

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

I Background Information:

A 510(k) Number

K192064

B Applicant

DiaSorin Inc.

C Proprietary and Established Names

LIAISON[®] Vitamin B12

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:

New Device

B Measurand:

Vitamin B12

C Type of Test:

Quantitative, chemiluminescent immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The DiaSorin LIAISON® Vitamin B12 assay uses chemiluminescent immunoassay (CLIA) technology for the quantitative determination of Vitamin B12 in human serum, SST serum and lithium heparin plasma. Measurements obtained by this device are used in the diagnosis and treatment of anemia's of gastrointestinal malabsorption. Assay results should be used in conjunction with other clinical or laboratory data to assist the clinician in making individual patient management decisions.

The assay must be performed on the LIAISON® XL Analyzer.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

For in vitro diagnostic use only

D Special Instrument Requirements:

LIAISON® XL Analyzer

IV Device/System Characteristics:

A Device Description:

The LIAISON® Vitamin B12 reagent contains:

- Magnetic particles - coated with Intrinsic Factor in phosphate buffer, stabilizers and preservatives; 2.4 mL
- Calibrator 1 - Vitamin B12 free human serum containing cyanocobalamin (Vitamin B12), and preservatives; 2.4 mL
- Calibrator 2 - Vitamin B12 free human serum containing cyanocobalamin (Vitamin B12), and preservatives; 2.4 mL
- Conjugate - Proprietary polymer conjugated with Vitamin B12 and an isoluminol derivative, in MES buffer with surfactant and preservatives; 12 mL
- Assay Buffer 1 – Glycine buffer containing TCEP (tris(2-carboxyethyl) phosphine) and preservatives; 3.0 mL
- Assay Buffer 2 – Potassium hydroxide buffer containing 0.05% potassium cyanide and preservatives; 12 mL

- Assay Buffer 3 – Potassium phosphate with dicyanocobinimide, surfactant and preservatives; 22 mL

B Principle of Operation:

The LIAISON® Vitamin B12 assay is a competitive chemiluminescence immunoassay (CLIA) for quantitative determination of Vitamin B12 in serum, SST serum and lithium heparin plasma. During the first incubation, Vitamin B12 is dissociated from its binding protein. After the initial incubation of 10.5 minutes, Vitamin B12 binds to an intrinsic factor on the solid phase. After a second incubation of 10.5 minutes, a Vitamin B12 linked to an isoluminol derivative is added to compete with the Vitamin B12 in the sample. After a third incubation of 5.25 minutes, the unbound material is removed with a wash cycle. Subsequently, the starter reagents are added to initiate a flash chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLU) and is inversely proportional to the concentration of Vitamin B12 present in samples.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Access Vitamin B12 Assay

B Predicate 510(k) Number(s):

K140496

C Comparison with Predicate(s):

Device & Predicate Device(s):	<u>K192064</u>	<u>K140496</u>
Device Trade Name	LIAISON® Vitamin B12	Beckman Coulter Access Vitamin B12
General Device Characteristic Similarities		
Intended Use/Indications For Use	For the quantitative determination of Vitamin B12 in serum and plasma.	Same
Assay Type	Chemiluminescence immunoassay	Same
Results	Quantitative	Same
Operation	Automated	Same
General Device Characteristic Differences		
Sample Type	Human serum, serum-separating tubes (SST)	uman serum and

Device & Predicate Device(s):	<u>K192064</u>	<u>K140496</u>
	and lithium heparin plasma	heparin plasma
Sample Size	100 µL	45 µL
Reagent Storage	2-8°C	2-10 °C
Analytical Measuring Range	55-1500 pg/mL	50-1500 pg/mL
Solid Phase	Magnetic particles coated intrinsic factor	Paramagnetic particles coated with goat anti-mouse IgG
Conjugate	Polymer conjugated with Vitamin B12	Porcine intrinsic factor-alkaline phosphate (bovine) conjugate

VI Standards/Guidance Documents Referenced:

- CLSI Guideline EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline.
- CLSI Guideline EP15-A3, User Verification of Precision and Estimation of Bias; Approved Guideline.
- CLSI Guideline EP07-A2, Interference Testing in Clinical Chemistry, Approved Guideline.
- CLSI Guideline EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures; A Statistical Approach; Approved Guideline.
- CLSI Guideline EP 17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline.
- CLSI Guideline EP28-A3C, Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

A 20-day reproducibility/precision study was performed following CLSI EP05-A3 guideline. Testing was performed using a coded panel comprised of 6 serum samples, spanning the assay range was tested, along with the LIAISON® Vitamin B12 kit controls (2 levels). Each

sample was tested in duplicate per run in two separate runs per day, over 20 operating days (N=80). The results are summarized in the below table.

Sample ID	Mean (pg/mL)	Intra-Run Precision		Total Precision	
		SD	%CV	SD	%CV
Control 1	642	21.56	3.4%	42.99	6.7%
Control 2	1262	48.79	3.9%	86.28	6.8%
Serum #1	240	10.16	4.2%	20.36	8.5%
Serum #2	417	21.85	5.2%	39.76	9.5%
Serum #3	646	24.83	3.8%	57.55	8.9%
Serum #4	773	29.36	3.8%	60.12	7.8%
Serum #5	1039	39.87	3.8%	103.87	10.0%
Serum #6	1093	47.96	4.4%	93.89	8.6%

2. Linearity:

One sample of each specimen type (serum, SST serum, lithium heparin plasma) containing endogenous and/or spiked Vitamin B12 above the measuring range of the assay at 1500 pg/mL was diluted with a low-level sample to create 13 evenly distributed concentrations spanning the measuring range (< 50 - >1500 pg/mL). Each sample was tested in duplicate following CLSI EP06-A guideline. The results for each sample were analyzed by regression of observed concentration (y) versus expected concentration (x). The regression results are summarized below for each sample type:

Serum: $y = 1.076x + 6.896$; $R = 0.998$

SST Serum: $y = 1.009x + 0.224$; $R = 0.999$

Lithium Heparin plasma: $y = 1.029x - 0.095$; $R = 0.996$

3. Analytical Specificity/Interference:

Interference testing was conducted following CLSI-EP07-A2 guideline. Potential interfering substances were spiked to serum samples with normal and high level of Vitamin B12 (200 and 800 pg/mL). The same samples without interferent but containing the “vehicle” were tested as controls. Interference was defined as more than 10% difference between the spiked and the un-spiked samples.

The substances and the highest concentrations tested without interference are summarized in the below table:

Drug/Substance	Concentration Tested
Hemoglobin	300 mg/dL
Bilirubin (conjugated)	40 mg/dL
Bilirubin (unconjugated)	40 mg/dL
Triglycerides	3,000 mg/dL
Cholesterol	500 mg/dL
Albumin	12 g/dL
Human IgG	12 g/dL

Drug/Substance	Concentration Tested
HAMA	1387.5 ng/mL
Rheumatoid Factor	1035 IU/mL
Acetaminophen	20 mg/dL
Acetylsalicylic Acid	65 mg/dL
Ibuprofen	50 mg/dL
Biotin	2 mg/mL

Under the limitations of the procedure in the labeling, the sponsor listed the substances that are known to interfere with the assay:

- Heterophilic antibodies in human serum can react with reagent immunoglobulins or other reagent material, interfering with in vitro immunoassays.
- Patients routinely exposed to animals, animal serum products, or other immunogenic products that may elicit heterophilic antibody production against the assay's reagents can be prone to this interference and anomalous values may be observed.
- Approximately 50% of patients with pernicious anemia have Intrinsic factor antibodies. During the reaction, the denaturation should inactivate intrinsic factor blocking antibodies. However, any remaining anti-intrinsic factor antibody or extremely high titer of the intrinsic factor antibody could cause erroneous results.

Cross-reactivity

Cross-reactivity was evaluated following CLSI-EP07-A2 guideline. The % cross reactivity is calculated as follows:

$$\% \text{ cross reactivity} = (\text{Corrected Assay Value} / \text{Cross-reactant Concentration}) * 100.$$

The result is summarized in the below table.

Cross-Reactant	Spiked Concentration	% Cross Reactivity
Dicyanocobinamide	10,000pg/mL	-0.19%

4. Assay Reportable Range:

55-1500 pg/mL

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

Traceability

The LIAISON[®] Vitamin B12 Calibrators are traceable to an in-house standard preparation of Vitamin B12 (pg/mL).

6. Detection Limit:

To determine the limit of blank (LoB), five blank samples were tested using 2 lots of reagent on 1 analyzer by two operators. Each sample was run in 2 replicates /run for 6 runs on three days yielding 60 results per reagent lot.

LoB was calculated as: $LoB = \mu (\text{Blank}) + 1.653 * \sigma (\text{Blank})$

The limit of detection (LoD), was determined by testing four samples in the range of approximately 25 to 100 pg/mL on 2 analyzers using two reagent lot by two operators. Each sample was tested in 2 replicates per run for over 6 runs over 3 days (yielding a minimum of 96 results).

LoD was calculated for each lot as: $LoD = LoB + 1.655 * \sigma_s (\text{Low Sample})$

For limit of quantitation (LoQ), eight samples (4 of which were used in the LoD determination) in the range of 25 to approximately 250 pg/mL were tested using 2 reagent lots on 2 analyzers by 2 operators. Each sample was tested in 2 replicates per run for 6 runs over 3 days, yielding a minimum of 144 results. LoQ was defined as the lowest concentration at which the regression line crosses the 20% CV line and was calculated for each of the reagent lots using a curve fit best suited to the data set.

The claimed LoB, LoD and LoQ are summarized in the below table using the highest concentration resulted from the different lots tested.

LoB	LoD	LoQ
38.7 pg/mL	51.2 pg/mL	55 pg/mL

7. Assay Cut-Off:

Not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

method comparison study was performed using a total of 156 serum samples (147 native, 9 spiked). The samples were tested in singlicate using the candidate device and predicate device, and the results were compared.

One sample was removed from the analysis as it was below the measuring range of the assay. The samples ranged from 71.2 - 1315 pg/mL pg/mL.

The result of the Passing-Bablok regression analysis is summarized in the below table:

N	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient
155	0.97 (0.94-1.00)	6.25 (-3.50-18.29)	0.985

2. Matrix Comparison:

Forty-eight (48) matched patient sets of serum, SST serum and lithium heparin plasma samples were tested to determine if these sample types provide equivalent results on the LIAISON® Vitamin B12 assay.

The results of the regression analysis are summarized below:

SST serum compared to Serum

N	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient
48	0.97 (0.94-0.99)	8.21 (2.14-16.56)	0.996

Lithium Heparin compared to Serum

N	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient
48	1.10 (1.05 to 1.15)	14.78 (1.09-29.87)	0.988

The study results support the sponsor's claim that human serum and lithium heparin plasma are acceptable sample types to be used with this assay.

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):

None.

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

A prospective reference range study was performed with serum samples from 166 apparently healthy adults aged 21 - 59 years of age from mixed ethnic backgrounds (30% Caucasian, 31% African Americans, 39% Hispanics) who were fasting for at least 8 hrs. Apparently healthy status was defined as subjects who had no history of anemias, folic acid or B12 deficiency, inflammatory bowel disease (IBD) or suspected IBD, celiac disease, gastrointestinal malabsorption disorders, or eating disorders. Inclusion criteria also include no oral contraceptive use within 3 months, and no alcohol within 48 hrs of blood draw or excessive alcohol usage. Pregnant women or anyone taking B12 supplement were excluded from the study.

Based on the 95% confidence interval, the following values were established following CLSI guideline EP28-A3c.

Observed Reference Ranges		
Population (n=166)	Median	Observed Range 2.5 th to 97.5 th Percentile
United States	318.5 (pg/mL)	107.2 pg/mL – 653.3 pg/mL

The sponsor recommended in the labeling that each laboratory establish its own range of expected values for the population taken into consideration.

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.