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

A. 510(k) Number:

k121946

B. Purpose for Submission:

New Device

C. Measurand:

Holotranscobalamin (HoloTC)

D. Type of Test:

Quantitative, Enzyme Immunoassay

E. Applicant:

Axis-Shield Diagnostics, Ltd.

F. Proprietary and Established Names:

Axis-Shield Active-B12 (Holotranscobalamin)

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
CDD	Class II	§862.1810 Vitamin B12 test system	Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:

   The Axis-Shield Active-B12 (Holotranscobalamin) assay is an enzyme-immunoassay (EIA) for the quantitative determination of holotranscobalamin (HoloTC) in human serum. HoloTC (vitamin B_{12} bound to transcobalamin) is used as an aid in the diagnosis and treatment of vitamin B_{12} deficiency.

3. Special conditions for use statement(s):

   For *in vitro* diagnostic use.

   For prescription use.

4. Special instrument requirements:

   No special instrument is required.

   Any microplate (96-well plate/strip) reader with 405nm filter can be used to read the developed yellow color at 405nm wavelength.

I. Device Description:

The Axis-Shield Active-B12 (Holotranscobalamin) EIA kit contains the following components:

Assay Reagents:

i) Microtitre Plate: 12 x 8-well breakapart microtitre strips coated with anti-holotranscobalamin murine monoclonal antibody, in a resealable foil pack with desiccant. Ready-to-use.
ii) Conjugate: 1 x 15 mL alkaline phosphatase-labelled murine monoclonal antibody to human transcobalamin in Tris buffer with protein stabilizer. Preservative: <0.1% (w/v) sodium azide. Ready-to-use.
iii) Wash Buffer 8x: 2 × 25 mL Phosphate buffer solution. Preservative: 0.72% (w/v) sodium azide. Dilute before use with distilled/deionized water.
iv) Substrate: 1 x 15 mL para-NitroPhenyl Phosphate (pNPP) buffer solution. Ready-to-use. Do not expose to light during storage.
v) Stop Solution: 1 × 15 mL 1M sodium hydroxide solution (pH > 10). Ready-to-use.
vi) Pre-treatment: 1 x 25 mL Citrate buffer solution. Preservative: < 0.1% (w/v) sodium azide. Ready-to-use.

Assay Calibrators:

i) Calibrator A, 1 x 1.0 mL of phosphate buffer solution with protein (bovine) stabilizer. Preservative < 0.1% (w/v) sodium azide. Ready-to-use. Do not expose to light during storage.
ii) Calibrators B to F, 5 x 1.0 mL of phosphate buffer solution with protein (bovine) stabilizer and recombinant HoloTC. Preservative < 0.1% (w/v) sodium azide. Ready-to-use. Do not expose to light during storage.

Assay Controls:

i) Low Kit Control: 1 × 1.0 mL Phosphate buffer with protein (bovine) stabilizer containing recombinant HoloTC. Preservative: < 0.1% (w/v) sodium azide. Ready-to-use. Do not expose to light during storage. Concentration: 25 pmol/L.
ii) High Kit Control: 1 x 1.0 mL Phosphate buffer with protein (bovine) stabilizer containing recombinant HoloTC. Preservative: < 0.1% (w/v) sodium azide. Ready-to-use. Do not expose to light during storage. Concentration: 60 pmol/L.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   ARCHITECT Active-B12 (HoloTranscobalamin)

2. Predicate 510(k) number(s):
   k112443

3. Comparison with predicate:

Similarities and differences between the predicate and candidate devices		
Item	ARCHITECT Active-B12 (k112443) Predicate Device	Axis-Shield Active-B12 EIA Candidate Device
Intended use / Indications for use	For the quantitative determination of holotranscobalamin (HoloTC) in human serum. HoloTC (Active B12, vitamin B12 bound to transcobalamin) is used as an aid in the diagnosis and treatment of vitamin B12 deficiency.	Same
Instrument	ARCHITECT *i* System	Microplate reader with 405nm filter
Capture antibody	Murine monoclonal antibody 3C4	Same
Conjugate antibody	Murine monoclonal antibody 3-11	Same
Substrate/signal generation	Acridinium tracer	para-NitroPhenyl Phosphate/ alkaline phosphatase
Storage conditions	Reagent pack must be stored at 2 to 8ºC.	Same
Onboard reagent stability	Reagents can be stored onboard of the ARCHITECT *i* System for a maximum of 30 days.	Not applicable.
Measuring range	5.0 to 128.0 pmol/L	10.0 to 128.0 pmol/L
Assay dilution	1:2 autodilute or manual dilution with ARCHITECT *i* Multi-Assay Manual Diluent	Not applicable
Calibration	6-point calibration curve. 4PLC Y-weighted curve-fit.	6-point calibration curve. Linear regression curve-fit.
Calibrator range	Target assigned values on product labeling as follows: Calibrator A (0.0 pmol/L) to Calibrator F (128.0 pmol/L).	Levels are lot-specific. Representative levels are as follows: Calibrator A (0.0 pmol/L) to Calibrator F (154.0 pmol/L).
Sample type	Human serum including serum collected in serum separator tubes.	Same
Controls	2 levels	Same
Detection limits	Limit of Blank = <0.4 pmol/L Limit of Detection = 1.9 pmol/L Limit of Quantitation = 5.0 pmol/L	Limit of Blank = 4.9 pmol/L Limit of Detection = 8.1 pmol/L Limit of Quantitation = 8.3 pmol/L

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

CLSI Guideline, EP5-A2 Evaluation of Precision Performance of Clinical Chemistry Devices

CLSI Guideline, EP9-A2 Method Comparison and Bias Estimation Using Patient Samples

CLSI Guideline, EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach

CLSI Guideline, EP17-A Protocols for demonstration, Verification, and Evaluation of Limits of Detection and Quantitation

CLSI Guideline, EP7-A2, Interference Testing in Clinical Chemistry

CEN, 13640, Stability Testing of In Vitro Diagnostic Reagents

L. Test Principle:

The enzyme immunoassay is a two step assay. The microtitre wells are coated with a highly specific monoclonal antibody for Active-B12 (Holotranscobalamin). During the first incubation, holotranscobalamin in serum specifically binds to the antibody-coated surface. In the second incubation, the murine anti-human transcobalamin alkaline phosphatase conjugate binds to any captured holotranscobalamin. The wells are then washed using the wash buffer to remove unbound components. The bound holotranscobalamin is detected by incubation with the substrate pNPP. Addition of the stop solution terminates the reaction, resulting in a colored end-product. The concentration of holotranscobalamin in pmol/L is directly related to the color generated and can be estimated by interpolation from a dose-response curve based on the calibrators. All calibrators, controls, and patient samples are assayed in duplicate and the mean results will be used to generate the calibration curve and obtain the final patient results.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

   Precision study was performed following the CLSI EP5-A2 guidelines. Six human serum samples and two controls were assay at Axis-Shield site using three lots of reagents. Samples were assayed by two operators in replicates of eight, once a day for five days (N = 80). The results are summarized in the following table:

Sample	n	Lot	Operator	Mean (pmol/l)	Intra-assay %CV	Total %CV
1	80	1	1	17.8	7.5%	8.2%
			2	17.5	3.1%	9.3%
		2	1	20.1	6.0%	6.6%

			2	20.3	6.9%	9.2%
		3	1	19.1	5.5%	8.0%
			2	18.9	8.5%	11.0%
2	80	1	1	21.8	5.5%	9.9%
			2	21.8	3.9%	7.5%
		2	1	22.6	5.6%	8.7%
			2	23.5	9.0%	10.3%
		3	1	23.9	7.0%	10.2%
			2	23.2	5.8%	8.9%
3	80	1	1	28.8	3.8%	7.8%
			2	30.7	4.3%	9.6%
		2	1	31.0	6.8%	8.0%
			2	31.4	4.3%	6.1%
		3	1	31.5	4.5%	6.4%
			2	32.2	4.0%	9.2%
4	80	1	1	49.3	3.9%	7.4%
			2	52.6	4.1%	6.7%
		2	1	50.8	5.6%	10.0%
			2	51.7	4.7%	5.9%
		3	1	52.6	4.6%	4.8%
			2	55.0	5.5%	6.1%
5	80	1	1	68.4	4.0%	7.6%
			2	73.2	3.7%	7.5%
		2	1	74.8	4.3%	8.2%
			2	75.9	4.6%	6.4%
		3	1	75.1	4.4%	7.9%
			2	76.3	4.9%	6.2%
6	80	1	1	115.9	4.2%	5.9%
			2	121.1	3.6%	7.0%
		2	1	123.2	4.3%	10.2%
			2	124.0	4.2%	6.4%
		3	1	127.0	4.8%	10.1%
			2	129.5	3.2%	5.6%
Low Control	80	1	1	23.7	9.4%	10.9%
			2	23.8	5.1%	11.5%
		2	1	20.0	6.0%	7.5%
			2	18.6	5.8%	8.5%
		3	1	20.3	8.3%	9.7%
			2	20.1	8.3%	10.0%
High Control	80	1	1	61.2	6.3%	6.4%
			2	58.8	4.5%	8.9%
		2	1	50.3	6.3%	8.1%
			2	50.2	5.9%	8.4%
		3	1	52.2	7.7%	9.2%
			2	50.8	5.8%	8.5%

b. Linearity/assay reportable range:

Linearity study was performed according to the CLSI EP6-A guidelines. One

high real patient serum sample at approximately 156.0 pmol/L HoloTC concentration and a low human serum based buffer at approximately 5.0 pmol/L were use to prepare a dilution series of a total of ten samples ranging in concentration from 5.28 to 156.04 pmol/L. The samples were tested in replicates of four with one lot of reagent by one operator. The linear regression analysis of the data suggested a linear fit. The results are summarized below:

Range tested (pmol/L)	Correlation Coefficient (R^2)	Slope (95% CI)	Intercept (95% CI)
5.28 to 156.04	1.00	1.00 (0.98 to 1.02)	1.30 (-0.42 to 3.01)

Based on the results, the measuring range of the Axis-Shield Active-B12 assay is claimed from 10.0 to 128.0 pmol/L. Samples read at concentrations greater than 128.0 pmol/L should be reported out as >128.0 pmol/L.

A high dose hook effect was performed and showed no hook effect with HoloTC concentration up to 2236 pmol/L.

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

Traceability

There is currently no international recognized reference method or reference material. HoloTC calibrators are prepared gravimetrically from a stock solution of recombinant HoloTC following an internal procedure. The purity of the recombinant HoloTC stock solution is determined (i) by HPLC and (ii) spectroscopically from the ratio between the protein component and the cobalamin component.

Value assignment

HoloTC calibrators are value assigned against internal reference standards and concentrations values are lot specific and may vary between lots.

HoloTC Controls are prepared from HoloTC calibrator stock solution and HoloTC calibrator diluent, and assigned values with reference to the HoloTC calibrators.

Stability

The Axis-Shield Active-B12 (Holotranscobalamin) EIA assay kit was evaluated for shelf life and in-use storage stability. A shelf-life of 52 weeks when stored at 2 to 8ºC was assigned to the Axis-Shield Active-B12 (Holotranscobalamin) EIA assay kit based on an accelerated stability study performed at 25ºC. The real-time storage stability studies are on-going to support the shelf-life. Based on the in-use kit storage stability studies, a kit once opened can be reused on three occasions over a three month period. In addition, the sponsor has provided suggestion on the handling and storage of

the kit in the labeling. Stability study protocols and acceptance criteria has been reviewed and found to be adequate.

d. Detection limits:

Analytical detection limits studies were performed according to the CLSI EP17-A guidelines.

For LoB estimation, two serum based buffer samples were tested by two operators in replicates of 15 in two runs over two days using two reagent lots (N=120 per reagent lot). The LoB was determined to be 4.9 pmol/L.

For LoD estimation, five patient samples at holotranscobalamin concentrations between LoB and 4 x LoD were tested in replicates of six in two runs over two days using two reagent lots (N=120 per reagent lot). LoD was calculated using the formula: LoD = LoB + (1.653 x pooled SD). The LoD was determined to be 8.1 pmol/L.

For LoQ estimation, the LoD study data from the five patient samples were used to estimate LoQ. A precision plot was generated to determine the inter-assay precision at the low end concentration range. LoQ is defined as the concentration where the inter-assay precision is <20%CV. LoQ was determined to be 8.3 pmol/L.

The claimed measuring range of the Axis-Shield Active-B12 assay is 10.0 to 128.0 pmol/L.

e. Analytical specificity:

Interference study was performed following the CLSI EP7-A2 guidelines. Serum samples at three HoloTC concentrations (approximately 30, 72 and 120 pmol/L) were spiked with potentially interfering substances at a range of target concentrations and tested in replicates of five in the Axis-Shield Active-B12 (HoloTC) assay. Non-significant interference is defined by a percent difference of within or equal to ±10% between the control non-spiked sample and the test samples spiked with interfering substances.

Potentially Interfering Substances	**Highest level with non-significant interference**
Bilirubin	30 mg/dL
Hemoglobin	500 mg/dL
Total Protein	9000 mg/dL
Triglyceride	3000 mg/dL
Rheumatoid Factor	75 IU/mL

A cross-reactivity study with vitamin B12 binding proteins apotranscobalamin

and haptocorrin was performed using the HoloTC assay. Apotranscobalamin at 500 pmol/L concentration was added to an Active B-12 negative serum-based buffer. Haptocorrin at 5000 pmol/L concentration was added to three serum samples at approximately 35, 71 and 125 pmol/L HoloTC concentrations. Parallel control samples were prepared in the respective base matrices with no added apotranscobalamin and haptocorrin. The test and control samples were assayed in replicates of ten using one lot of reagents, calibrators and controls. The following vitamin B12 binding proteins did not cross-react in the Axis-Shield Active-B12 (HoloTC) assay as defined by a percent cross-reactivity of within or equal to ±10% from the corresponding non-spiked control samples.

Cross reactant	**Cross reactant concentration (pmol/L)**	**Percent Cross-reactivity**
Apotranscobalamin	500	0.12%
Haptocorrin	5000	Up to -4.6 %

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Method comparison study was performed following the CLSI EP9-A2 guidelines. 120 patient serum samples ranging in holotranscobalamin concentration from 10.0 to 128.0 pmol/L were tested in duplicate over three days using three lots of reagents by multiple operators. Only 111 sample results were analyzed since nine samples measured >128.0 pmol/L either in the predicate or the candidate assay. The mean of duplicate results were analyzed by Passing & Bablok analysis to determine the slope, intercept, and coefficient of regression respectively. The results are shown below:

No. of Observations	111
Correlation Coefficient (95% Cl)	0.93 (0.90 to 0.95)
Slope (95% Cl)	0.95 (0.89 to 1.01)
Intercept (95% Cl)	8.39 (5.73 to 11.77)

b. Matrix comparison:

Matrix comparison study was performed to evaluate the suitability of the plastic serum separator tubes (SST) for use in the Axis-Shield Active-B12 assay. Thirty six fresh blood samples were collected in plastic serum tubes (control) and the SST tubes and processed for serum following manufacturer's instructions. Each serum sample was tested in replicates of four by one

operator using one reagents lot. The mean results were analyzed by Passing & Bablok analysis to determine the slope, intercept, and coefficient of regression. The results are shown below:

No. of Observations	36
Sample Range (pmol/L)	22.59 to 112.3
Correlation Coefficient (r2)	0.98
Slope (95% Cl)	0.97 (0.92 to 1.01)
Intercept (95% Cl)	0.16 (-2.48 to 2.61)

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):

   Not applicable

4. Clinical cut-off:

   Not applicable

5. Expected values/Reference range:

   In a reference range study, serum specimens from 135 healthy donors were tested using the Axis-Shield Active-B12 (HoloTC) assay. The mean HoloTC concentration was 72.0 pmol/L with a range from 15.0 to 147.0 pmol/L. The central 95% of the population defined the expected range of 21.0 to 123.0pmol/L.

   In the labeling the sponsor recommends that each laboratory establishes their own expected reference range based upon its unique population characteristics including geographical, patient, dietary and environmental factors.

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.