510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K233541

B Applicant

Axis-Shield Diagnostics, Ltd

C Proprietary and Established Names

ARCHITECT Active-B12 (Holotranscobalamin)

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
CDD	Class II	21 CFR 862.1810 - Vitamin B12 Test System	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

Modified Device

B Measurand:

Holotranscobalamin (Active-B12)

C Type of Test:

Quantitative, chemiluminescent microparticle immunoassay (CMIA)

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

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.

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

D Special Instrument Requirements:
ARCHITECT i2000SR system

IV Device/System Characteristics:

A Device Description:
The ARCHITECT Active-B12 (Holotranscobalamin) device includes the following:

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

Other materials needed to run the assay, but not included in the kit include: ARCHITECT Active-B12 (Holotranscobalamin) calibrator kit and ARCHITECT Active-B12 (Holotranscobalamin) control kit.

B Principle of Operation:
The ARCHITECT Active-B12 (Holotranscobalamin) assay is a two-step immunoassay for the quantitative determination of Holotranscobalamin in human serum. In the first step, 70µL of sample with 40µL buffer and anti-holotranscobalamin coated paramagnetic microparticles are combined. Holotranscobalamin present in the sample binds to the anti-holotranscobalamin coated microparticles. After washing, anti-transcobalamin 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 instrument.

V Substantial Equivalence Information:

A Predicate Device Name(s):
ARCHITECT Active B-12 (Holotranscobalamin)

B Predicate 510(k) Number(s):
K112443

C Comparison with Predicate(s):
ARCHITECT Active B-12 (Holotranscobalamin)

Device & Predicate Device(s):	K233541	K112443
Device Trade Name	Architect Active-B12 (Holotranscobalamin)	Same
General Device Characteristic Similarities		
Intended Use/Indications For Use	Quantitative determination of Holotranscobalamin in human serum. Active-B12 (Holotranscobalamin) is used as an aid in the diagnosis and treatment of vitamin B12 deficiency	Same
Assay Type	Chemiluminescent microparticle immunoassay (CMIA)	Same
Results	Quantitative	Same
Sample Type	Serum	Same
Analytical Measuring Range	5.0-128.0 pmol/L	Same
General Device Characteristic Differences		
Sample volume	70µL with 40µL buffer chase	110µL
Reagent Composition	Formulation changes to the Microparticles and Conjugate reagents to accommodate elimination of Triton X-405	Previous formulations of reagents included Triton X-405
Interference	≤10% bias in the presence of the following substances; Bilirubin (unconjugated): 40 mg/dL Bilirubin (conjugated): 40 mg/dL Hemoglobin: 1000 mg/dL Total Protein: 15 g/dL Biotin: 4250 mg/dL	≤10% bias in the presence of the following substances; Bilirubin: 20 mg/dL Hemoglobin: 200 mg/dL Total Protein: 10 g/dL Biotin: No biotin interference data
Precision	Total %CV ≤ 6.2 % Within-run %CV ≤ 5.8 %	Total %CV ≤ 5.8 % Within-run %CV ≤ 4.4 %

VI Standards/Guidance Documents Referenced:

CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition

CLSI EP06 Evaluation of the Linearity of Quantitative Measurement Procedures – Approved Guideline – Second Edition

CLSI EP07 Interference Testing in Clinical Chemistry; Approved Guideline – Third Edition

CLSI EP28-A3c Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory – Approved Guideline – Third Edition

CLSI document EP17-A2:2012, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures – Approved Guideline – Second Edition

CLSI document EP09c, Measurement Procedure Comparison and Bias Estimation Using Patient Samples – Partial Recognition – Third Edition

CLSI EP35 Assessment of Equivalence or Suitability of Specimen Types for Medical Laboratory Measurement Procedures – Approved Guideline – First Edition

CLSI EP37 Supplemental Tables for Interference Testing in Clinical Chemistry – Approved Guideline – First Edition

CLSI EP39 A Hierarchical Approach to Selecting Surrogate Samples for the Evaluation of In Vitro Medical Laboratory Tests – Approved Guideline – First Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

A study was performed according to CLSI EP05-A3. Testing was conducted using 1 lot of ARCHITECT Active-B12 (Holotranscobalamin), Calibrators, and Controls and 2 instruments. Two levels of controls and 7 pooled native human serum samples spanning the analytical measuring interval were assayed in 2 replicates per run, 2 runs per day, for 20 different days. The data are summarized in the following table.

Sample	n	Mean	Within-Run (Repeatability)		Between-Run		Between-Day		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 1	80	19.62	0.49	2.50	0.16	0.80	0.34	1.70	0.62	3.10
Sample 2	80	40.04	0.86	2.20	0.00	0.00	0.86	2.10	1.22	3.00
Sample 3	80	56.03	1.21	2.20	0.00	0.00	0.96	1.70	1.54	2.70

Sample	n	Mean	Within-Run (Repeatability)		Between-Run		Between-Day		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 4	80	72.63	1.49	2.10	0.70	1.00	1.02	1.40	1.94	2.70
Sample 5	80	87.88	1.45	1.70	1.30	1.50	1.01	1.20	2.20	2.50
Sample 6	80	94.74	2.35	2.50	0.92	1.00	4.00	4.20	4.74	4.97
Sample 7	80	117.81	2.78	2.40	0.00	0.00	5.15	4.40	5.85	5.00
Low Control	80	16.61	1.37	8.30	0.20	1.20	0.57	3.40	1.50	9.03
High Control	80	48.95	2.85	5.80	0.00	0.00	1.04	2.10	3.04	6.20

2. Linearity:

A study was performed based on CLSI EP06-Ed2. One high holotranscobalamin native serum sample was diluted using calf serum across 11 dilution levels from 2.26 to 134.17 pmol/L. All dilutions were assayed in replicates of five (5). Linearity was evaluated using linear regression analysis. The deviation from linearity did not exceed 10% for samples across the range of LoQ to 128.0 pmol/L. The results of the linearity study support the claimed analytical measuring range of 5.0-128.0 pmol/L.

Dilution Verification

A sample dilution study was conducted to support the claim that samples with holotranscobalamin (HoloTC) concentrations >128 pmol/L can be diluted 1:2, either manually or automatically by the device. The dilution study results support the sponsor's labeling claims that samples with HoloTC concentrations above 128 pmol/L may be diluted following the instructions for use.

3. Analytical Specificity/Interference:

Interference studies were performed according to CLSI EP07-ED3. Serum samples were spiked into a minimum of two samples with HoloTC concentrations ranging from 20 to 45 pmol/L and 70 to 90 pmol/L. The samples were assayed, and the HoloTC concentrations of the spiked samples were compared to control samples. No significant interference was defined as less than 10% difference between the mean of control and spiked samples.

The substances and the highest concentration tested which did not cause significant interference are listed below.

Potential Interfering Substance	Highest concentration tested at which no significant interference is observed
Bilirubin (conjugated)	40 mg/dL
Bilirubin (unconjugated)	40 mg/dL
Biotin	4250 ng/mL
Hemoglobin	1000 mg/dL
Total Protein	15 g/dL
Triglycerides	850 mg/dL
Rheumatoid Factor	70 IU/mL

Cross-Reactivity Study:
A cross reactivity study was performed to evaluate the potential cross-reactants, apotranscobalamin and haptocorrin. Apotranscobalamin and haptocorrin were spiked into control samples at approximately 500 pmol/L and 5000pmol/L, respectively. The samples were assayed and the resulting % cross-reactivity was calculated using the following formula: % cross reactivity = [(mean concentration of spiked samples in pmol/L) – (mean concentration of control samples in pmol/L) / (concentration of cross reactant in pmol/L)] * 100%. The data are summarized in the following table.

Cross Reactant	Sample	Cross-Reactant Concentration (pmol/L)	Mean Spiked Concentration (pmol/L)	Mean Unspiked Concentration (pmol/L)	% Cross Reactivity
Apotranscobalamin	1	500	29.51	30.40	0.18
	2	500	74.61	74.49	0.02
	3	500	87.57	84.02	0.71
Haptocorrin	1	5000	29.53	29.20	0.01
	2	5000	73.84	72.78	0.02
	3	5000	87.72	87.65	0.00

The sponsor has the following statements in their labeling:

"Do not use hemolyzed samples. Hemolyzed samples will cause erroneous results."

"Samples containing concentrations of triglycerides at > 850 mg/dL, total protein at > 15 g/dL, and/or rheumatoid factor at > 70 IU/mL may interfere with ARCHITECT Active-B12 (Holotranscobalamin) assay 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 (Holotranscobalamin) 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 values may be observed. Additional information may be required for diagnosis."

4. Assay Reportable Range:

5.0-128.0 pmol/L.

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

The ARCHITECT Active-B12 (Holotranscobalamin) assay is traceable to an internal reference material which is traceable to another commercially available assay.

6. Detection Limit:

The Limit of Blank, Limit of Detection, and Limit of Quantitation (LoB, LoD and LoQ) for the ARCHITECT Active-B12 (Holotranscobalamin) assay were evaluated following guidance in the CLSI EP17-A2. The LoQ was defined as the lowest concentration of analyte which has within-laboratory imprecision less than or equal to 10%. Results are summarized below.

LoB (pmol/L)	LoD (pmol/L)	LoQ (pmol/L)
< 0.1	0.2	0.7

7. Assay Cut-Off:
Not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:
A comparison study between the candidate ARCHITECT Active-B12 (Holotranscobalamin) assay and the predicate ARCHITECT Active-B12 (Holotranscobalamin) assay was conducted according to the CLSI EP09C-ED3 guideline.

A total of 108 samples with HoloTC ranging from 5.4 to 128.0 pmol/L were collected and evaluated: 105 samples were individual native serum samples and 3 samples were diluted using calf serum to cover the low end of the measuring range. The data were analyzed using Passing-Bablok regression and the comparison of the candidate ARCHITECT Active-B12 (Holotranscobalamin) assay (y) and the predicate ARCHITECT Active-B12 (Holotranscobalamin) assay (x), is summarized below:

N	**Slope (95% CI)**	**Intercept (95% CI)**	**Correlation Coefficient**	**Sample Range Tested (pmol/L)***
108	0.9 (0.85, 0.94)	-0.50 (-3.44, 2.58)	0.95 (0.92, 0.96)	5.4 – 128.0

*As measured by the predicate.

2. Matrix Comparison:
A matrix comparison study was conducted to support the use of the ARCHITECT Active-B12 assay with different sample matrices. Specifically, the study compared values obtained from samples drawn into serum tubes with gel (SST) and serum tubes without gel (Serum clot). Paired SST and Serum clot samples, with HoloTC concentrations ranging from 7.3 to 119.5 pmol/L, were collected and tested. The following results were determined using Passing-Bablok regression analysis: $y=1.025X-0.819$, $R^2=0.985$.

The results support the sponsor's claims that the assay is suitable for use with serum tubes with gel (SST) and serum tubes without gel (Serum clot).

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:

Reference Range
A study was performed to verify the transference of the Reference Range established on the predicate ARCHITECT Active-B12 assay (reported as 25.1 – 165.0 pmol/L) to the candidate ARCHITECT Active-B12 assay. The study was performed using serum samples from 40 apparently normal human serum donors. The data from the study supports the transference validity of the predicate device reference range to the candidate device.

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.