

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

A. 510(k) Number:

k030655

B. Analyte:

Vitamin B12

C. Type of Test:

RADIOASSAY, VITAMIN B12

D. Applicant:

AXIS-SHIELD BIOCHEMICALS, ASA

E. Proprietary and Established Names:

HOLO TC RIA

F. Regulatory Information:

1. Regulation section:
21CFR§ - 862.1810 Vitamin B12 test system.
21CFR§ - 862.1150 Calibrator.
21CFR§ - 862.1660 Quality control material (assayed and unassayed).
2. Classification:
II, II, I (reserved)
3. Product Code:
CDD, JIS, JJX
4. Panel:
Chemistry (75)

G. Intended Use:

1. Indication(s) for use:
The Axis-Shield HoloTC RIA is an in-vitro diagnostic assay for quantitative measurement of the fraction of cobalamin (vitamin B12) bound to the carrier protein transcobalamin in human serum or plasma. Measurements obtained by this device are used in the diagnosis and treatment of vitamin B12 deficiency.
2. Special condition for use statement(s):
Not Applicable
3. Special instrument Requirements:
Not Applicable

H. Device Description:

The Axis-Shield Holo TC RIA is a competitive binding immunoassay in which a specific monoclonal antibody is used to capture transcobalamin from the patient sample. Thereafter the procedure is as commonly used in vitamin B12 assays. The cobalamin (vitamin B12) is released from the transcobalamin using dithiothreitol and sodium hydroxide. The released cobalamin (vitamin B12) then competes for a limited amount of intrinsic factor with added ⁵⁷Co labeled vitamin B12. The Axis-Shield HoloTC RIA differs from the predicate device in two main aspects:

- 1) The use of a transcobalamin specific antibody, this allows the quantitation of only the cobalamin bound to the protein transcobalamin as opposed to measurement of cobalamin bound to all proteins in the predicate device and
- 2) The detection signal is radioactivity (^{57}Co) as opposed to chemiluminescence in the predicate device.

I. Substantial Equivalence Information:

1. Predicate device name(s):
BAYER DIAGNOSTICS ACS:180 VB12, BAYER DIAGNOSTICS ADVIA CENTA
2. Predicate K number(s):
K993571
3. Comparison with predicate:

DEVICE	PREDICATE
A. Similarities	
<ul style="list-style-type: none"> • Used in the diagnosis and treatment of vitamin B12 deficiency. 	<ul style="list-style-type: none"> • Used in the diagnosis and treatment of vitamin B12 deficiency.
B. Differences	
<ul style="list-style-type: none"> • Holo-TC (vitamin B12 bound to transcobalamin) • Manual RIA 	<ul style="list-style-type: none"> • B12 (total serum cobalamin) • Automated immuno assay

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

NCCLS EP5-T2

K. Test Principle:

The Axis-Shield HoloTC RIA is a competitive binding radioimmunoassay. The patient sample is incubated with magnetic particles coated with monoclonal anti-human TC antibodies (Capturing Reagent). Following separation of the bound TC a reducing reagent (Reductant) is added, as well as a denaturing reagent (Extractant) and ^{57}Co labeled vitamin B12 (Tracer). The vitamin B12 released from TC and Tracer compete for the Binder (Intrinsic Factor, IF). Unbound Tracer is then removed by centrifugation and the measured radioactivity bound is inversely proportional to the amount of vitamin B12 bound to TC in the sample. The concentration of vitamin B12 bound to TC is interpolated from a dose response curve constructed using recombinant HoloTC Calibrators.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:
 - a. *Precision/Reproducibility:*
Precision studies were done according to NCCLS - Evaluation of Precision Performance of Clinical Devices EP5-T2. Precision of the system was demonstrated by using three levels of serum samples, low, medium and high (14, 67 and 139 pmol/L HoloTC, respectively). Within-run CV for duplicate measurements of serum low, medium and high were 11%, 5% and 8%, respectively and total precision was 11%, 6% and 8%, respectively.

- b. *Linearity/assay reportable range:*
Dilution Linearity within the working range of 10-160 pmol/L was demonstrated to meet all criteria for linearity. Recovery test of HoloTC spiked serum samples was demonstrated to be within acceptance criteria. Dilution of high HoloTC sample into six dilutions showed a correlation coefficient $r^2 = 0.998$, slope = 1.01 ± 0.02 and y-intercept = -5 ± 2 pmol/L for dilutions ranging between 13 - 182 pmol/L HoloTC.
- c. *Traceability (controls, calibrators, or method):*
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; The requirement is that the ratio $A_{280}/A_{362} = 2.3 \pm 0.1$ which shows >97% purity (Quadros et al, J Biol Chem, 1986; 261:15455-60). The HoloTC stock solution concentration is determined (i) from the absorbance at 362 nm, using an extinction coefficient of 30,000 M⁻¹ cm⁻¹ (Fedosov et al, J Biol Chem, 1999; 274 :26015-20) and (ii) by determination in a vitamin B12 assay (the stock solution has a high enough concentration to be measured). HoloTC Controls are prepared from human serum and assigned values with reference to the HoloTC calibrators.
- d. *Detection limit:*
Limit of Quantification defined as the concentration range over which the CV of the assay remained $\leq 15\%$ is 8-160 pmol/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	Concentration
Bilirubin	40 mg/dl
Haptocorrin	70,000 pg/mL
Haemoglobin	1,000 mg/mL
Triglycerides	1,500 mg/dL
Total serum protein	8,000 mg/dL

- f. *Assay cut-off:*

Based on a Finnish population of normal individuals (n=303, age 22-88 years) the 95% central reference interval was found to be 37-171 pmol/L.

Analysis of covariance demonstrated that HoloTC levels depended on gender (but not age) in this reference population. The 90 % confidence intervals for the lower limit of the reference range are for the whole population, males and females 36-37 pmol/L, 37-39 pmol/L and 35-36 pmol/L, respectively.

2. Comparison studies:a. *Method comparison with predicate device:*

As expected Holotranscobalamin (B12 bound to transcobalamin) to cobalamin (B12) shows poor correlation.

The Axis-Shield HoloTC RIA was compared to the Bayer Advia Centaur VB12 assay (K993571) using 392 patient samples with vitamin B12 concentrations ranging from 114-821 pmol/L.

Linear regression (least squares) yielded the following statistics:

HoloTC pmol/L = 0.55 VB12-2 pmol/L

$r^2 = 0.52$

See clinical findings below

b. *Matrix comparison:*

Serum/EDTA Plasma comparison 50 paired serum and EDTA plasma samples collected from the same donors were analyzed and compared. No significant difference in measured HoloTC level was demonstrated between serum sample and EDTA sample. In-use stability of the reagent and calibrator kit showed stability up to three months after opening.

3. Clinical studies:

Estimated Concordance

Using a decision threshold for holoTC of ≤ 37 pmol/L indicating deficiency the estimated concordance and relative sensitivity and specificity of HoloTC was calculated using samples from 3 study sites that were classified as being likely and not likely vitamin B12 deficient in the following way.

Likely Deficient	B12	Vitamin B12 level below lower reference limit of normal, MMA > 0.7 μ mol/L	n=112
Not Likely Deficient	B12	Vitamin B12 level above lower reference limit of normal, MMA below lower reference limit of abnormal.	n=313

The limitation of this approach is that a significant number of samples remain unclassified; this reflects the absence of an accepted diagnostic algorithm using laboratory tests for diagnosing vitamin B12 deficiency.

A general parametric approach was used to combine the results of all 3 studies an approach using: *Whitehead A and Whitehead J. A general parametric approach to the meta-analysis of randomized clinical trials. Stat Med 1991;10:1665-77.*

The estimated concordance, sensitivity, and specificity for each study was weighted and then all were combined and presented in the below table.

Estimated Concordance, relative Sensitivity and Specificity for HoloTC across 3 studies.

	Estimate (%)	95% C.I. (%)	Width 95% C.I. (%)
Concordance	80	76.3-83.7	7.4
Relative Sensitivity	99.5	97.3-100	2.7
Relative Specificity	76.3	71.8-80.8	9.0

These results may indicate a decreased specificity of HoloTC for vitamin B12 deficiency or may be indicative of increased sensitivity, as might be expected from HoloTC levels being the earliest reflection of changes in vitamin B12 status.

a. Clinical sensitivity:

99.5 %

b. Clinical specificity:

76.3%

4. Clinical cut-off:
HoloTC of ≤ 37 pmol/L indicating deficiency
5. Expected values/Reference range:

M. Conclusion:

The information and data provided by AXIS-SHIELD BIOCHEMICALS, ASA supports a Substantial Equivalence (SE) determination to the predicate device and other assays regulated under 21 CFR - 862.1810, 862.1150, and 862.1660.