spectrometry.

The comparison with the predicate included 298 samples, which included 205 adult males and 93 adult females. The range of data as measured by the AxSYM was 0.05 – 12.05 ng/mL and by the predicate was 0.14 – 9.79 ng/mL. A Passing-Bablok regression analysis was done on the data, which produced the following line equation and correlation coefficient:

Slope = 1.03 (95% Confidence Interval 1.01 to 1.06)

y-intercept = -0.121 (95% Confidence Interval -0.14 to -0.09)

Correlation Coefficient 0.98

For the second comparison, 100 samples were chosen from the group of 298 and analyzed by GC-MS. This group included 50 males and 50 females. The range of data as measured by the AxSYM was 0.05 – 12.05 and by GC-MS was 0.10 – 12.82 ng/mL. A Passing-Bablok regression analysis was done on the data, which produced the following line equation and correlation coefficient:

Slope = 1.10 (95% Confidence Interval 1.01 to 1.16)

y-intercept = -0.06 (95% Confidence Interval -0.10 to -0.02)

Correlation Coefficient 0.97

*b. Matrix comparison:*

The sponsor recommends the use of serum or plasma with this assay. To demonstrate equivalence, 10 matched human serum and plasma specimens

were collected in the following tube types: serum clot tube with no additive (control), serum separator tube, EDTA plasma, sodium heparin plasma, and lithium heparin plasma. Results were as follows:

Sample #	Testosterone Concentration (ng/mL)					% Difference				
	Serum Clot Tubes	Serum SST	Plasma EDTA	Plasma Li Heparin	Plasma Na Heparin	Serum Clot Tubes	Serum SST	Plasma EDTA	Plasma Li Heparin	Plasma Na Heparin
FS1	0.414	0.405	0.412	0.413	0.427	-----	-2.2	-0.6	-0.3	3.1
FS2	0.203	0.198	0.193	0.148	0.192	-----	-2.4	-4.7	-27.0	-5.2
FS3	0.211	0.188	0.241	0.218	0.196	-----	-10.9	13.8	3.1	-7.3
FS4	0.178	0.200	0.222	0.186	0.200	-----	12.4	24.7	4.5	12.4
FS5	0.377	0.356	0.386	0.374	0.397	-----	-5.6	2.4	-0.8	5.3
MS1	2.088	2.061	2.096	1.968	1.921	-----	-1.3	0.4	-5.7	-8.0
MS2	7.758	7.872	7.870	7.298	7.811	-----	1.5	1.4	-5.9	0.7
MS3	7.014	6.959	6.962	7.578	7.048	-----	-0.8	-0.7	8.0	0.5
MS4	8.054	7.890	7.731	7.399	7.695	-----	-2.0	-4.0	-8.1	-4.5
MS5	2.734	2.700	2.634	2.456	2.636	-----	-1.2	-3.7	-10.2	-3.6
Mean	2.90	2.88	2.87	2.81	2.85	-----	-1.3	2.9	-4.2	-0.7

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):*

4. Clinical Cutoff:

Not applicable.

5. Expected Values/Reference Range:

The sponsor performed their own reference range study with samples from 296 apparently healthy adult donors. The study included 92 females (age range 18 – 62) and 204 males (age range 18 – 70). Results were as follows:

					Percentile	
					2.5%	97.5%
Testosterone (ng/mL)					(ng/mL)	(ng/mL)
Specimen	n	Median	Minimum	Maximum		
Females	92	0.23	0.05	0.73	0.05	0.62
Males	204	4.21	1.95	11.38	2.25	9.72

**N. Conclusion:**

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