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

A. 510(k) Number:

k160757

B. Purpose for Submission:

New device

C. Measurand:

Holotranscobalamin (Active-B12)

D. Type of Test:

Quantitative, chemiluminescent immunoassay

E. Applicant:

Axis-Shield Diagnostics Ltd.

F. Proprietary and Established Names:

ADVIA Centaur Active-B12 (Holotranscobalamin) (AB12) assay
ADVIA Centaur Active-B12 (AB12) Master Curve Material (MCM)
ADVIA Centaur Active-B12 (AB12) Quality Control

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.1810, Vitamin B12 test system
   21 CFR 862.1660, Quality Control Material

2. Classification:

   Class II
   Class I, reserved

3. Product code:

   CDD, JJX

4. Panel:

   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   Refer to Indications for Use below

2. Indication(s) for use:

   The ADVIA Centaur® Active-B12 (Holotranscobalamin) (AB12) assay is for in vitro diagnostic use in the quantitative measurement of holotranscobalamin (holoTC) in human serum using the ADVIA Centaur XP system. Active-B12 (holotranscobalamin) is used as an aid in the diagnosis and treatment of vitamin B12 deficiency.

   Quality Control
   The ADVIA Centaur® Active-B12 (AB12) quality control is for in vitro diagnostic use to monitor the precision and accuracy of the ADVIA Centaur AB12 (Holotranscobalamin) assay using the ADVIA Centaur systems.

   Master Curve Material (MCM)
   The ADVIA Centaur® Active-B12 (AB12) Master Curve Material (MCM) is for in vitro diagnostic use in the verification of calibration and reportable range of the ADVIA Centaur AB12 (Holotranscobalamin) assay using the ADVIA Centaur systems.

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   For use on the ADVIA Centaur XP system only.

I. Device Description:

The following components are included with the assay:

ADVIA Centaur AB12 ReadyPack® primary reagent pack; Lite Reagent	5.0 ml per reagent pack murine anti-transcobalamin (TC) monoclonal antibody 3-11 (0.5 μg/mL) labeled with acridinium ester in buffer with surfactant and preservatives
ADVIA Centaur AB12 ReadyPack primary reagent pack; Solid Phase Reagent	15.0 ml per reagent pack streptavidin coated paramagnetic microparticles preformed with biotinylated murine anti-holoTC monoclonal antibody 3C4 (~0.4 mg/ml) in buffer with surfactant
ADVIA Centaur Wash 1	1500 or 2500 mL per pack phosphate-buffered saline with sodium azide (< 0.1%) and surfactant
ADVIA Centaur ReadyPack ancillary reagent pack; Multi-Diluent 13	10 ml per pack buffer with surfactant and sodium azide (< 0.1%)
ADVIA Centaur Active B-12 Calibrators	One vial (4 mL) AB12 ADVIA Centaur low calibrator with target value of 19 pmol/L and one vial AB12 ADVIA Centaur high calibrator with target value of 121 pmol/L. Calibrators are 2 mL/vial and contain Recombinant Holotranscobalamin, bovine serum albumin, and sodium azide (<0.1%)

The following are required and provided separately:

ADVIA Centaur Active B-12 Controls: Each kit contains two vials (Control 1 and Control 2, 7 mL each) containing Holotranscobalamin in bovine serum albumin, buffer, and sodium azide at target concentrations of 15.60 and 60.40 pmol/L.

ADVIA Centaur Active B-12 Master curve materials: Each kit contains five vials (MCM 1 – 5, 7 mL each) of Holotranscobalamin in bovine serum albumin, buffers, and sodium azide at target concentrations of 0, 19.00, 44.00, 95.11, and 133.00 pmol/L.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   ARCHITECT Active-B12 (Holotranscobalamin), including quality control
   SIEMENS ADVIA Centaur Vitamin B12 Master Curve Material

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

   k112443
   k140505

3. Comparison with predicate:

a. Comparison of the Active B12 test/assay:

Similarities (Assay)		
Item	**Candidate Device ADVIA Centaur Active-B12**	**Predicate Device ARCHITECT Active-B12 k112443**
Intended use	Same	For the quantitative determination of Holotranscobalamin in human serum
Assay Technology	Same	Chemiluminescent microparticle immunoassay (CMIA)
Substrate / Signal Generation	Same	Acridinium Tracer
Specimen type	Same	Serum and Serum Separator
Conjugate antibody	Same	Murine anti-transcobalamin (TC) monoclonal antibody 3-11
Storage conditions	Same	Reagent Pack must be stored at 2-8ºC.
Unit of Measure	Same	pmol/L
Cross- Reactivity	Same	≤ 10% with Apotranscobalamin (500 pmol/L) and Haptocorrin (5000 pmol/L)
Assay dilution protocol	1:2 autodilute with ADVIA Centaur Multi-Diluent 13	1:2 autodilute or manual with ARCHITECT *i* Multi-Assay manual diluent
Detection Limit	Same	Limit of Quantitation of ≤ 5.0 pmol/L

Differences (Assay)		
Item	**Candidate Device ADVIA Centaur Active-B12**	**Predicate Device ARCHITECT Active-B12 k112443**
Calibration	2-point Calibration using 2 level calibrators	6-point calibration curve. 4PLC Y-weighted
Expected Values	The mean Holotranscobalamin concentration for the group was established at 81.91 pmol/L with a 95% central reference interval from 28.96 to 168.90 pmol/L	The mean Holotranscobalamin concentration was 71.9 pmol/L with a range from 20.6 to 196.7 pmol/L. The central 95% of the population defined the expected range of 25.1 to 165.0 pmol/L
Interference	≤ 10% with; Bilirubin at 40 mg/dL Unconjugated bilirubin at 60 mg/dL Haemoglobin at 500 mg/dL Triglyceride at 1000 mg/dL Rheumatoid Factor at 200 IU/mL Total protein at 12 g/dL Biotin at 100 mg/dL Human IgG at 12 g/dL Cholesterol at 500 mg/dL Silwet L720 at 0.2 mg/dL Methotrexate at 91 mg/dL Perimethamine at 75 µg/mL	≤ 10% with; Bilirubin at 20 mg/dL Haemoglobin at 200 mg/dL Triglycerides at 850 mg/dL Rheumatoid Factor at 70 IU/mL Total protein at 10 g/dL
Cross- Reactivity	No detectable cross-reactivity with; Apotranscobalamin at 500 pmol/L or Haptocorrin at 5000 pmol/L	No detectable carryover with; Apotranscobalamin at 500 pmol/L or Haptocorrin at 5000 pmol/L
Measuring Range	5.0 to 146.0 pmol/L	5.0 to 128.0 pmol/L
Calibration Range	0 to 146.0 pmol/L	0 to 128.0 pmol/L
On-board Reagent Stability	Reagents can be stored onboard the Centaur XP instrument for a maximum of 44 days.	Reagents can be stored onboard the ARCHITECT instrument for a maximum of 30 days.
Calibration Frequency	44 days	30 days

Differences (Assay)		
Item	**Candidate Device ADVIA Centaur Active-B12**	**Predicate Device ARCHITECT Active-B12 k112443**
Sample Stability	Separated specimens are stable for 16 hours at room temperature, 3 days at 2-8ºC For longer storage, specimens may be frozen for 3 months at -20 ºC or colder. Avoid more than one freeze/thaw cycle. Do not store in a frost-free freezer	Up to 16 hours at room temperature Up to 3 days at 2-8ºC (for longer than 3 days store at -20ºC or colder for up to 6 months). Avoid more than three freeze-thaw cycles.

b. Comparison of the Active B12 quality control

Similarities - quality control		
Item	**Candidate Device ADVIA Centaur Active-B12 Quality Control**	**Predicate Device ARCHITECT Active-B12 (Holotranscobalamin) Controls (Low and High) k112443**
Intended Use	Same	For quality control monitoring of the Active-B12 assay when used for the quantitative determination of holotranscobalamin (HoloTC)
Format	Same	Liquid, ready-to-use
Levels	Same	Two

Differences – quality control		
Item	**Candidate Device ADVIA Centaur Active-B12 Quality Control**	**Predicate Device ARCHITECT Active B12 (Holotranscobalamin) Controls (Low and**
Stability	Shelf-life of 56 weeks	Shelf-life of 52 weeks

c. Comparison of the Active B12 Master Curve Materials (MCM):

Similarities - MCM		
Item	**Candidate Device ADVIA Centaur®Active-B12 Master Curve Materials**	**Predicate Device ADVIA Centaur Vitamin B12 Master Curve Material k140505**
Intended Use	Intended for in vitro diagnostic use in the verification of calibration and reportable range	Intended for in vitro diagnostic use in the verification of calibration and reportable range of the ADVIA Centaur Vitamin B12 assay
System Compatibility	Same	Advia Centaur Systems
Intended Storage	Same	2 – 8° C
Stability	Same	Stable unopened until the expiration date

Differences - MCM		
Item	**Candidate Device ADVIA Centaur®Active-B12 Master Curve Materials**	**Predicate Device ADVIA Centaur Vitamin B12 Master Curve Material k140505**
Levels	Five	Six

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

1. CLSI EP5-A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition
2. CLSI EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline
3. CLSI EP7-A2: Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition
4. CLSI EP9-A3: Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Third Edition
5. CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline -- Second Edition
6. CLSI EP25-A: Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline
7. CLSI EP28-A3: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline- Third Edition

L. Test Principle:

The ADVIA Centaur AB12 assay is a fully automated, two-step direct immunoassay using chemiluminescent technology. The assay utilizes an acridinium ester-labeled anti - transcobalamin antibody as the Lite Reagent. The Solid Phase consists of biotinylated anti - holotranscobalamin antibody coupled to streptavidin-coated magnetic latex microparticles. A direct relationship exists between the amount of Active-B12 present in the sample and the amount of signals detected by the system.

M. Performance Characteristics (if/when applicable):

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

   a. Precision/Reproducibility:

   Precision was determined following guidance from CLSI Document EP5-A2. One Centaur XP instrument and two lots of ADVIA Centaur® AB12 reagents were used for the precision study. Pooled serum samples (three levels designated sample 1, sample 4 and sample 5) and internal serum quality controls (QCs, two levels – designated sample 2 and sample 3) were used to assess the precision across the ADVIA Centaur AB12 assay range from approximately 16.82 to 123.10 pmol/L.

   The five samples were assayed for imprecision assessments in replicates of two (each replicate from a different sample cup), twice daily, for 20 non-consecutive days (n=80* observations for each sample per reagent lot). At least 2 hours were left between assay runs.

   *Note: on the second assay on testing day 20 for sample 5 (Lot 1) one sample

replicate was missed in error therefore this day of testing for sample 5 for Lot 1 was excluded from the final analysis (n=78 observations respectively). Results are summarized below:

Reagent Lot	ID #	Mean (pmol/L)	Within-Run		Between-Run		Between-Day		Total	
			SD	CV	SD	CV	SD	CV	SD	CV
1	1	19.71	0.46	2.3%	0.32	1.6%	0.46	2.3%	0.72	3.7%
	2	39.26	1.39	3.6%	0.57	1.5%	1.04	2.6%	1.83	4.7%
	3	74.61	2.18	2.9%	1.52	2.0%	1.56	2.1%	3.08	4.1%
	4	103.81	2.11	2.0%	1.45	1.4%	1.64	1.6%	3.05	2.9%
	5	122.36	4.65	3.8%	2.62	2.1%	1.60	1.3%	5.57	4.6%
2	1	16.82	0.30	1.8%	0.65	3.9%	0.32	1.9%	0.79	4.7%
	2	38.12	1.12	2.9%	1.22	3.2%	0.19	0.5%	1.66	4.4%
	3	74.24	1.64	2.2%	2.23	3.0%	1.31	1.8%	3.06	4.1%
	4	104.76	1.92	1.8%	3.24	3.1%	1.78	1.7%	4.16	4.0%
	5	123.10	3.92	3.2%	4.02	3.3%	0.00	0.0%	5.61	4.6%

b. Linearity/assay reportable range:

The sponsor conducted a linearity study according to the CLSI EP6-A guideline.

A serum sample with a high holotranscobalamin concentration (greater than 146.00 pmol/L) was selected as the high sample was diluted with a compatible zero-analyte serum matrix to eleven different concentrations. A total of 11 concentrations were tested with samples ranging from 0.09 to 162 pmol/L.

Linear regression of the observed vs. expected values produced the following:

Slope (95% CI)	Intercept (95% CI)	r^2
0.98 (0.93 – 1.02)	-1.52 (-5.71 – 2.68)	1.00

Based on the results of the linearity study the sponsor claims that the assay is linear from 5.00 to 146.00 pmol/L.

An additional dilution study was performed to support the sponsor's claim that results >146 pmol/L can be automatically diluted X2 by the analyzer. Ten samples with holotranscobalamin concentrations ranging from approximately 90 to 190 pmol/L were tested by diluting X2 manually and by the instrument. Results obtained manually were compared to the results obtained automatically and % differences

were ≤10% for all the samples tested. The sponsor claims in the labeling that samples >146 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 no internationally recognized Standard preparation or material available for calibration of Active-B12 (Holotranscobalamin). The ADVIA Centaur Active-B12 Master Standards and ADVIA Centaur Active-B12 Calibrators (Calibrators) are expressed in pmol/L and are prepared gravimetrically from commercially available purified human holotranscobalamin. The assay is traceable to the recombinant Holo-Transcobalamin using Spectrophotometric measurement by absorbance at 280 nm.

Value Assignment

ADVIA Centaur Active-B12 Low and High Calibrators, Quality Control Material, and Master Curve Material (MCM) are prepared from a commercially available purified reference material. If the concentration is outside the specified range, then the levels are adjusted accordingly, as per an internal protocol, and re-tested. Below are the target concentrations and target control ranges of the calibrator, control, and master curve material. Target concentrations and target control ranges are lot specific.

ADVIA Centaur Active-B12 Calibrators:

Calibrator	Target Concentration
Low	19
High	121

ADVIA Centaur Active-B12 Quality Control:

Control	Target Concentration (pmol/L)	Concentration Range (pmol/L)
Level 1	15.60	11.70 – 19.50
Level 2	60.40	45.30 – 75.50

ADVIA Centaur Active-B12 Master Curve Material:

MCM	Target Concentration (pmol/L)	Concentration Range (pmol/L)
MCM 1	0	<5.00
MCM 2	19.00	14.25 – 23.75
MCM 3	44.00	33.00 – 55.00
MCM 4	95.00	71.25 – 118.75
MCM 5	133.00	99.75 – 166.25

Stability

The protocols and acceptance criteria for the stability studies were reviewed and found to be acceptable. Real-time stability studies were performed to support the following storage conditions/claims:

ADVIA Centaur Active-B12 Low and high calibrators, Master Curve Material (MCM), and Quality Controls:
Shelf life when stored at 2 – 8° C: 52 weeks
Opened vial when stored at 2 – 8° C: 14 days

d. Detection limit:

The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) was determined following guidance from CLSI document EP17-A2. The LoB is defined as the highest measurement result that is likely to be observed for a blank sample. The LoD is defined as the lowest concentration of Active B12 that can be detected with 95% probability. The LoQ is defined as the lowest concentration of Active B12 that can be detected at a total CV of 8%.

In order to determine the LoB, LoD and LoQ serum samples were used and low concentrations were obtained by diluting with a compatible zero-analyte serum matrix.

- For LoB determinations, five serum samples were spiked into a zero-analyte serum matrix to give Holotranscobalamin concentrations of <1.20 pmol/L. Sixty replicates of each sample were run on each of two reagent lots for a total of 120 measurements.
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- For LoD determinations, five serum samples were spiked into a zero-analyte serum matrix give Holotranscobalamin concentrations of approximately 2.00 pmol/L. Sixty replicates of each sample were run on each of two reagent lots for a total of 120 measurements.

- For LoQ determinations, five serum samples were diluted with a zero-analyte serum matrix to give Holotranscobalamin concentrations of <6.00 pmol/L. Forty replicates of each sample were run on each of two reagent lots for a total of eighty measurements.

As a result of the studies, the sponsor concluded that the ADVIA Centaur Active-B12 assay has an LoB of 0.74 pmol/L, and LoD of 1.08 pmol/L and an LoQ of 5.00 pmol/L.

The ADVIA Centaur Active-B12 assay has a measuring range from 5.00 to 146.00 pmol/L.

e. Analytical specificity:

Interference

An interference study was performed according to the CLSI guideline EP7-A2. Serum samples with low and high Holotranscobalamin concentrations (30-40 and 60-80 pmol/L) were spiked with potentially interfering substances at high concentrations at or exceeding those recommended by EP7-A2. The sponsor's definition of non-significant interference is ≤ 10% difference between the test and control samples. The sponsor evaluated the potential interferents below and claims that the effect on assay results is insignificant at the concentrations listed:

Endogenous and exogenous potential interferents	Highest concentration at which no significant interference (≤10%) was observed
*Biotin	100 mg/dL
Cholesterol	500 mg/dL
Conjugated Bilirubin	40 mg/dL
Hemoglobin	500 mg/dL
*Human IgG	12 g/dL
Methotrexate	91 mg/dL
*Perimethamine	75 µg/mL
*Rheumatoid Factor	200 IU/mL
*Silwet L720	0.2 mg/dL
Total Protein	12 g/dL
Unconjugated Bilirubin	60 mg/dL
Triglyceride	1000 mg/dL

*CLSI EP7-A2 does not specify a concentration to evaluate for these substances. Concentrations to test for this evaluation were drawn from other sources.

Cross-reactivity

Cross-reactivity with other B12 binding proteins, apotranscobalamin and haptocorrin was tested in the presence and absence of Active-B12 according to CLSI EP7-A2 using the ADVIA Centaur Active-B12 assay.

Percent cross-reactivity is calculated as:

% cross-reactivity = (concentration of spiked sample - concentration of unspiked sample) x 100 / concentration of cross-reactant

The following results were obtained:

Cross-reactant	Concentration (pmol/L)	% Cross-reactivity
Apotranscobalamin	250	0.2
	500	-0.1
Haptocorrin	2500	-0.4
	5000	-0.4

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

- The ADVIA Centaur Active-B12 assay is limited to the detection of active-B12 in human serum
- The performance of the ADVIA Centaur Active-B12 assay has not been established with cord blood, neonatal specimens, cadaver specimens, heat-inactivated specimens, or body fluids other than serum, such as saliva, urine, amniotic, or pleural fluids.
- The performance of the assay has not been established for populations of immunocompromised or immunosuppressed patients.
- Heterophilic antibodies in human serum can react with the immunoglobulins included in the assay components causing interference with in vitro immunoassays. Samples from patients routinely exposed to animals or animal serum products can demonstrate this type of interference, which can potentially cause an anomalous result. Additional information may be required for diagnosis.
- Potential interferences from monoclonal gammopathies were not investigated.

Hook Effect:

The potential for a high-dose hook effect was evaluated. Patient samples having concentrations up to 1867.80 pmol/L do not demonstrate a hook effect and will be reported as >146.00 pmol/L.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

One hundred and four (104) human serum samples were measured in singlicate on the ARCHITECT analyzer using the predicate device (X) and on the ADVIA Centaur XP analyzer using the candidate device (Y). Ten of the 104 samples were diluted with a compatible serum matrix in order to achieve concentrations at the lower end of the measuring range. No spiked samples were used for this study. Sample concentrations ranged from 5.56 to 142.58 pmol/L were used for the data analysis.

The following Passing Bablok regression statistics with 95% Confidence Intervals were calculated as follows

Slope (95% CI)	Intercept (95% CI)	Correlation coefficient Pearson (r)
0.97 (0.92 – 1.04)	-0.99 (-3.23 – 0.84)	0.95 (0.93 – 0.97)

NOTE: Holotranscobalamin can be reported in units of pg/mL or pmol/L. In the US, the pg/mL unit is used. The sponsor has included both units of Holotranscobalamin concentration in their labeling. The following formula may be used to convert between the units:

Holotranscobalamin (pmol/L) = Holotranscobalamin (pg/mL) ×43

b. Matrix comparison:

Not applicable. Serum is the only acceptable matrix for the assay, and samples may be collected into a plain plastic serum tube or serum separator tube.

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:

   The ADVIA Centaur AB12 assay results were obtained on 241 apparently healthy males (n = 103) and females (n = 138). The age range was 21 - 67 years. The mean holoTC concentration for the group was established at 81.91 pmol/L with a 95% central reference interval from 28.96 – 168.90 pmol/L according to the CLSI EP28-A3c guideline

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.