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

A. 510(k) Number:

k062467

B. Purpose for Submission:

New device

C. Measurand:

Vitamin B_{12}

D. Type of Test:

Quantitative microparticle enzyme immunoassay (MEIA)

E. Applicant:

Axix-Shield Diagnostics, Ltd.

F. Proprietary and Established Names:

Axis-Shield HoloTC assay

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
CDD	II	§862.1810 Vitamin B_{12} test system	Chemistry
JIT	II	§862.1150 Calibrator secondary	Chemistry
JJX	I	§862.1660 Single (specified) analyte controls (assayed and unassayed)	Chemistry

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:

Indications for Use:

Reagents

AxSYM HoloTC assay is a Microparticle Enzyme Immunoassay (MEIA) for the quantitative determination of holotranscobalamin (vitamin B12 bound to transcobalamin) in human serum and plasma on the AxSYM System. HoloTC is used as an aid in the diagnosis and treatment of vitamin B_{12} deficiency.

Calibrators

The AxSYM HoloTC Standard Calibrators are for the standard calibration of the AxSYM System when used for the quantitative determination of holotranscobalmin (HoloTC) in human serum and plasma.

Controls

The AxSYM HoloTC Controls are for the use in quality control to monitor the accuracy and precision of the HoloTC assay when used for the quantitative determination of holotranscobalamin (HoloTC) in human serum and plasma on the AxSYM System.

For *in vitro* diagnostic use.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

Abbott AxSYM®

I. Device Description:

The AxSYM® HoloTC Reagents, Calibrators and Controls are designed to be used in the AxSYM® HoloTC assay on the AxSYM® system.

Reagents:

The AxSYM® HoloTC assay is a Microparticle Enzyme Immunoassay (MEIA) for the quantitative determination of HoloTC in human plasma on the AxSYM System.

Each HoloTC Reagent kit contains 1 bottle of each component of Wash Buffer, Conjugate, Microparticles and Matrix Cell Wash.

- Microparticles – 1 Bottle (8.4 mL) Anti-HoloTC (Mouse, Monoclonal) Antibody Coated Microparticles in TRIS buffer with protein (Bovine) stabilizers.

Minimum concentration: 0.025% solids (w/v). Preservative: Sodium Azide. (Reagent Bottle 1).

- Conjugate - 1 Bottle (13.3 mL) Anti-TC Antibody: Alkaline Phosphatase Conjugate in TRIS buffer with protein (Bovine) stabilizers. Minimum concentration: 0.1 μg/mL. Preservative: Sodium Azide. (Reagent Bottle 2).
- Wash Buffer – 1 Bottle (14.2 mL) Wash Buffer containing detergent. Preservative: ProClin® 300. (Reagent Bottle 3).
- Matrix Cell Wash – 1 Bottle (33.0mL) Matrix Cell Wash. Preservatives: Sodium Azide and Antimicrobial Agents. (Reagent Bottle 4).

Calibrators:

The AxSYM® HoloTC Standard Calibrators are for the standard calibration of the AxSYM System when used for the quantitative determination of HoloTC in human serum and plasma.

Each HoloTC Standard Calibrator kit contains 6 bottles of AxSYM HoloTC Standard Calibrators (4.3mL each). Calibrator A is phosphate buffer with protein (Bovine) stabilizers. Calibrators B-F contains HoloTC in phosphate buffer with protein (Bovine) stabilizers to yield the concentrations (pmol/L) shown below:

Cal A - 0 pmol/L
Cal B - 8 pmol/L
Cal C - 16 pmol/L
Cal D - 32 pmol/L
Cal E - 64 pmol/L
Cal F - 128 pmol/L

Preservative: Sodium Azide.

Controls:

The AxSYM® HoloTC Controls are for use in quality control to monitor the accuracy and precision of the AxSYM HoloTC assay when used for the quantitative determination of holotranscobalamin (HoloTC) in human serum and plasma on the AxSYM System. Each HoloTC Control kit contains 2 bottles (8.3 mL each) of AxSYM HoloTC Controls containing HoloTC in processed human serum/phosphate buffer to yield the concentrations (pmol/L) as follows:

Control	HoloTC Concentration (pmol/L)	Range (pmol/L)
Control Low	21	9 - 33
Control High	48	26 - 70

The human serum is nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, and anti-HCV or HCV RNA. Preservative: Sodium Azide.

J. Substantial Equivalence Information:

Axis-Shield AxSYM® HoloTC assay
Predicate: Axis-Shield HoloTC RIA (K030655)

SIMILARITIES between the submission (test) and predicate are:
1. Intended use statements.
2. The operating principles are similar in that they both employ antibody/antigen interactions.
3. Both assays are quantitative.
4. Both assays are for use with human serum and plasma.
5. Both assays report concentrations of analyte in pmol/L

DIFFERENCES between the submission (test) and predicate are:
1. The test is an enzyme immunoassay and the predicate is a radioimmunoassay.
2. The test is a sandwich format; the predicate is a competitive binding format.
3. The test uses specific anti-HoloTC antibody-coated microparticles whereas the predicate device uses anti-TC antibody.
4. The test quantitates holotranscobalamin (vitamin B12 bound to transcobalamin) directly whereas the predicate quantitates the cobalamin that was bound to transcobalamin (in the form of cyanocobalamin).
5. The test is an automated assay; the predicate is a manual assay.

Parameter	**Submission Device Axis-Shield AxSYM® HoloTC**	**Predicate Device Axis-Shield HoloTC RIA**
510 (k) No.	k062467	k030655
Intended use	AxSYM HoloTC assay is a Microparticle Enzyme Immunoassay (MEIA) for the quantitative determination of holotranscobalamin (HoloTC) in human serum and plasma on the AxSYM System. HoloTC is used as an aid in the diagnosis and treatment of vitamin B_{12} deficiency.	The Axis-Shield Radioimmunoassay (RIA) is an *in vitro* diagnostic assay for the quantitative measurement of holotranscobalamin (vitamin B_{12} bound to transcobalamin) in human serum or plasma. Measurements obtained by this device are used in the diagnosis and treatment of vitamin B_{12} deficiency.
Technology Format	Automated. Sandwich format. Microparticle Enzyme Immunoassay (MEIA).	Manual. Competitive binding format. Radioimmunoassay (RIA).

Capture Antibody	Anti-HoloTC (mouse, monoclonal) antibody coated microparticles.	Anti-TC (mouse, monoclonal) antibody coated microparticles
Detection Antibody	Anti-TC: Alkaline Phosphatase conjugate.	Not applicable
Substrate	4-Methylumbelliferyl phosphate.	Not applicable
Assay End-Point	Fluorescence	Radioactivity
Protocol steps/principle of procedure	● Incubate the sample with the anti-HoloTC antibody-coated microparticles. ● Add anti-TC: alkaline phosphatase conjugate and incubate. ● Transfer to matrix cell. ● Wash to remove unbound substances ● Add substrate. ● Measure fluorescent product.	● Incubate the sample with anti-TC antibody coated particles. ● Following separation of the bound TC a reducing reagent (Reductant), denaturing reagent (Extractant) and ^{57}Co labeled vitamin B_{12} (cobalamin) Tracer are added. ● The vitamin B_{12} cobalamin) released from (TC competes with the tracer for binding to intrinsic factor (IF) ● Unbound tracer is removed by centrifugation. ● The measured radioactivity bound is inversely proportional to the vitamin B_{12} bound to TC in the sample

Parameter	Submission Device Axis-Shield AxSYM® HoloTC	Predicate Device Axis-Shield HoloTC RIA
Quantitation	Results are determined from a standard calibration curve (0, 8, 16, 32, 64, 128 pmol/L) generated and stored on the instrument.	Results are determined from a calibration curve (0, 10, 20, 40, 80, 160 pmol/L).
Standardization	AxSYM HoloTC calibrators (made from the same recombinant HoloTC as in the RIA) were value assigned (using regression analysis) by running 204 samples in both the RIA and AxSYM. Calibrators in the RIA were prepared gravimetrically from a stock solution of recombinant HoloTC.	Calibrators are prepared gravimetrically from a stock solution of recombinant HoloTC.
Standard Calibrator Range	0-128 pmol/L	0-160 pmol/L
Analytical Sensitivity	≤1 pmol/L	6 pmol/L
Imprecision	Within run CV of 3.4% to 5.1%. Total CV of 6.3% to 8.5%.	Within run CV of 5% to 10% Total CV of 6% to 12%.
Reagent stability	Up to stated expiration date when stored at 2-8°C.	Up to stated expiration date when stored at 2-8°C.
Sample stability	28 days at 2-8°C: store at -20°C or colder if testing will be delayed beyond 28 days. Specimens stored at -20°C or colder for 6 months did not show performance differences.	7 days at 2-8°C; store frozen if testing is delayed more than 7 days.
Expected values	In a representative study: N = 281 healthy subjects (age range 21-70), reference interval = 19.1-119.3 pmol/L (central 95% of population).	In a representative study: N = 303 healthy subjects (age range 22-88), reference interval = 37-171 pmol/L (central 95% of population).

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

STANDARDS
Title and Reference Number
How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline - Second Edition (C28-A2)

Other Standards

GUIDANCE			
Document Title	**Office**	**Division**	**Web Page**
Points to Consider Guidance Document on Assayed and Unassayed Quality Control Material; Draft	**OIVD**		**http://www.fda.gov/cdrh/ode/99.html**
Format for Traditional and Abbreviated 510(k) s - Guidance for Industry and FDA Staff	**OIVD**		**http://www.fda.gov/cdrh/ode/guidance/1567.html**

L. Test Principle:

The assay is based upon microparticle enzyme immunosaay technology (MEIA). When human HoloTC antigen is present in the sample; it binds to the coated microparticles, forming antigen-antibody complexes on the microparticles. An enzymatic reaction yields a fluorescent product which is measured by the analyzer.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*

   Precision studies were done according the National Committee for Clinical Laboratory Standards (NCCLS) Protocol EP5-A2. This study assessed repeatability (within-run precision), reproducibility (total precision based on between-day, between run, within-day and between instrument precision).

   Precision of the system was demonstrated by assaying the low and high kit controls for the AxSYM HoloTC assay. Assays were run twice daily for 20 days (leaving at least 2 hours between assay runs) on each of two AxSYM instruments. In the event of a lost day, additional day(s) were added to the study in order to generate 20 days worth of complete data.

   A calibration curve was generated on each AxSYM during Run 1 on Day 1,

calibrators and controls were run as samples in replicates of two (n=80 for each control).

Results:
Analysis included the with-in run and total SD and %CV. Summary results are shown below.

Precision Summary Statistics, HoloTC Low Control and High Control

Low Control		Total		Within Run	
AxSYM	Mean (pmol/ L)	SD	%CV	SD	%CV
1	23.3	1.48	6.3	1.03	4.4
	22.8	1.49	6.5	0.77	3.4

High Control		Total		Within Run	
AxSYM	Mean (pmol/ L)	SD	%CV	SD	%CV
2	49.7	3.87	7.8	2.51	5.1
	48.2	4.09	8.5	2.36	4.9

Precision (both reproducibility and repeatability) met the sponsors established criteria specification of ≤ 10% CV.

b. Linearity/assay reportable range:

The reported or measurable range of the AxSYM HoloTC assay was defined by the analytical sensitivity and the highest calibrator concentration. The sponsor claims an analytical sensitivity of ≤ 1 pmol/L and a high calibrator concentration of 128pmol/L.

Dilution linearity within the working range of the assay was demonstrated to meet all criteria for linearity (acceptable range is 80% to 120%). Recovery test of HoloTC spiked serum samples was demonstrated to be within acceptance criteria of 80% to 120%. Dilution of three commercially available pools of a high HoloTC sample into six dilutions showed correlation coefficients of: pool 1 = R^2 = 0.9747 y = 1.0507 + 1.0222; pool 2 = R^2 = 0.978 y = 0.9613 x + 6.1325; and pool 3 = R^2 = 0.9948 y = 1.0345x – 0.3515. The above least square regression plot results for diluted AxSYM HoloTC samples demonstrated linearity over the range 19.8 – 103pmol/L.

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

HoloTC calibrators are prepared gravimetrically from a stock solution of recombinant HoloTC. The purity of the recombinant HoloTC is determined (i) by SDS electrophoresis and (ii) spectroscopically from the ratio between the protein component and the cobalamin component.

HoloTC Controls are prepared from human serum and assigned values with reference to the HoloTC calibrators.

d. Detection limit:

The sponsor claims an analytical sensitivity of ≤ 1pmol/L. The sponsor defined the analytical sensitivity of the AxSYM HoloTC assay as the concentration at two standard deviations above the AxSYM HoloTC Standard Calibrator A (0.0pmol/L) and represents the lowest concentration of HoloTC that can be distinguished from zero. In a representative study, the analytical sensitivity was evaluated by repeated testing (n=12 runs in replicates of 10 across two reagent lots) of the AxSYM HoloTC Standard Calibrator A. In this study, the mean analytical sensitivity was calculated as 0.08pmol/L. (observed range 0.03 to 0.14pmol/L).

e. Analytical specificity:

Serum samples were spiked with potentially interfering substances and tested in the HoloTC assay. the interference was ≤ 10% for all the following:

Interfering Substance	Pool ID	Replicates tested	Interfering Substance conc.	Control HoloTC conc. (pmol/L)	Supplemented Sample HoloTC conc. (pmol/L)	Mean Interference (%)
Bilirubin	Low	3	0.4 mg/mL	20.8	19.1	-8
	High	3		47.9	47.9	0
Hemoglobin	Low	3	5 mg/mL	24.4	25.6	5
	High	3		63.2	64.4	2
Triglycerides	Low	3	15 mg/mL	25.3	25.4	0
	High	3		58.9	57.3	-3
Total Protein	Low	3	95 g/L	12.9	14.2	10
	High	3		37.3	36.7	-2

The AxSYM HoloTC assay was also evaluated for analytical specificity in a study where cross-reactivity with B_{12} binding proteins transcobalamin and haptocorrin were measured by the assay. 500pmol/L transcobalamin was added to line diluent and 5000pmol/L haptocorrin was added to Calibrator A and then assayed. (n = 5 replicates) Data from this study are summarized in the table below.

Cross-reactant	Cross-reactant Concentration (pmol/L)	% Cross-reactivity
Transcobalamin	500	Not detected
Haptocorrin	5000	Not detected

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A study was performed comparing the AxSYM HoloTC assay to the predicate Axis Shield HoloTC RIA assay. The results from the Passing-Babcock linear regression analysis are summarized in the following table.

Passing-Bablok Comparison					
n	slope	95% CI on slope	intercept	95% CI on intercept	r
204	1.02	0.95 to 1.10	2.1	-0.91 to 4.84	0.90

Sample range for RIA: 7.1 – 144.3 pmol/L
Sample range for AxSYM: 8.9 – 123.3 pmol/L

b. Matrix comparison:

Matrix comparison studies to determine which anticoagulants tube types are suitable for collection of samples to be measured with the AxSYM HoloTCC assay was performed. One tube of plasma was collected in each of the following collection tubes from 10 in-house volunteers: serum, Lithium Heparin, serum gel separator and EDTA plasma tubes. The tubes were processed according to the manufacturer's guidelines and the plasma removed from each tube and stored. Each sample was tested in replicates of 3 for each tube type on one instrument. The comparison results are presented in the table below.

Sample No.	Serum (glass)	Lithium Heparin	Serum Gel Separator	EDTA	Overall % Difference from Serum (glass)		
					Lithium Heparin	Serum Gel Separator	EDTA
1	25.22	26.70	24.01	31.48	5.89	-4.79	24.84
2	25.58	25.56	23.71	34.27	-0.09	-7.3	33.94
3	30.14	26.31	23.35	37.46	-12.72	-22.53	24.26
4	31.81	31.82	30.02	44.34	0.03	-5.65	39.36
5	31.11	35.15	31.63	43.59	12.97	1.66	40.11
6	37.97	39.71	34.73	55.53	4.58	-8.54	46.23
7	36.63	40.39	33.58	57.39	10.25	-8.32	56.64
8	19.92	21.61	18.45	27.90	8.48	-7.34	40.1
9	23.02	23.93	21.59	31.67	3.92	-6.2	37.56
10	65.87	60.34	57.86	94.11	-8.4	-12.17	42.86
Grand Mean	32.73	33.15	29.89	45.77	2.49	-812	38.59

The sponsors established acceptance criteria of grand mean % difference ≤ / ± 10% to serum was met for Lithium Heparin plasma and gel-separator Serum collection tubes. EDTA Plasma is not recommended for use in this assay.

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 cut-off:

   Not applicable

5. Expected values/Reference range:

   In a representative study, serum specimens from 281 healthy donors were tested using the AxSYM HoloTC assay. The mean HoloTC concentration was 47.7 pmol/L with a range from 8.9 to 123.3 pmol/L. The central 95% of the population defined the expected range of 19.1 to 119.3 pmol/L.

In the labeling the sponsor recommends that each laboratory determine its own reference range based upon its particular locale and population characteristics.

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.