

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

A. 510(k) Number:

k112443

B. Purpose for Submission:

New device

C. Measurand:

Holo transcobalamin (HoloTC)

D. Type of Test:

Quantitative, immunoassay

E. Applicant:

Axis-Shield Diagnostics, Ltd.

F. Proprietary and Established Names:

ARCHITECT Active-B12 (Holo transcobalamin) Reagents

ARCHITECT Active-B12 (Holo transcobalamin) Calibrators (A-F)

ARCHITECT Active-B12 (Holo transcobalamin) Controls (Low and High)

G. Regulatory Information:

1. Regulation section:

862.1810 Vitamin B12 test system,

862.1150 Calibrator, secondary,

862.1660 Single analyte controls (assayed and unassayed)

2. Classification:

Class II, class II, and class I reserved, respectively

3. Product code:

CDD, JIT, and JJX, respectively

4. Panel:

Chemistry (75)

H. Intended Use:

1. Intended use(s):

See Indications for use below

2. Indication(s) for use:

Reagents

The ARCHITECT Active-B12 (Holotranscobalamin) assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of holotranscobalamin in human serum on the ARCHITECT i System. Active-B12 (Holotranscobalamin) is used as an aid in the diagnosis and treatment of vitamin B12 deficiency.

Calibrators

The ARCHITECT Active-B12 (Holotranscobalamin) Calibrators are for the calibration of the ARCHITECT i System when used for the quantitative determination of holotranscobalamin in human serum.

Controls

The ARCHITECT Active-B12 (Holotranscobalamin) Controls are for the estimation of test precision and the detection of systematic analytical deviations of the ARCHITECT i system (reagents, calibrators, and instrument) when used for the quantitative determination of holotranscobalamin in human serum.

3. Special conditions for use statement(s):

For prescription use only.

The sponsor has the following limitations stated in their labeling:

Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA).

Specimens containing HAMA may produce anomalous values when tested with assay kits such as ARCHITECT Active-B12 (Holo transcobalamin) that employ mouse monoclonal antibodies.

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 results may be observed. Additional information may be required for diagnosis.

4. Special instrument requirements:

ARCHITECT *i* 2000_{SR} system

I. Device Description:

Reagent:

Each HoloTC Reagent kit contains 1 bottle of each component of conjugate and microparticles. Reagent kit contains enough reagents for either 100 tests or 500 tests.

1. Microparticles - 1 Bottle (6.5 ml/26.5 mL) Anti-HoloTC (mouse, monoclonal) antibody coated microparticles in HEPES buffer with surfactants. Minimum concentration: 0.1% solids. Preservative: sodium azide.
2. Conjugate - 1 Bottle (5.8 mL/25.8 mL) Anti-transcobalamin antibody acridinium-labeled conjugate in MES buffer with surfactants. Minimum concentration: 0.15 µg/mL. Preservatives: Sarafloxacin and Nipasept.

Calibrators:

Each HoloTC calibrator kit contains 6 bottles of calibrators (4 mL 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) as follows: 8, 16, 32, 54, and 128 pmol/L

Controls:

Each HoloTC control kit contains 2 bottles (8 mL each) of controls containing HoloTC in phosphate buffer to yield the concentrations (pmol/L) as follows: 15 and 48 pmol/L.

J. Substantial Equivalence Information:

1. Predicate device name(s):

AxSYM Active-B12 (Holo transcobalamin)

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

k062467

3. Comparison with predicate:

Reagent:

Similarities and differences between the predicate and candidate devices		
Item	AxSYM Active-B12 Predicate Device (k062467)	ARCHITECT Active-B12 Candidate Device
Intended use/Indications for use	For the quantitative determination of holotranscobalamin in human serum. Holotranscobalamin (HoloTC) is used as an aid in the diagnosis and treatment of vitamin B ₁₂ deficiency.	Same
Instrument	Abbott AxSYM	ARCHITECT <i>i</i> 2000 _{SR} system
Method	Microparticle Enzyme Immunoassay (MEIA)	Chemiluminescent microparticle immunoassay (CMIA)
Measuring range	1.0 to 128 pmol/L	5.0 to 128 pmol/L
Sample type	Serum or lithium heparin plasma	Serum
Expected values	Central 95%: 19.1 to 119.3 pmol/L	Central 95%: 25.1 to 165 pmol/L

Calibrators:

Similarities and Differences		
Item	AxSYM Active-B12 calibrators Predicate device (k062467)	ARCHITECT Active-B12 calibrators Candidate device
Intended Use/Indications for Use	For calibration of the Active-B12 assay when used for the quantitative determination of Holotranscobalamin (HoloTC)	Same
Format	Liquid, ready to use	Same
Level	6 levels	Same
Stability	Shelf-life of 104 weeks	Shelf-life of 52 weeks

Controls:

Similarities and Differences		
Item	AxSYM Active-B12 controls Predicate device (k062467)	ARCHITECT Active-B12 controls Candidate device
Intended Use/ Indications for Use	For quality control monitoring of the Active-B12 assay when used for the quantitative determination of holotranscobalamin (HoloTC)	Same
Format	Liquid, ready to use	Same
Levels	Two levels	Same
Stability	Shelf-life of 104 weeks	Shelf-life of 52 weeks

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

1. CLSI Guideline, EP5-A2 *Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline Second edition*
2. CLSI Guideline, EP6-A *Evaluation of the Linearity of Quantitative Analytical Methods; Approved Guideline*
3. CLSI Guideline, EP7-A2 *Interference Testing in Clinical Chemistry; Approved Guideline- Second edition*
4. CLSI Guideline, EP9-A2 *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline Second edition*
5. CLSI Guideline, EP17-A *Protocols for Demonstration, Verification, and Evaluation of Limits of Detection and Quantitation; Approved Guideline*

L. Test Principle:

The ARCHITECT Active-B12 (Holotranscobalamin) assay is a two step immunoassay using chemiluminescent microparticle immunoassay (CMIA) technology. In the first step, sample and anti-holotranscobalamin coated paramagnetic microparticles are combined. Holotranscobalamin present in the sample binds to the anti- holotranscobalamin coated microparticles. After washing, anti- holotranscobalamin acridinium-labeled conjugate is added to create a reaction mixture in the second step. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of holotranscobalamin in the sample and the RLUs detected by the ARCHITECT i system optics.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

All performance characteristics were performed on the ARCHITECT i 2000_{SR} system.

a. *Precision/Reproducibility:*

A precision study was performed according to the CLSI EP5-A2 guideline. Testing was conducted using 2 lots of reagents, calibrators, controls, and 2 instruments. Two levels of controls and 5 levels of human serum samples were assayed in a minimum of 2 replicates at 2 separate times per day for 20 different days (N=80). Precision results are shown in the table below:

Sample	Instrument	Reagent Lot	N	Mean (pmol/L)	Within Run		Total	
					SD	%CV	SD	%CV
Low Control	1	1	80	15.42	0.64	4.2	0.76	4.9
	2	2	80	15.56	0.59	3.8	0.63	4.1
High Control	1	1	80	49.82	1.87	3.8	2.65	5.3
	2	2	80	47.75	1.80	3.8	2.13	4.5
Sample 1	1	1	80	18.56	0.36	1.9	0.97	5.2
	2	2	80	16.81	0.46	2.8	0.86	5.1
Sample 2	1	1	80	24.62	0.56	2.3	1.02	4.2
	2	2	80	22.40	0.51	2.3	0.74	3.3
Sample 3	1	1	80	52.95	0.86	1.6	2.01	3.8
	2	2	80	48.36	1.33	2.7	2.07	4.3
Sample 4	1	1	80	81.34	1.83	2.2	3.83	4.7
	2	2	80	72.15	2.17	3.0	3.37	4.7
Sample 5	1	1	80	113.74	2.07	1.8	4.85	4.3
	2	2	80	100.08	4.39	4.4	5.83	5.8

b. *Linearity/assay reportable range:*

The sponsor conducted a linearity study according to the CLSI EP6-A guideline. The linearity study used a spiked high serum pool (129.5 pmol/L) and a low serum pool (2.5 pmol/L) to create additional intermediate levels of samples. A total of 11 samples with HoloTC concentration ranging from 2.5 to 129.5 pmol/L were tested in replicates of 5 on the ARCHITECT i 2000 SR system. The linear regression generated by plotting the expected values against the observed values was: $Y=1.009X - 1.46$, $R^2=0.99$

Based on the results of the linearity study the sponsor claimed that the assay is linear from 5.0 to 128 pmol/L.

An additional dilution study was performed to support the sponsor's claim that results >128 pmol/L can be diluted 1:2 automatically. 5 samples with HoloTC concentrations ranging from 152 to 254 pmol/L were tested by diluting 1:2 manually and by the instrument. Results obtained manually were compared to the results obtained automatically and % differences were <8% for all the samples tested. The sponsor claimed in the labeling that samples >128 pmol/L can be diluted in a 1:2 ratio by the instrument.

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

Traceability:

There is currently no internationally recognized reference method or reference material for HoloTC. The ARCHITECT HoloTC calibrators are traceable to an internal reference material which is traceable to another commercially available assay. The ARCHITECT Active-B12 (Holotranscobalamin) Primary Reference Calibrators were manufactured by gravimetric methods using recombinant Holotranscobalamin.

Value Assignment:

The ARCHITECT Active B-12 Calibrators are value assigned against the primary reference calibrators with multiple testing on the ARCHITECT i analyzer at the following target concentrations:

Calibrator	Concentration (pmol/L)	Calibrator	Concentration (pmol/L)
CAL A	0.0	CAL D	32.0
CAL B	8.0	CAL E	64.0
CAL C	16.0	CAL F	128.0

The ARCHITECT Active-B12 Controls are prepared using the ARCHITECT Active-B12 calibrator stock solution and diluent with multiple testing on the ARCHITECT i analyzer at the following target concentrations:

Control	Concentration Target (pmol/L)	Concentration Range (pmol/L)
Control L	15.0	9.6 – 21.8
Control H	48.0	29.6 – 66.7

Stability:

Stability of the calibrators and controls are determined based on accelerated studies. Real-time stability studies are on-going. The calibrators and controls have a shelf-life stability of 12 months and an open-vial stability of 6 months when stored at 2-8°C. Stability study protocols and acceptance criteria are provided and found to be adequate.

d. *Detection limit:*

A detection limits study was performed according to the CLSI guideline EP17-A. Several blank samples and four low concentrations of HoloTC were used to determine the LoB and LoD. An additional 6 low samples (range from 1.4 to 5.7 pmol/L) were assayed to determine the LoQ. All samples were tested in 2 runs over 2 days using two lots of reagents and two instruments over 5 days. LoB was

determined to be 0.4 pmol/L. LoD was determined to be 1.90 pmol/L. LoQ was determined to be 5.0 pmol/L based on an interassay precision of <10%.

The sponsor claimed that the candidate assay has a measuring range of 5.0 to 128 pmol/L.

e. Analytical specificity:

An interference study was performed according to the CLSI guideline EP7-A. Serum samples were spiked with potentially interfering substances and tested using the HoloTC assay in replicates of 5 to 6. Three different concentrations of HoloTC (20, 70, 109 pmol/L) were tested for various concentrations of the interfering substances. All these tested samples were compared to those obtained from control samples containing no potential interfering substances. The sponsor's definition of non-significant interference is $\leq 10\%$ difference between the tested and the control samples. The percent difference between the control sample and the sample spiked with the potential interfering substances was no greater than $\pm 10\%$ for concentrations at or below those listed in the following table.

Potential interfering substance	Concentration at which no significant interference ($\leq 10\%$) was observed
Bilirubin	≤ 20 mg/dL
Hemoglobin	≤ 200 mg/dL
Triglyceride	≤ 850 mg/dL
Total protein	≤ 10 g/dL
Rheumatoid Factor	≤ 70 IU/mL

In the package insert the manufacturer has stated the following limitations:

“Do not use hemolyzed samples. Hemolyzed samples will cause erroneous results”

“Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Specimens containing HAMA may produce anomalous values when tested with assay kits such as ARCHITECT Active-B12 (Holo transcobalamin) that employ mouse monoclonal antibodies.”

“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 results may be observed. Additional information may be required for diagnosis.”

A cross reactivity study was performed to evaluate the potential cross-reactants,

apotranscobalamin and haptocorrin. Apotranscobalamin was spiked into each of the two serum samples listed in the table below. Haptocorrin was spiked into each of the three serum samples listed in the table below. The samples were assayed and results are summarized in the following table:

Cross-Reactant	Sample	Cross-Reactant Concentration (pmol/L)	Mean Spiked Conc. (pmol/L)	Mean Unspiked Conc. (pmol/L)	% Cross-Reactivity
Apotranscobalamin	1	250	6.2	6.3	-0.9
		500	6.3	6.1	2.0
	2	250	28.8	27.1	6.1
		500	28.5	29.8	-4.5
Haptocorrin	1	2500	6.5	6.5	0.2
		5000	6.7	6.4	3.6
	2	2500	21.2	21.8	-2.9
		5000	22.6	21.9	3.2
	3	2500	95.9	96.1	-0.2
		5000	100.9	94.7	6.5

A high dose hook effect was performed and showed no hook effect when HoloTC up to 1757 pmol/L was tested.

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. *Method comparison with predicate device:*

Method comparison studies were performed according to the CLSI EP9-A2 guideline. 125 serum samples with HoloTC ranging from 8.1 to 124.4 pmol/L were analyzed in singlet on both the candidate device and the predicate device. 20 samples were diluted to cover the low end measuring range. Results of the method comparison study yielded the following linear regression correlation:

$$Y = 0.97X - 0.36, r^2 = 0.94. (X = \text{predicate method}, Y = \text{candidate method})$$

Confidence interval for slope is (0.92 to 1.03), CI for intercept is (-3.20 to 2.02).

b. Matrix comparison:

Not applicable

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):

See 2.a. above

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

The sponsor conducted an expected range study using 181 apparently healthy subjects. A total of 83 men and 98 women age between 18 to 65 years old with no known diseases were evaluated. The results showed that the central 95% range is 25.1 to 165.0 pmol/L. It is recommended that each laboratory establish its own reference range, which may be unique to the population it serves depending upon geographical, patient, dietary, or 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.