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

A. 510(k) Number:

k110579

B. Purpose for Submission:

New device

C. Measurand:

Vitamin B12

D. Type of Test:

Quantitative chemiluminescent microparticle immunoassay

E. Applicant:

Abbott Laboratories

F. Proprietary and Established Names:

ARCHITECT B12 Reagent Kit, ARCHITECT B12 Controls, ARCHITECT B12
Calibrators

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
CDD	II	21 CFR 862.1810, Vitamin B12 Test System	Chemistry (75)
JIT	II	21 CFR 862.1150, Calibrator	Chemistry (75)
JJX	I, reserved	21 CFR 862.1660, Quality Control Material	Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See Indications for use below.

2. Indication(s) for use:

   The ARCHITECT B12 assay is a chemiluminescent microparticle Intrinsic Factor
   assay for the quantitative determination of vitamin B12 in human serum on the
   ARCHITECT *i* System. Measurements obtained by this device are used in the
   diagnosis and treatment of anemias of gastrointestinal malabsorption.

The ARCHITECT B12 Controls are used for the estimation of test precision and the detection of systematic analytical deviations of the ARCHITECT *i* System when used for the quantitative determination of vitamin B12 in human serum when using the ARCHITECT B12 Reagent Kit.

The ARCHITECT B12 Calibrators are used to calibrate the ARCHITECT *i* System when the system is used for the quantitative determination of vitamin B12 in human serum using the ARCHITECT B12 Reagent Kit.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

ARCHITECT *i* 2000$_{SR}$ instrument

I. Device Description:

ARCHITECT B12 Reagents:

- 1 Bottle (6.6 mL per 100-test bottle / 27.0 mL per 500-test bottle) Intrinsic Factor (porcine) coated Microparticles in borate buffer with protein (bovine) stabilizers.

- 1 Bottle (5.9 mL per 100 test bottle / 26.3 mL per 500 test bottle) B12 acridinium-labeled Conjugate in MES buffer.

- 1 Bottle (10.0 mL per 100 test bottle / 51.0 mL per 500 test bottle) B12 Assay Diluent containing borate buffer with EDTA.

- 1 Bottle (27.0 mL per 100 test bottle / 50.4 mL per 500 test bottle) B12 Pre-Treatment Reagent 1 containing 1.0 N sodium hydroxide with 0.005% potassium cyanide.

- 1 Bottle (5.5 mL per 100 test bottle / 25.9 mL per 500 test bottle) B12 Pre-Treatment Reagent 2 containing alpha monothioglycerol and EDTA.

- 1 Bottle (5.5 mL per 100 test bottle /25.9 mL per 500 test bottle) B12 Pre-Treatment Reagent 3 containing cobinamide dicyanide in borate.

ARCHITECT B12 Controls:

- 1 Bottle (8 mL) of ARCHITECT B12 Low Control contains cyanocobalamin in borate buffer with protein (human albumin) stabilizer. Target concentration is 251 pg/mL.

- 1 Bottle (8 mL) of ARCHITECT B12 Medium Control contains cyanocobalamin in human serum. Target concentration is 454 pg/mL.

- 1 Bottle (8 mL) of ARCHITECT B12 High Control contains cyanocobalamin in borate buffer with protein (human albumin) stabilizer. Target concentration is 915 pg/mL.

ARCHITECT B12 Calibrators:

6 Bottles (4 mL each) of ARCHITECT B12 Calibrators. Calibrator A contains borate buffer with protein (human albumin) stabilizer. Calibrators B through F contain gravimetrically prepared cyanocobalamin in borate buffer with protein (human albumin) stabilizer. Calibrators have the following approximate concentrations: 0, 110, 250, 500, 1000, and 2000 pg/mL.

The human albumin donor units used in the ARCHITECT B12 Calibrators and Controls were tested with FDA licensed assays and found to be nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2, and anti-HCV.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Roche Elecsys Vitamin B12 Immunoassay

2. Predicate K number(s):

   k060755

3. Comparison with predicate:

Similarities		
Item	Proposed Device	Predicate Device (k060755)
Intended Use/Indications for use	For the quantitative determination of vitamin B12 in human serum and plasma. Measurements obtained by this device are used in the diagnosis and treatment of anemias of gastrointestinal malabsorption.	Same

Differences		
Item	Proposed Device	Predicate Device (k060755)
Platform	ARCHITECT i 2000$_{SR}$ instrument (immunoassay analyzer)	Roche Elecsys 2010 and MODULAR ANALYTICS E170 (Elecsys module) immunoassay analyzers
Methodology	Chemiluminescence	Electrochemiluminescence

Differences		
Item	Proposed Device	Predicate Device (k060755)
Specimen type	Serum	Serum and plasma
Measuring range	146 – 2000 pg/mL	30 - 2000 pg/mL
Calibrator Levels	6 levels: A: 0 pg/mL B: 110 pg/mL C: 250 pg/mL D: 500 pg/mL E: 1,000 pg/mL F: 2,000 pg/mL	2 levels: Cal 1: approximately 250 pg/mL Cal 2: approximately 1,500 pg/mL
Control Matrix and Components	Low and high control contain cyanocobalamin in borate buffer with protein stabilizers (human albumin). Medium control contains cyanocobalamin in human serum.	Human serum
Control Levels	3 levels Targets: Low: 251 pg/mL Medium: 454 pg/mL High: 915 pg/mL	3 levels PC A1: < 350 pg/mL PC A2: approximately 560 pg/mL PC A3: approximately 1160 pg/mL

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

- Clinical and Laboratory Standards Institute (CLSI) Guideline EP5-A2: *Evaluation of Precision Performance of Quantitative Measurement Methods*

- CLSI Guideline EP 17-A: *Protocols for Determination of Limits of Detection and Limits of Quantitation*

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

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

- CLSI Guideline EP7-A2: *Interference Testing in Clinical Chemistry*

- CLSI Guideline C28-A2: *How to Define and Determine Reference Intervals in the Clinical Laboratory*

L. Test Principle:

The ARCHITECT B12 assay is a two-step assay with an automated sample pretreatment, for determining the presence of B12 in human serum using chemiluminescent microparticle immunoassay (CMIA) technology. Sample and Pre-Treatment Reagent 1, Pre-Treatment Reagent 2, and Pre-Treatment Reagent 3 are combined. An aliquot of the pre-treated sample is aspirated and transferred into a new reaction vessel (RV). The pre-treated sample, assay diluent, and intrinsic factor coated paramagnetic microparticles are combined. B12 present in the sample binds to the intrinsic factor coated microparticles. After washing, B12 acridinium-labeled conjugate is added in the second step. Pre-Trigger and Trigger Solutions are then added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative light units (RLUs). An inverse relationship exists between the amount of B12 in the sample and the RLUs detected by the ARCHITECT *i* System optics.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*

   This study was designed based on the CLSI document EP5-A2. Three control levels (low, medium, and high) and 1 serum panel were tested.

   Testing was performed using 3 lots of reagents, 2 lots of calibrators, and 1 lot of controls on one ARCHITECT i 2000$_{SR}$ instrument. One calibrator lot was paired with 1 reagent lot and the other calibrator lot was paired with the remaining 2 reagent lots. The 3 control levels and the serum panel were each tested in replicates of 3, twice daily (a minimum of 2 hours apart), on each of 20 days, using 3 reagent lots on one ARCHITECT i 2000$_{SR}$ instrument, for a total of 360 replicates. Results are summarized in the table below:

Samples	N	Mean (pg/mL)	Within-Run SD	Within-Run %CV	Total SD	Total SD Upper 95% CL	Total %CV	Total %CV Upper 95% CL
Serum panel	357	248	11.6	4.7	13.3	14.3	5.4	5.8
Low control	356	241	10.4	4.3	12.9	14.0	5.4	5.8
Medium control	352	408	13.3	3.3	15.5	16.7	3.8	4.1
High control	355	885	23.9	2.7	29.7	32.2	3.4	3.6

   b. *Linearity/assay reportable range:*

   The study was performed and analyzed based on the CLSI document EP6-A. One ARCHITECT i 2000$_{SR}$ instrument and 1 lot each of ARCHITECT B12 Reagent Kit, Calibrators, and Controls were used.

   A high sample was prepared to a concentration of approximately 2,200 pg/mL by spiking B12 stock solution into a serum sample. The high sample (Stock A) was mixed with serum containing various levels of low B12 concentration (Stock B). Stock B was diluted with ARCHITECT Wash Buffer (0 pg/mL, phosphate buffered saline) or Calibrator A diluent because a B12-deficient (0

pg/mL) specimen cannot be obtained. Each sample was tested in replicates of 4 using the ARCHITECT B12 assay. Data was analyzed using 1st order, 2nd order, and 3rd order least square regression according to the CLSI EP6-A guideline and the results are summarized below:

1st order: $y = 1.0234x + 4.18$
2nd order: $y = 1.1676x - 0.0001x^2 - 28.79$
3rd order: $y = 1.0196x + 0.0001x^2 - 0x^3 - 8.01$

The 3rd order cubic model was the best fit. The percent deviation (DL) from linearity was within ± 10% for all samples tested (18 samples ranged from 109 to 2075 pg/mL).

The B12 assay has a measuring range of 146 – 2000 pg/mL.

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

Calibrators: Abbott manufactures B12 internal standards (primary calibrators) gravimetrically using cyanocobalamin (B12) USP Reference Standard. The B12 calibrators (market calibrators) are manufactured and tested against these internal standards.

Primary B12 Calibrator A (0 pg/mL) is prepared with sodium borate, sodium chloride, water and human serum albumin. The pH of the solution is checked and adjusted, if necessary. Sodium azide is added, and the solution is diluted to final batch size with water, mixed, and filtered. The calibrator diluent is stored at 2 to 8°C.

Preparation of Primary B12 Calibrators B through F: The cyanocobalamin USP reference standard is gravimetrically diluted with filtered water to create a concentrated reference solution. The concentrated reference solution is gravimetrically diluted with calibrator diluent to create a B12 primary stock solution.   Each Primary B12 Calibrator B through F is gravimetrically prepared with B12 primary stock solution and calibrator diluent to target concentrations of 100, 250, 500, 1,000, and 2,000 pg/mL, respectively.

Secondary and Market versions of B12 Calibrator A is prepared the same way as the Primary B12 Calibrator A. Each Secondary and Market version of B12 Calibrator B through F is prepared with ARCHITECT B12 stock solution (cyanocobalamin diluted in calibrator diluent) and calibrator diluent to target concentrations of 110 , 200, 500, 1,000, and 2,000 pg/mL, respectively.

Value assignment: The concentrations of each Secondary B12 Calibrator B through F are determined by comparison of the relative light unit (RLU) values against the corresponding primary calibrators using the ARCHITECT *i* System. The on-test calibrator is compared to the corresponding primary calibrator using a sample/reference ratio of the grand mean RLU results. The concentrations are adjusted, if necessary, by adding either ARCHITECT B12 stock solution or calibrator diluent, to be within ± 1% of the primary calibrator

RLU values. Market calibrators are similarly assigned values by comparison via RLU values to the secondary calibrators.

Controls: Primary and secondary controls are made similarly to calibrators (above). The B12 primary stock solution is mixed with calibrator diluent to target concentrations of 200, 400 and 800 pg/mL. The secondary controls are assigned values via the same process described above for secondary calibrators. The ARCHITECT B12 Low and High Control are prepared with ARCHITECT B12 stock solution (cyanocobalamin diluted in calibrator diluent) and calibrator diluent to target concentrations of 251 and 915 pg/mL. The ARCHITECT B12 Medium Control is prepared by spiking human serum containing B12 with ARCHITECT B12 stock solution to a target concentration of 454 pg/mL. Value assignment for market controls is similar to that described above for the market calibrators.

Stability: Protocols and acceptance criteria for shelf-life and in-use (open vial) stability were reviewed and found to be adequate. Currently, real-time stability data supports 8 months at 2-8˚C for controls and 11 months at 2-8˚C for calibrators; for in use (open vial) stability; in use (open vial) stability for calibrators is one month and for controls is two months. Reagents are stable on board the ARCHITECT *i* System for 26 days.

d. *Detection limit:*

The detection limits study and statistical analysis was performed based on the CLSI document EP17-A. Testing was performed using at least two ARCHITECT *i* 2000$_{SR}$ instruments and 2 reagent lots. Both reagent lots were tested on each instrument, along with 1 lot each of ARCHITECT B12 Calibrators and Controls. Four zero-level and 9 low-level samples were tested. Samples were prepared at the following concentrations: 58, 85, 97, 116, 126, 136, 146, 168, and 200 pg/mL. All samples were tested in 1 replicate for the zero-level samples and a minimum of 3 replicates for the low-level samples on 5 runs over 3 days for each reagent lot and instrument combination for a total of at least 60 replicates for each analyte level.

Limit of Blank (LoB) was calculated to be 66 pg/mL. Limit of Detection (LoD) was calculated to be 88 pg/mL. Limit of Quantitation (LoQ) is the imprecision where the %CV is less than or equal to 10%. The LoQ was determined to be 146 pg/mL.

The B12 assay has a measuring range of 146 – 2000 pg/mL.

e. *Analytical specificity:*

Interference: A study was conducted to evaluate the susceptibility of the ARCHITECT B12 assay to potentially interfering substances: total bilirubin, hemoglobin, total protein, and triglycerides. The study was performed based on the CLSI document EP7-A2. One ARCHITECT *i* 2000$_{SR}$ instrument and 1 lot each of ARCHITECT B12 Reagent Kit, Calibrators, and Controls were used.

A serum specimen pool containing 150 - 250 pg/mL B12 (low sample) and a serum specimen pool containing > 500 pg/mL B12 (higher sample) were obtained. The sponsor defines no significant interference if bias is less than 10% between the spiked and unspiked sample. Total bilirubin (conjugated and unconjugated), total protein, and triglycerides showed no significant interference in the ARCHITECT B12 assay for low samples (concentration range: 150 pg/mL to 250 pg/mL) and higher samples (concentration range: > 500 pg/mL) at the following concentrations:

- Bilirubin   ≤ 20 mg/dL
- Total Protein   ≤ 12 g/dL
- Triglycerides   ≤ 3000 mg/dL

At a hemoglobin concentration of approximately 400 mg/dL, the ARCHITECT B12 assay showed an average interference of –17.5% with low samples (concentration range: 150 pg/mL to 250 pg/mL) and –12.5% with higher samples (concentration range: > 500 pg/mL). Therefore, the sponsor put the following limitation in their package insert.

"Hemolysis has been demonstrated to exhibit negative interference in this B12 assay. Hemolyzed specimens should not be analyzed."

Cross reactivity: A study was conducted to evaluate the susceptibility of the ARCHITECT B12 assay to cross-reactivity with cobinamide. The study was performed using one ARCHITECT i 2000$_{SR}$ instrument and 1 lot each of ARCHITECT B12 Reagent Kit, Calibrators, and Controls.
A serum specimen pool containing approximately 230 pg/mL B12 was obtained. A test sample was prepared by adding a 360 ng/mL cobinamide stock solution to the serum specimen pool, targeting a cobinamide concentration of ≥ 9,000 pg/mL. A reference sample was prepared by adding a volume of distilled water, equivalent to the volume of cobinamide stock solution used in the test sample, to the serum specimen pool.

The test and reference samples were each tested once in replicates of 14 using the ARCHITECT B12 assay. Analysis of the test vs. reference sample testing showed 1.8% bias between the test and reference sample. The sponsor claimed that there is no cross reactivity with cobinamide based on the data.

f. *Assay cut-off:*

Not applicable.

2. Comparison studies:

a. *Method comparison with predicate device:*

The study was performed based on the CLSI document EP9-A2. The Roche Elecsys E170 Vitamin B12 assay was used as the predicate device.

A total of 198 human serum specimens were tested with both the ARCHITECT B12 and Roche Elecsys Vitamin B12 assays. For the ARCHITECT B12 assay, 2 lots of reagents, 1 lot each of calibrators and

controls, and 1 ARCHITECT *i* 2000SR instrument were used for testing, with specimen testing divided over the 2 reagent lots. For the Roche Elecsys Vitamin B12 assay, 1 lot each of reagents, calibrators, and controls and 1 Roche Elecsys instrument were used for testing. Specimens were tested in replicates of 2 with each assay over a period of 6 days. A specimen tested using the ARCHITECT B12 assay was tested within 24 hours using the Roche Elecsys Vitamin B12 assay. 47 samples were excluded from analysis because they were outside the claimed range of the assay (24 below the LoQ, 23 above 2000 pg/mL). Results of the study are summarized in the table below.

First Replicate ARCHITECT B12 vs. Mean Roche Elecsys Vitamin B12, ARCHITECT B12 sample range tested: 147 pg/mL - 1980 pg/mL

| N | Correlation Coefficient (r) | | Regression Method | Intercept | | Slope | |
	r	95% CL (Lower One-Sided)		Estimate	95% CI	Estimate	95% CI
151	0.991	0.988	Passing-Bablok	-20.23	-30.88, -5.61	1.06	1.03, 1.08
			Least squares	-41.35	-64.30, -18.39	1.09	1.06, 1.11

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):

   Not applicable.

4. Clinical cut-off**:**

Not applicable.

5. Expected values/Reference range:

A study was performed based on the CLSI document C28-A2. One ARCHITECT *i* 2000SR instrument, 2 lots of ARCHITECT B12 Reagents, and 1 lot each of ARCHITECT B12 Calibrators and Controls were used. Serum specimens from 121 individuals with normal mean corpuscular volume, homocysteine, and folate results were assayed for B12 using the ARCHITECT B12 assay.

The B12 concentration range for this population was < 146 - 1218 pg/mL with a median of 409 pg/mL. The central 95% of the sample population (expected range) is defined as: Expected Range 213 - 816 pg/mL (157 - 602 pmol/L)

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.