

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

A. 510(k) Number:

K151786

B. Purpose for Submission:

New device

C. Measurand:

Vitamin B12

D. Type of Test:

Quantitative, Electrochemiluminescence Immunoassay

E. Applicant:

Roche Diagnostics

F. Proprietary and Established Names:

Elecsys Vitamin B12 II assay

Elecsys Vitamin B12 II CalSet

G. Regulatory Information:

1. Regulation section:

21 CFR 862.1810, Vitamin B12 test System

21 CFR 862.1150, Calibrator

2. Classification:

Class II

3. Product code:

CDD

JIX

4. Panel:

Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

See indication for use below.

2. Indication(s) for use:

Binding assay for the in vitro 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.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and cobas e immunoassay analyzers.

Vitamin B12 II CalSet is used for calibrating the quantitative Elecsys Vitamin B12 II assay on the Elecsys and cobas e immunoassay analyzers.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Roche cobas e411 analyzer

I. Device Description:

The Elecsys Vitamin B12 II assay consists of the following items:

A. The reagent working solutions:

- 1) The rackpack (kit placed on instrument)
- 2) Streptavidin coated microparticles: 0.72 mg/mL; preservative, 1 bottle, 6.5 mL
- 3) Reagent 1 (ruthenium labeled intrinsic factor). Ruthenium labeled recombinant porcine intrinsic factor 4 µg/L; cobinamide dicyanide 15µg/L; stabilizer, human serum albumin: phosphate buffer, pH 5.5; preservative, 1 bottle, 10mL
- 4) Reagent 2 (vitamin B12 labeled biotin). Biotinylated vitamin B12 25 µg/L; biotin 3 µg/L, phosphate buffer, pH 7.0, preservative, 1 bottle, 8.5 mL
- 5) Pretreatment 1, Dithiothreitol 1.028 g/L; stabilizer, pH 5.5, 1 bottle, 4 mL
- 6) Pretreatment 2, sodium hydroxide 40 g/L, sodium cyanide 2.205 g/L, 1 bottle, 4 mL

B. Vitamin B 12 II CalSet contains the following:

- 1) Cal 1 (approximately 250 pg/mL vitamin B12 in a Human serum matrix), comes in lyophilized form.
- 2) Cal 2 (approximately 1500 pg/mL vitamin B12 in a Human serum matrix), comes in lyophilized form.

All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV using FDA approved/cleared or equivalent methods.

J. Substantial Equivalence Information:

1. Predicate device name(s):
Roche Elecsys Vitamin B12 Immunoassay
Roche Elecsys Vitamin B12 CalSet II
2. Predicate 510(k) number(s):
k060755
3. Comparison with predicate:

Comparison with B12 assay

Similarities		
Item	Predicate: Elecsys Vitamin B12 (k060755)	Candidate Device: Elecsys Vitamin B12 II Assay
Intended Use	Binding assay for the in vitro 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
Assay Method	The Elecsys Vitamin B12 assay employs a competitive test principle using intrinsic factor specific for vitamin B12. Vitamin B12 in the sample competes with the added vitamin B12 labeled with biotin for the binding sites on the ruthenium-labeled intrinsic factor complex.	Same
Detection Protocol	Electrochemiluminescent Assay	Same
Instrument Platform	Elecsys and cobas e immunoassay analyzers.	Same

Similarities		
Item	Predicate: Elecsys Vitamin B12 (k060755)	Candidate Device: Elecsys Vitamin B12 II Assay
Sample Volume	15 µL	Same
Total duration of assay	27 minutes	Same
Sample Type	Human serum and plasma.	Same
Calibration Interval	Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows: <ul style="list-style-type: none"> • After 1 month (28 days) when using the same reagent lot. • After 7 days (when using the same reagent kit on the analyzer). • As required: e.g. quality control findings outside the defined limits 	Same
Controls	Elecsys PreciControl Varia	Same

Differences		
Item	Predicate: Elecsys Vitamin B12 (k060755)	Candidate Device: Elecsys Vitamin B12 II Assay
Reagent	Native Porcine Intrinsic Factor	Recombinant Porcine Intrinsic Factor
Traceability	Standardized against the Elecsys Vitamin B12 assay (k973702)	Standardized against the Elecsys Vitamin B12 assay (k060755).
Reagent Stability	Unopened: 2-8°C - Up to the stated expiration date. After Opening at 2-8°C - 12 weeks. On the Analyzers – 5 weeks	Same Same On the Analyzers – 5 weeks onboard or 60 days when stored alternatively in the refrigerator and on the analyzer, with the total time onboard on the analyzer not exceeding 10 x 8 hours

Differences		
Item	Predicate: Elecsys Vitamin B12 (k060755)	Candidate Device: Elecsys Vitamin B12 II Assay
Measuring Range	30.0 – 2000 pg/mL	150- 2000 pg/mL
Analytical Sensitivity	Lower detection limit = 30.0 pg/mL	Limit of Blank (LoB) = 50 pg/mL Limit of Detection (LoD) = 100 ng/mL Limit of Quantitation (LoQ) = 150 pg/mL

Comparison with CalSet:

Item	Predicate device: Elecsys Vitamin B12 CalSet II (k060755)	Candidate device: Elecsys Vitamin B12 II CalSet
Similarities		
Intended Use	For calibrating the quantitative Elecsys Vitamin B12 assay on the Elecsys and cobas e immunoassay analyzers.	Same
Matrix	Human serum matrix	Same
Levels	Two	Same
Target Ranges	Cal 1: 250 pg/mL Cal 2: 1500 pg/mL	Same
Format	Lyophilized	Same
Handling	Reconstitute in 1.0 mL of distilled or deionized water before use	Same
Differences		
Stability of reconstituted calibrator if kept at: - -20°C - 2-8°C - On the analyzer 20-25°C	3 days 3 months Use only once	84 days 72 hrs. Use only once

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-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures

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

L. Test Principle:

The Elecsys Vitamin B12 II assay is an electrochemiluminescence immunoassay (ECLIA) for the quantitation of Vitamin B12 in serum or plasma. Total duration of assay is 27 minutes as follow:

- 1) 15 µL of the sample incubates with the vitamin B12 pretreatment 1 and pretreatment 2, bound vitamin B12 is released.
- 2) By incubating the pretreated sample with the ruthenium labeled intrinsic factor, a vitamin B12-binding protein complex is formed, the amount of which is dependent upon the analyte concentration in the sample.
- 3) Addition of streptavidin-coated microparticles and vitamin B12 labeled with biotin, the still-vacant sites of the ruthenium labeled intrinsic factor become occupied, with formation of a ruthenium labeled intrinsic factor vitamin B12 biotin complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- 4) The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- 5) Results are determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Precision was evaluated on the cobas e 411 according to CLSI EP5-A2. Five human serum samples (native and spiked) plus 3 quality control materials (Preci Control Varia samples) were assayed using 1 lot of reagent in duplicate (2 aliquots), twice per day for 21 days (n = 84).

Within Run and Total precision results are summarized in the table below:

Sample	n	Mean (pg/mL)	Within Run		Total precision	
			SD (pg/mL)	CV%	SD (pg/mL)	CV%
Serum 1	84	176	8.86	5.0	12.7	7.2
Serum 2	84	405	13	3.2	17.5	4.3
Serum 3	84	960	19.7	2.1	31	3.2
Serum 4	84	1230	27.4	2.2	96.4	3.8
Serum 5	84	1940	40.9	2.1	72.6	3.7
PC Varia 1	84	229	8.96	3.9	12.4	5.4
PC Varia 2	84	447	12.2	2.7	18.6	4.2
PC Varia 3	84	934	20.2	2.2	38.4	4.1

b. Linearity/assay reportable range:

Linearity of Vitamin B12 II assay was evaluated according to CLSI EP6-A. A high analyte serum sample (spiked) was serially diluted into 10 concentrations (from 2239 pg/mL to 110 pg/mL) with a buffered human serum albumin matrix. Samples were measured 3 times (n=3) within a single run on cobas e 411. The measured values were plotted against the target values and the regression results support the sponsor's claimed measuring range of 150-2000 pg/mL. The linear regression equation is summarized in the table below.

Range tested (pg/mL)	Correlation Coefficient(R ²)	Slope	Intercept
110 to 2239	0.998	1.0713	-14.5517

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

Traceability:

The Elecsys Vitamin B12 II assay is traceable to the Elecsys Vitamin B12 assay (k060755).

Vitamin B12 II CalSet Value assignment:

A human serum sample panel set (Master Calibrators) is available and traceable to the Elecsys Vitamin B12 assay (predicate device). The Kit Calibrators (two levels) are prepared from Vitamin B12 spiked in human serum matrix. Reagent comparison is performed using at least 3 instruments of each platform with at least 6 runs. The Master Calibrators and Kit Calibrators are run in duplicate. The values of PreciControl Varia (level 1&2) are read off from the calibration curve and should be

within the pre-defined acceptance criteria for PreciControl Varia to release the assigned values for Vitamin B12 II CalSet.

For Vitamin B12 II CalSet, the following production targets for the CalSet levels are defined in Table below.

	Target value (pg/mL)	Target range (pg/mL)
Cal 1	250	<300
Cal 2	1500	1450-1650

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:

Reagent:

Open-vial, stored at 2-8°C: 84 days

On-board/room temperature: 35 days

On-board/refrigerated: 60 days

Shelf-life, at 2-8°C: the study is on-going to support a claim of 24 months. Currently, the shelf life claim is 12 months based on the real-time stability data.

Calibrators:

Stability of Reconstituted Vitamin B12 II Cal Set	
-20 °C	84 days
2-8 °C	72 hours
On-board 20-25 °C	At least 5 hours, use only once

Additionally, the sponsor provided studies supporting the following calibration stability claim: 1 month for lot calibration and 7 days for on-board calibration.

d. Detection limit:

The Limit of Blank (LoB), Limit of Quantitation (LoQ) and Limit of Detection (LoD) were determined according to CLSI EP17-A.

Limit of Blank (LoB): A blank sample in buffered human serum albumin matrix was measured in 5 replicates/run, 2 runs/day for 3 days, using 3 reagent lots and on two instruments (n= 180). The LoB was calculated according to CLSI EP17-A2 (non-parametric approach).

Limit of Detection (LoD): Five low level serum samples (native and dilutes) were measured in singles, 2 runs/day for 3 days, using 3 reagent lots and on two instruments (n=60). $LoD = LoB + 1.653 \times SD \text{ total}$.

Limit of Quantitation (LoQ): The limit of quantification (LoQ) is defined as the

lowest analyte concentration that can be reproducibly measured with a total %CV of $\leq 20\%$. 10 human serum samples were measured in 5 replicates/run, one run/day for 5 days, using 3 reagent lots on one instrument (n=25 per reagent lot).

The results of the detection limits are summarized in the table below:

LoB	LoD	LoQ
44.3 pg/mL	89.2 pg/mL	129 pg/mL

Based on the detection limits studies, sponsor claimed that the measuring range of the candidate device is 150 pg/mL to 2000 pg/mL.

e. *Analytical specificity:*

Cross reactivity:

Cobinamide was tested at 4 levels of 30, 60, 120 and 210 ng/mL in serum samples with low and high levels of vitamin B12 (approximately 129 pg/mL and 550 pg/mL). No significant cross-reactivity was observed based on the study results. The results are summarized in the table below:

Cross reactant	Max. concentration Tested (ng/mL)	Highest cross reactivity observed (%)
Cobinamide dicyanide	210	0.003

Endogenous Interference:

Interference testing was performed according to CLSI EP7-A2. Three human serum samples containing low, medium and high concentrations of Vitamin B12 were used on cobas e 411 Immunoassay analyzer using the Elecsys Vitamin B12 II assay. One aliquot of each low, medium and high samples were spiked with these interfering substances: Rheumatoid Factor, Biotin, Lipemia, Hemoglobin (two hemoglobin levels of 60 mg/dL and 1000 mg/dL were used), Bilirubin, IgG, IgM, and IgA. Another aliquot was spiked with the same volume of isotonic saline solution. The spiked sample was then diluted in 10% increments. The recovery was determined by testing a control sample without the interferent and comparing it to the value obtained from a test sample in which the potential interferent had been added. The sponsor's defined acceptance criteria for non-significant interference are:

Samples ≤ 200 pg/mL: Recovery within ± 20 pg/mL of un-spiked reference value

Samples > 200 pg/mL: Recovery within $\pm 10\%$ of un-spiked reference value.

The highest tested concentration of endogenous substance without significant interference are summarized in the table below and stated in the product insert:

Endogenous substance	None significant Interference seen up to	
Biotin	50	ng/mL
Intralipid (Lipemia)	1500	mg/dL
Hemoglobin	100	mg/dL
Bilirubin	65	mg/dL
Rheumatoid Factor	1500	IU/mL
Human IgG	28	g/L
Human IgM	10	g/L
Human IgA	16	g/L

Based on hemoglobin testing, a falsely depressed (-17% bias) result from hemoglobin of >100 mg/dL was observed; therefore sponsor has the following limitations in the specimen collection and preparation section of the package insert:

“Do not use any hemolyzed samples because samples showing visible signs of hemolysis will have falsely depressed results.”

In addition, the sponsor states the following in the limitation section in the package insert:

“Samples should not be taken from patients receiving therapy with high biotin doses (i.e. >5 mg/day) until at least 8 hours following the last biotin administration.”

Exogenous Interferences/Drugs:

Sixteen commonly used pharmaceuticals were examined for potential effect on the Vitamin B12 II assay. Each drug was spiked into 2 serum samples (approximate concentration of the samples 200 and 1200 pg/mL) and was tested in 8 replicates one cobas e 411 Immunoassay Analyzer. The drug concentrations tested are according to the recommendation (if available) given in the CLSI guideline EP7-A2. The sponsor defined significant interference is $\pm 10\%$ bias in the spiked sample as compared to the control sample.

For common drug interferences, results of the concentration tested that did not show significant interference are summarized in the table below.

Substance tested	Concentration tested that showed non-significant interference (mg/mL)
Acetylcystein	1660
Acetaminophen	200
Acetylsalicylic acid	1000
Ampicillin-Na	1000

Ascorbic Acid	300
Cefoxitin	2500
Cyclosporin	5
Doxycycline	30
Heparin	5000 U
Ibuprofen	500
Levodopa	20
Methyldopa+1.5	20
Metronidazole	120
Phenylbutazone	400
Rifampicin	60
Theophylline	100

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

112 human serum samples (all single donors, native as well as 11 spiked samples) were measured in singleton covering Vitamin B12 values ranged between 153 –1812 pg/mL. The study was performed on the cobas e 411 analyzer using the predicate device (X) and the candidate device (Y).

The following is the Passing Bablok regression result:

$$y = 0.953x - 11.1$$

Correlation coefficient Pearson(r) = 0.942

n=112

b. *Matrix comparison:*

Serum, Li-Heparin, Na-Heparin, K₂-EDTA- plasma, K₃-EDTA- plasma tubes and Li-Heparin plasma Gel separation tubes are the recommended specimen types for the Vitamin B12 II assay. A minimum of 90 serum/plasma pairs (native, spiked) were tested in singleton with one reagent lot on one cobas e 411 immunoassay analyzer.

Passing- Bablok regression was used to fit the vitamin B12 results of each of the plasma types against serum. The data and regression analysis are summarized below:

	Li-Heparin	Na-Heparin	K ₂ -EDTA	K ₃ -EDTA	Li-Heparin plasma Gel
n	93	93	95	95	90
Slope	0.992	1.01	0.984	1.00	1.00

Y- Intercept(pg/mL)	7.42	1.09	1.21	-10.0	1.15
Correlation Coefficient	0.995	0.993	0.996	0.991	0.997

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 table below shows reference range from samples on four sites of an apparently healthy population in the United States, using the Elecsys Vitamin B12 II assay. The samples tested include 120 sera (66 men, 54 women, age range of 22 and 79 years). All the samples were native serum samples, measured in singleton, over 2 run for 2 days with one reagent lot and on one cobas e 411 analyzer. The reference range was determined using the median value and the 2.5th -97.5th percentile (pg/mL).

The sponsor states that these values should be used as guidelines; each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

n	Median (pg/mL)	Range (2.5 th -97.5 th percentile) (pg/mL)
120	443	232-1245

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.