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

**A. 510(k) Number:**

k051327

**B. Purpose for Submission:**

New device (significant modification of previously cleared reagent – k965186)

**C. Measurand:**

Vitamin B12

**D. Type of Test:**

Quantitative, Chemiluminescent microparticle intrinsic factor assay

**E. Applicant:**

Abbott Laboratories

**F. Proprietary and Established Names**:

Abbott AxSYM B12

**G. Regulatory Information:**

1. Regulation section
   21CFR§ - 862.1810 Vitamin B12 test system.

2. Classification:
   Class II

3. Product code:
   CDD

4. Panel:
   75 – Clinical Chemistry

**H. Intended Use:**

1. Intended use:
   See Indications for use

2. Indications for use:
The AxSYM B12 reagent is a microparticle enzyme intrinsic factor assay for the quantitative determination of vitamin $B_{12}$ in human serum or plasma on the AxSYM System. Measurements obtained by this device are used in the diagnosis and treatment of anemias of gastrointestinal malabsorption.

The AxSYM B12 Specimen Diluent is used for manually diluting specimens for testing using the AxSYM B12 Assay.

3. Special conditions for use statement:
Prescription Use Only

4. Special instrument requirements:
AxSYM System

**I. Device Description:**

The AxSYM B12 assay is supplied as a 100 test Reagent Pack (Dual Pack) consisting of the following reagents:

**Reagent Pack A**

| | |
|---|---|
| Reagent Bottle 1 | Extractant 1: Cobinamide Dicyanide in borate buffer with protein (avian) stabilizer. The preservative is Sodium Azide. |
| Reagent Bottle 2 | Extractant 2: Alpha Monothioglycerol in EDTA |
| Reagent Bottle 3 | Monoclonal (mouse) anti-intrinsic factor antibody complexed with intrinsic factor (porcine) coated microparticles in borate buffer with protein (bovine) stabilizer. Minimum concentration: 0.0013% solids. The preservative is Sodium Azide. |

**Reagent Pack B**

| | |
|---|---|
| Reagent Bottle 1 | Alkaline Phosphatase (bovine) Conjugate in TRIS buffer with protein (avian) stabilizer. Minimum concentration: 0.1 µg/mL. The preservative is Sodium Azide. |
| Reagent Bottle 2 | Denaturant 0.8 N Sodium Hydroxide with 0.005% Potassium Cyanide. |
| Reagent Bottle 3 | Monoclonal (mouse) anti-intrinsic factor antibody complexed with intrinsic factor (porcine) coated microparticles in borate buffer with protein (bovine) stabilizer. Minimum concentration: 0.0013% solids. The preservative is Sodium Azide. |

**J. Substantial Equivalence Information:**

1. Predicate device name:
Abbott ARCHITECT® B12 assay

2. Predicate 510(k) number:
   k981408

3. Comparison with predicate:

| Similarities | | |
|---|---|---|
| **Characteristics** | **Abbott AxSYM B12** | **Predicate device Abbott ARCHITECT® B12** |
| Assay Principle | Microparticle Enzyme Intrinsic Factor assay | Chemiluminescent Microparticle Intrinsic Factor assay |
| Analyte Measured | Vitamin $B_{12,}$ | Same |
| Specimens | Serum (including separator tubes)<br><br>Plasma (lithium heparin [including separator tubes], sodium heparin, dipotassium EDTA) | Serum (including separator tubes)<br><br>Plasma (tripotassium EDTA) |

| Differences | | |
|---|---|---|
| **Characteristic** | **Abbott AxSYM B12** | **Predicate device Abbott ARCHITECT® B12** |
| Technology | MEIA – uses carboxylate microparticles | Chemiluminescent microparticle immunoassay (CMIA) uses carboxylate magnetic microparticles |
| Detection of Analyte | Enzyme-labeled B12 binds to unoccupied intrinsic factor binding sites which are coupled to the microparticle with anti-intrinsic factor monoclonal antibody. | Acridinium-labeled B12 binds to unoccupied intrinsic factor binding sites coupled to microparticles. |
| Instrumentation | AxSYM System | ARCHITECT *i* System |

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

NCCLS Guidance Documents:

EP-5A, Evaluation of Precision Performance of Quantitative Measurement Methods

EP-7A, Interference Testing in Clinical Chemistry
EP-9A2, Method Comparison and Bias Estimation Using Patient Samples
C28-A2, How to Define and Determine Reference Intervals in the Clinical Laboratory

**L. Test Principle:**

The AxSYM B12 is a two-step microparticle enzyme intrinsic factor based on microparticle enzyme immunoassay (MEIA) technology. Sample and reagents required for one test are pipetted into various wells of a reaction vessel (RV). Extractant 1 and extractant 2 are combined in one RV well. Sample, denaturant, and a portion of the extractant mixture are combined in another RV well. The RV is immediately transferred into the processing center. Anti-intrinsic factor antibody complexed with intrinsic factor coated microparticles is added to the reaction mixture. B12 present in the sample bind to the intrinsic factor coated microparticles forming a B12-intrinsic factor-microparticle complex. An aliquot of the reaction mixture is transferred to the matrix cell. The microparticles bind irreversibly to a glass fiber matrix. The matrix cell is washed to remove materials not bound to the microparticles. The B12:alkaline phosphate conjugate is dispensed onto the matrix cell forming a B12-intrinsic factor-microparticle-conjugate complex. The matrix cell is washed to remove the unbound conjugate. The substrate, 4-methylumbelliferyl phosphate is added to the matrix cell and the fluorescent product is measured by the MEIA optical assembly.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

*a. Precision/Reproducibility:*

Precision was evaluated as described in NCCLS EP-5A. Three B12 control levels-low, medium, and high-were assayed in replicates of two at two separate times of day for 20 days (n = 80). The targeted B12 concentration for the low control is 200, for the medium control is 424 and for the high control is 881. The results of the study are:

| Control Level | Instrument | Reagent Lot | n | Mean pg/mL | Within-run SD | Within-run %CV | Total SD | Total %CV |
|---|---|---|---|---|---|---|---|---|
| Low | A | 1 | 80 | 205.9 | 11.94 | 5.8 | 13.93 | 6.8 |
| Low | A | 2 | 80 | 193.6 | 12.94 | 6.7 | 19.72 | 10.2 |
| Low | B | 1 | 80 | 212.5 | 14.23 | 6.7 | 16.94 | 8.0 |
| Low | B | 2 | 80 | 206.9 | 10.61 | 5.1 | 14.09 | 6.8 |
| Medium | A | 1 | 80 | 396.7 | 11.05 | 2.8 | 13.07 | 3.3 |
| Medium | A | 2 | 80 | 405.9 | 12.98 | 3.2 | 20.67 | 5.1 |
| Medium | B | 1 | 80 | 405.8 | 11.20 | 2.8 | 12.22 | 3.0 |
| Medium | B | 2 | 80 | 397.1 | 15.72 | 4.0 | 17.73 | 4.5 |
| High | A | 1 | 80 | 832.5 | 23.96 | 2.9 | 40.97 | 4.9 |
| High | A | 2 | 80 | 879.5 | 28.78 | 3.3 | 31.69 | 3.6 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| High | B | 1 | 80 | 837.7 | 18.77 | 2.2 | 27.08 | 3.2 |
| High | B | 2 | 80 | 845.8 | 20.58 | 2.4 | 24.86 | 2.9 |

*b. Linearity/assay reportable range:*

A study was conducted to evaluate the capability of the assay to recover known concentrations of spiked B12 using ten human serum specimens supplemented with known amounts of cyanocobalamin. Control samples were prepared by supplementing with an equivalent volume of the diluent used to prepare the B12 stock solution. The mean B12 concentration was calculated for each set of four replicates. The difference between the means of each test sample and its corresponding control sample was calculated. The percent recovery was calculated using the following equation:

$$\% \text{ Recovery} = \frac{\text{Difference}}{\text{Concentration of B12 added}} \times 100$$

Where Difference = Test mean B12 concentration - Control mean concentration

The results are summarized in the following table:

| **Specimen ID** | **Control Mean pg/mL** | **Test Mean pg/mL** | **Added Analyte pg/mL** | **% Recovery** |
|---|---|---|---|---|
| 01 | 692.2 | 910.4 | 199.42 | 109.4 |
| 02 | 183.6 | 370.4 | 199.42 | 93.7 |
| 03 | 669.4 | 910.3 | 199.42 | 120.8 |
| 04 | 470.4 | 638.0 | 199.42 | 84.0 |
| 06 | 186.3 | 631.2 | 398.84 | 111.5 |
| 07 | 723.8 | 1094.2 | 398.84 | 92.9 |
| 08 | 318.9 | 787.8 | 398.84 | 117.6 |
| 09 | 570.7 | 1009.2 | 398.84 | 110.0 |
| 10 | 613.9 | 1010.1 | 398.84 | 99.4 |
| 11 | 482.8 | 718.2 | 199.42 | 118.0 |
| **Mean % Recovery** | | | | **105.7** |

A study was conducted to evaluate the capability of AxSYM B12 assay to measure linearly diluted specimens containing B12 concentrations greater than 1200 pg/mL. Three human serum specimens with known B12 concentrations were spiked with a 100,000 pg/mL B12 stock solution to yield a target B12 concentration of approximately 4800 pg/mL. Each spiked specimen was then serially diluted with the diluent (Calibrator A) at 1:4, 1:8, and 1:16. Each sample was tested in replicates of two and the mean B12 concentration was calculated for each sample dilution. The percent recovery was calculated for each dilution using the following equation:

% Recovery = Mean observed concentration x 100
Expected concentration

The results are presented in the following table. The data supports the 1:4 manual dilution.

| Specimen | Dilution | Expected pg/mL | Lower Limit (-20%) pg/mL | Upper Limit (+20%) pg/mL | Observed pg/mL |
|---|---|---|---|---|---|
| **1** | Neat | | | | >1200 |
| | 1:4 | 1200 | 960 | 1440 * | 1119 |
| | 1:8 | 560 | 448 | 672 | 626 |
| | 1:16 | 280 | 224 | 336 | 298 |
| **2** | Neat | | | | >1200 |
| | 1:4 | 1200 | 960 | 1440 * | 1143 |
| | 1:8 | 571 | 457 | 686 | 629 |
| | 1:16 | 286 | 229 | 343 | 290 |
| **3** | Neat | | | | >1200 |
| | 1:4 | 1200 | 960 | 1440 * | 1190 |
| | 1:8 | 595 | 476 | 714 | 675 |
| | 1:16 | 298 | 238 | 357 | 317 |

* Note: This is a calculated value. The AxSYM instrument would report >1200.

The assay reportable range is 60 - 1200 pg/mL.

Deleted: l

c. *Traceability, Stability, Expected Values (controls, calibrators, or method):* The on board reagent stability was performed using one reagent kit lot. The time points were approximately 24 hours after the calibration run and at approximately 8 and/or 16 hour increments thereafter. One run was performed at each time point. A run consisted of two replicates each of the low, medium, and high controls. The stability of the reagent kit while stored on board the AxSYM system was determined to be 224 cumulative hours.

The stability of the reformulated microparticles was tested with the existing reagent components (denaturant, extractant 1, extractant 2, conjugate). Three kits of microparticles were evaluated at 2-8ºC, under onboard storage conditions, and freeze/thaw. The stability performance was determined by performing analysis on two lots of calibrators and controls at time points up to 12 months. The reagent pack includes the conjugate, denaturant, extractant 1 and extractant 2. The dating is determined by the component with the shortest stable period in the reagent pack (the denaturant) which has 6 months of stability. Therefore, the reagent pack is stable for 6 months when stored at 2-8ºC.

NOTE: The calibrators and controls were cleared with the original 510(k) for this device, k965186.

d. *Detection limit*
Analytical sensitivity, defined as 2 standard deviations above the concentration of Calibrator A (0 pg/mL), was calculated to be 58 pg/mL at the 95th percentile. This is below the reportable range of the assay.

*e. Analytical specificity:*
The specificity of the assay was determined by studying the cross-reactivity with cobinamide. A study was performed based on NCCLS EP-7A. Normal serum specimens were supplemented with 9000 pg/mL cobinamide. The mean observed cross-reactivity was 0.1%.

The assay has a mean potential interference from bilirubin, hemoglobin, red blood cells, and triglycerides of ≤ 10% at the following levels as confirmed by a study based on NCCLS EP-7A.

- Bilirubin ≤ 20 mg/dL
- Hemoglobin ≤ 500 mg/dL
- RBCs ≤ 0.4% (v/v)
- Triglycerides ≤ 1000 mg/dL

2. Comparison studies:
*a. Method* comparison with predicate device:
The assay was compared to the predicate assay using a study based on NCCLS EP-A2. The sample ranges for the 441 specimens in the study were as follows: AxSYM (73.8 - 1177.0 pg/mL) and predicate (77 - 1210 pg/mL). Specimens were tested once with two reagent lots on two instruments. Data was generated using Least Squares and Passing-Bablok analyses. The results are as follows:

| **Regression Method** | **n** | **Intercept** | **Slope** |
|---|---|---|---|
| Least Squares | 441 | 3.7 | 1.09 |
| Passing-Bablok | 441 | -13.9 | 1.12 |
| **Correlation coefficient was 0.98** | | | |

*b. Matrix comparison:*
A study was conducted to compare the results of human specimens collected in different serum (glass, plastic, and serum separator tubes) and plasma collection tubes (lithium heparin, lithium heparin plasma separator tubes, sodium heparin and dipotassium EDTA). Matched draws of specimens were obtained from 19 donors. A separate study matched draws of specimens obtained from 20 donors in glass serum with no additive (control) and plastic SST with clot activator. The following specimen collection tubes may be used for the assay:

| | **Glass** | **Plastic** |
|---|---|---|
| Serum | • No additive | • With clot |

| | | |
|---|---|---|
| | • Serum separator with clot activators | activator<br>• Serum separator with clot activator |
| Plasma | | • Lithium Heparin<br>• Lithium Heparin Plasma Separator Tube<br>• Sodium Heparin<br>• Dipotassium EDTA |

Specimen Storage: A study was conducted to compare the performance of specimens stored under various conditions to their performance when the specimens were freshly collected. For serum and plasma specimens removed from cells, clot or separator gel and stored at 15-30ºC for 24 hours, the grand mean % difference for all tube types ranged from -1.8% to 3.7%. For serum and plasma specimens removed from the cells, clot or separator gel and stored at 2-8ºC for 10 days, the grand mean % difference values for all tube types ranged from -2.8% to 6.2%. The sponsor states that specimen performance is acceptable when serum and plasma specimens are removed from the cells, clot or separator gel or cells and stored at 15-30ºC for up to 24 hours before testing or stored up to 10 days at 2-8 ºC.

3. *Clinical studies:*

*a. Clinical sensitivity:*
Not applicable

*b. Clinical specificity:*
Not applicable

4. *Clinical cut-off:*
Not applicable

5. Expected values/Reference range:
B12 Normal Values
A study was performed based on NCCLS 28-A2. Serum specimens from 258 individuals with normal mean corpuscular volume (MCV), homocysteine and folate results were assayed. The median, minimum, maximum, and the central 95% confidence interval were calculated. The results are presented in the following table.

| **N** | **Median (pg/mL)** | **Minimum (pg/mL)** | **Maximum (pg/mL)** | **2.5 percentile (pg/mL)** | **97.5 percentile (pg/mL)** |
|---|---|---|---|---|---|
| 258 | 464.7 | 98.5 | >1200 | 208.0 | 963.5 |

The expected range was determined to be 208.0 to 963.5 pg/mL.

The sponsor recommends that each laboratory establish its own reference ranges.

B12 Indeterminates
The sponsor states in the labeling that levels above 300 or 400 pg/mL are rarely associated with B12 deficiency induced hematological or neurological diseases respectively. Further testing for folic acid, intrinsic factor blocking antibodies, homocysteine and/or methylmalonic acid is suggested in the labeling for symptomatic patients with the following B12 levels:

| | |
|---|---|
| Hematologic abnormalities | 100-300 pg/mL |
| Neurologic abnormalities | 100-400 pg/mL |

The references for the section on B12 Interdeterminates in the labeling are:

Klee, GC. Cobalamin and folate evaluation: measurement of methylmalonic acid and homocysteine vs vitamin B12 and folate. *Clin Chem* 46; 2000:1277-1283.

Snow, CF. Laboratory diagnosis of vitamin B12 and folate deficiency: A guide for the primary physician. *Arch Intern Med.* 1999; 159:1289-1298.

**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.