DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

ROCHE DIAGNOSTICS
KELLI TURNER
REGULATORY AFFAIRS PRINCIPAL
9115 HAGUE ROAD
INDIANAPOLIS IN 46250

September 24, 2015

Re: K151786
Trade/Device Name: Elecsys Vitamin B12 II Assay, Elecsys Vitamin B12 II CalSet
Regulation Number: 21 CFR 862.1810
Regulation Name: Vitamin B12 test system
Regulatory Class: II
Product Code: CDD, JIT
Dated: June 29, 2015
Received: July 1, 2015

Dear Ms. Turner:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Katherine Serrano -S

For: Courtney H. Lias
Director
Division of Chemistry and Toxicology Devices
Office of In Vitro Diagnostics and
Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number : k151786

Device Name
Elecsys Vitamin B12 II assay

Indications for Use *(Describe)*
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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number:*k151786*

Device Name
Elecsys Vitamin B12 II CalSet

Indications for Use *(Describe)*
Vitamin B12 II CalSet is used for calibrating the quantitative Elecsys Vitamin B12 II assay on the Elecsys and cobas e immunoassay analyzers.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

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Elecsys Vitamin B12 II Test System
510(k) Summary:k151786

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of 21 CFR 807.92.

Submitter Name	Roche Diagnostics
Address	9115 Hague Road Indianapolis, IN 46250
Contact	Kelli Turner Phone: (317) 521-4515 FAX: (317) 521-2324 Email: kelli.turner@roche.com
Date Prepared	06/29/2015
Proprietary Name	1. Elecsys Vitamin B12 II assay, 2. Elecsys Vitamin B12 II CalSet
Common Name	1. Vitamin B12 II assay 2. Vitamin B12 II CalSet
Classification Name	1. Radioassay, Vitamin B12 2. Secondary, calibrator
Product Codes	1. CDD, 862.1810 2. JIT, 862.1150
Predicate Devices	Elecsys Vitamin B12 Assay (k060755)
Establishment Registration	Roche Diagnostics GmbH in Mannheim, Germany, is 9610126 Roche Diagnostics GmbH in Penzberg, Germany, is 9610529 Roche Diagnostics in the United States is 1823260

1. DEVICE DESCRIPTION

The Elecsys Vitamin B12 II 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.

Results are determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via reagent barcode.

1.1. Reagents

The reagent working solutions include:

- the rackpack (kit placed on instrument)
- Streptavidin coated microparticles,
- Reagent 1 (ruthenium labeled intrinsic factor) and
- Reagent 2 (vitamin B12 labeled biotin).
- Pretreatment 1 (Dithiothreitol)
- Pretreatment 2 (sodium hydroxide, sodium cyanide)

1.2. Calibrator

The Vitamin B12 II CalSet is a lyophilized human serum matrix with added vitamin B12 in two concentration ranges.

The CalSet includes:

- Cal 1 (approximately 250 pg/mL vitamin B12 in a Human serum matrix)
- Cal 2 (approximately 1500 pg/mL vitamin B12 in a Human serum matrix)

2. INDICATIONS FOR USE

Elecsys B12 II assay
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.

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

3. TECHNOLOGICAL CHARACTERISTICS

Table 1: Assay Comparison: General Assay Features

Feature	Predicate Device: Elecsys Vitamin B12 (k060755)	Candidate Device: Elecsys Vitamin B12 II Assay
Intended Use/ Indications for Use	Binding assay for the in vitro quantitative determination of vitamin B12 in human serum and plasma. The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers. A Vitamin B12 test system is a device intended to measure Vitamin B12 in serum, plasma. Measurements obtained by this device are used in the diagnosis and treatment of anemias of gastrointestinal malabsorption.	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.
Assay Protocol	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.
Applications	27-minute application	Same.
Instrument Platform	Elecsys and cobas e immunoassay analyzers.	Same. (submission for **cobas e** 411)
Sample Volume	15 µL	Same.
Sample Type	Human serum and plasma.	Same.
Reagents	Competition principle. Total duration of assay: 27 minutes	Same.
Calibrator	Elecsys Vitamin B12 CalSet II	Elecsys Vitamin B12 II CalSet

Feature	Predicate Device: Elecsys Vitamin B12 (k060755)	Candidate Device: Elecsys Vitamin B12 II Assay
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: • 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
Traceability / Standardization	Standarized against the Elecsys Vitamin B12 assay (k973702)	Standardized against the Elecsys Vitamin B12 assay (k060755). Accuracy to WHO Standard 03/178
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	Unopened: 2-8°C - Up to the stated expiration date. After Opening at 2-8°C - 12 weeks 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
Measuring Range	30.0 – 2000 pg/mL	150- 2000 pg/mL

Feature	Predicate Device: Elecsys Vitamin B12 (k060755)			Candidate Device: Elecsys Vitamin B12 II Assay		
Precision	**cobas e** 411: Within-run Sample / Mean (pg/mL) / SD / CV HS 1 / 192 / 11.3 / 5.9% HS 2 / 358 / 20.1 / 5.6% HS 3 / 864 / 29.1 / 3.3% HS 4 / 1625 / 62.1 / 3.8% PCV0 / 221 / 10.2 / 4.6% PCV1 / 467 / 18.5 / 4.0% PCV2 / 958 / 31.1 / 3.3% Total Sample / Mean (pg/mL) / SD / CV HS 1 / 192 / 19.8 / 10.3% HS 2 / 358 / 27.7 / 7.7% HS 3 / 864 / 40.1 / 4.5% HS 4 / 1625 / 65.1 / 4.0% PCV0 / 221 / 10.2 / 4.6% PCV1 / 467 / 18.5 / 4.0% PCV2 / 958 / 31.1 / 3.3%			**cobas e** 411: Within-run (will be labeled Repeatability) Sample / Mean (pg/mL) / SD / CV HS 1 / 176 / 8.86 / 5.0% HS 2 / 405 / 13 / 3.2% HS 3 / 960 / 19.7 / 2.1% HS 4 / 1230 / 27.4 / 2.2% HS 5 / 1940 / 40.9 / 2.1% PCV0 / 229 / 8.96 / 3.9% PCV1 / 447 / 12.2 / 2.7% PCV2 / 934 / 20.2 / 2.2% Total (will be labeled Intermediate precision) Sample / Mean (pg/mL) / SD / CV HS 1 / 176 / 12.7 / 7.2% HS 2 / 405 / 17.5 / 4.3% HS 3 / 960 / 31.0 / 3.2% HS 4 / 1230 / 46.4 / 3.8% HS 5 / 1940 / 72.6 / 3.7% PCV0 / 229 / 12.4 / 5.4% PCV1 / 447 / 18.6 / 4.2% PCV2 / 934 / 38.4 / 4.1%		
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		
Analytical Specificity	Cross reactant	concentration tested (ng/mL)	Highest cross-reactivity observed (%)	Cross reactant	concentration tested (ng/mL)	Highest cross-reactivity observed (%)
	Cobinamide	200	0.024	Cobinamide	210	0.003
Linearity	30 to 2000 pg/mL			150 to 2000 pg/mL		

Feature	Predicate Device: Elecsys Vitamin B12 (k060755)	Candidate Device: Elecsys Vitamin B12 II Assay
Limitations	The assay is unaffected by: • Bilirubin < 65 mg/dL • Hemolysis < 1.0 g/dL • Lipemia < 1500 mg/dL • Biotin < 50 ng/mL • Rheumatoid factors < 1500 IU/mL • In vitro tests were performed on 54 commonly used pharmaceuticals. No interference with the assay was found. • In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design. For diagnostic purposes, the results should always be assessed in conjunction with RBC folate, the patient's medical history, clinical examination and other findings.	The assay is unaffected by: • Bilirubin ≤ 65 mg/dL • Hemolysis ≤ 0.1 g/dL • Lipemia ≤ 1500 mg/dL • Biotin ≤ 50 ng/mL • Rheumatoid factors < 1500 IU/mL • IgG ≤ 28 g/dL • IgM ≤ 10 g/dL • IgA ≤ 16 g/dL • In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found. • In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design. • Because intrinsic factor is typically used as the binding protein in serum vitamin B12 assays, anti-intrinsic factor antibodies (which are common in pernicious anemias) can lead to elevated vitamin B12 measurement values. • The Elecsys Vitamin B12 II assay is designed to avoid interference due to anti-intrinsic factor antibodies. For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings. Note: the presence of immunoglobulin-vitamin B12 complexes may cause unexpectedly high values of vitamin B12.

Table 2: Assay Comparison: Labeled Performance Characteristics

Feature	Predicate Device: Elecsys Vitamin B12 (k060755)						Candidate Device: Elecsys Vitamin B12 II Assay					
Reference range study	**Country**	**(N)**	**Median**		**2.5th-97.5th percentile**		**Subject cohort**	**(N)**	**Median**		**2.5th-97.5th percentile**	
	USA	178	pg/mL	Pmol/L	pg/mL	pmol/L	Apparently healthy male & female	120	pg/mL	pmol/L	pg/mL	pmol/L
			463	342	211-946	156-698			443	327	232-1245	171-919

Method Comparison			
	n = 120	**Passing/Bablok**	**Linear regression**
	Min = 156 pg/mL		
	Max = 1753 pg/mL		
	Slope	**0.950**	**0.984**
	Intercept	-9.83	-26.7
	Tau/r	0.941	0.996
	Bias at 200 pg/mL	-9.88	-15.0

HS= Human Serum

PCV0=PreciControl Varia level 0

PCV1=PreciControl Varia level 1

PCV2=PreciControl Varia level 2

Table 3: CalSet Comparison

Characteristic	Predicate device: Elecsys Vitamin B12 CalSet II	Candidate device: Elecsys Vitamin B12 II
Intended Use	Vitamin B12 CalSet II is used for calibrating the quantitative Elecsys Vitamin B12 assay on the 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.
Analyte	Vitamin B12	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

Characteristic	Predicate device: Elecsys Vitamin B12 CalSet II	Candidate device: Elecsys Vitamin B12 II
Handling	Carefully dissolve the contents of one bottle by adding exactly 1.0 mL of distilled or deionized water and allow to stand closed for 15 minutes to reconstitute. Mix carefully, avoiding foam formation. Transfer aliquots of the reconstituted calibrators into empty labeled snap‑ cap bottles (CalSet Vials). Attach the supplied labels to the additional bottles. Store the aliquots immediately at ‑ 20 °C. Perform only one calibration procedure per aliquot.	Same.

4. NON-CLINICAL PERFORMANCE EVALUATION

Non-clinical performance evaluations for the Elecsys Vitamin B12 II executed with the study briefly summarized.

4.1. Precision

The precision results were obtained using serum samples evaluated on the **cobas e** 411 Immunoassay Analyzer. Within-run precision (repeatability) and total imprecision (intermediate precision) were determined according to CLSI Guideline EP5-A2. The protocol included testing 2 replicates per run, 2 runs per day for 21 days.

Specifications:

	Repeatability	Intermediate precision
≤ 200 pg/mL	SD ≤ 14 pg/mL	SD ≤ 24 pg/mL
> 200 pg/mL	CV ≤ 7 %	CV ≤ 12 %

All results met the pre-defined acceptance criteria for repeatability and intermediate precision.

4.2. Limit of Blank

For the analytical sensitivity studies, 2 **cobas e** 411 analyzers and 3 lots of reagents were used. The Limit of Blank (LoB) was determined using a buffered human serum albumin matrix similar to human serum (no analyte added). A total of n = 60 LoB measurements were made (5 replicates, 2 runs per day on 2 instruments over 3 days). The LoB was calculated according to CLSI EP17-A2 (non-parametric approach). Acceptance criterion: LoB ≤ 50 pg/mL

4.3. Limit of Detection

For the analytical sensitivity studies, 2 **cobas e** 411 analyzer and 3 lots of reagents were used. The Limit of Detection (LoD) was determined using 5 low-level human serum samples (native and diluted). A total of n = 60 LoD measurements were made (5 samples, 2 runs per day on 2 instruments over 3 days). The LoD was calculated according to CLSI EP17-A2 (chapter 5.3.3.2). Acceptance criterion: LoD ≤ 100 pg/mL

4.4. Limit of Quantitation

The Limit of Quantitation (LoQ) was determined using a minimum set of seven human serum samples, three reagent lots on one **cobas e** 411 analyzer. The Limit of Quantitation (LoQ) was determined in accordance with CLSI Guideline EP17-A2. Each sample was analyzed in replicates of 5, one run per day over 5 days. Acceptance criterion: LoQ (Imprecision) ≤ 20 % at 150 pg/mL.

4.5. Dilution

To demonstrate the Vitamin B12 II assay dilution study, four different dilutions with dilution factors between 1:1.5 and 1:3 were prepared.

Five serum samples were spiked with vitamin B12 to concentrations exceeding the measuring range. The samples were diluted with Elecsys Diluent Universal and recovery was investigated on the cobas e 411. The vitamin B12 concentrations of the undiluted samples were calculated by multiplying the result of the diluted samples using the appropriate dilution factor.

4.6. Linearity

The linearity results were obtained with serum samples on the **cobas e** 411 Immunoassay analyzer. Linearity was determined according to CLSI Guideline EP6-A. All results met the pre-defined acceptance criteria for linearity. The linearity results support a claimed measuring range.

Acceptance criteria:

- Significant level for deviation to higher order polynomial: 5%
- Limits for deviation to higher order polynomial regression
    - ≤ 200 pg/mL: ± 20 pg/mL
    - > 200 pg/mL: ± 10 %

4.7. Analytical Specificity

The specificity was determined using two human serum samples (single donors, native) spiked with potential cross-reactant compounds. The analyte concentration of the samples was at 129 and 550 pg/mL Vitamin B12. Four levels of the cross-reactant were prepared and measured in duplicate. The spiked and non-spiked samples were tested in duplicates on the **cobas e** 411 Immunoassay Analyzer. Cross-reactivity results will be reported in the method sheet.

4.8. Endogenous Interferences

The effect on quantitation of analyte in the presence of endogenous interfering substances was determined on the **cobas e** 411 Immunoassay analyzer using human serum samples (single donors, native as well as spiked). For each interfering substance (Biotin, Lipemia, Hemoglobin, Bilirubin, Rheumatoid Factor, IgG/IgM/IgA) three human serum samples containing low, mid and high concentrations of Vitamin B12 were tested. The recovery for each sample was calculated by comparison to the reference sample. Predefined acceptance criterion was met. The claims included in the method sheet were set set to the concentration without observed interference.

Acceptance criterion:

- ≤ 200 pg/mL: ± 20 pg/mL of unspiked reference value
- > 200 pg/mL: ± 10% of unspiked reference value

4.9. Exogenous Interferences- Drugs

The effect on quantitation of analyte in the presence of drugs was determined by comparing values obtained from samples spiked with 16 pharmaceutical compounds spiked into two human serum samples (single donors, native) and tested on the **cobas e** 411 Immunoassay Analyzer. The analyte concentration of the samples were approximately 200 and 1200 pg/mL. The drug concentrations tested are in accordance with the recommendation (if available) given in the CLSI guideline EP7-A2. When concentrations are not given in the guideline, concentrations of at least 3-times of the maximum recommended daily dose were tested. The two serum samples were divided into aliquots and spiked with the potential interferents. The reference sample without interferent was spiked with the respective amount of solvent only.

The concentration of the spiked aliquots was determined in 8-fold determination and compared to the Vitamin B12 concentration determined for the reference aliquot (also in 8-fold determination). Acceptance criterion: ± 10% of the reference value (unspiked sample).

4.10. Exogenous Interferences- Anticoagulants

The effect on quantitation of analyte in the presence of anticoagulants with the Elecsys Vitamin B12 II Immunoassay was determined by comparing values obtained from samples (single donors, native as well as spiked) drawn into Serum, Li-Heparin, Na-Heparin, K2-EDTA- , K3-EDTA-plasma primary tubes and Li-Heparin Plasma Gel Separation Tubes. A minimum of 90 serum/plasma pairs per sample material were tested in singleton with one reagent lot on one **cobas e** 411 Immunoassay Analyzer. Potential effects are assessed by regression analysis.

Acceptance criterion:

- Slope (Passing/Bablok): 0.9 – 1.1
- Intercept (Passing/Bablok): < ± 100 pg/mL
- coefficient of correlation Pearson's r (linear regression): ≥ 0.95
- Bias at 200 pg/mL: ± 10 %

4.11. Method Comparison

A method comparison was performed using the current Elecsys Vitamin B12 assay (cleared under K060755) as predicate device.

A total of 120 human serum samples (all single donors, native as well as spiked) were measured in singleton covering the entire measuring range.

The study was performed on the **cobas e** 411 analyzer over 3 runs using the current Elecsys Vitamin B12 assay (X) and the updated Elecsys Vitamin B12 II assay (Y). Regression analysis was performed.

Acceptance criteria:

- Slope (Passing/Bablok): 1.00 ± 0.05
- Correlation (linear regression) Pearson's r: ≥ 0.95
- Intercept (Passing/Bablok): ≤ ± 30 pg/mL
- Bias at 200 pg/mL: ≤ ± 10 %

4.12. Reagent Stability

To test reagent stability, four studies were executed with three studies completed.

4.12.1. Study 1. Reagent stability refrigerator/onboard (60 days)

Reagent stability refrigerator / onboard was tested on one **cobas e** 411 Immunoassay Analyzer.

A fresh reagent rackpack (kit) was placed on the analyzer and calibrated. Reference values for the five human serum (HS) samples and three controls were determined in duplicate. After

measurement the rackpack was removed from the analyzer and kept at 2-8 °C for 64 days. During this period of time, the kit was stressed alternately at 20°C + 3°C (on-board condition) 10 times for 8 hours (80 hours in total). On day 8, 15, 29, 50 and day 64, the same samples were measured with this reagent kit kept under alternating storage conditions (refrigerator /on-board) using the calibration curves established on day 0, 8, 22, 43 and 57, respectively. Acceptance criterion for recovery was compared to day 0 value.

4.12.2. Study 2. Reagent stability after first opening at 2-8°C (84 days)

Reagent stability after first opening for the Elecsys Vitamin B12 II assay was tested on one **cobas e** 411 Immunoassay Analyzer.

A fresh reagent rackpack was placed on the analyzer and calibrated. Reference values for the five human serum (HS) samples and three controls were determined in duplicate. After measurement the kit was removed from the analyzer and kept at 2-8 °C up to 92 days. After 36, 64 and 92 days the kit was placed on the analyzer again, calibrated and the test samples were determined. Acceptance criterion for recovery was compared to day 0 value.

4.12.3. Study 3. On board reagent stability (35 days)

Reagent On-board Stability and Calibration Stability for the Elecsys Vitamin B12 II assay was tested on one **cobas e** 411 Immunoassay Analyzer. A fresh Reagent Rack-Pack was placed on the analyzer and calibrated. Reference values for the five human serum (HS) samples and three controls were measured in duplicate on day 0. On day 8, 22 and day 36, the same samples were measured with the same reagent kit kept at 20°C + 3°C (on-board condition) using the calibration curves established on day 0, 15 and 29, respectively. Acceptance criterion for recovery was compared to day 0 value.

4.12.4. Study 4: A real-time stability study is ongoing to support shelf-life stability claim.

In the ongoing real-time stability study, the Elecsys Vitamin B12 II reagent is stored at 2-8°C. The stored assay reagents are tested at time point T=0 (after manufacturing) and at specified intervals over the shelf life of the device up to the planned shelf life plus one month. Testing will be performed using PreciControl Varia 3, Level 0, 1 and 2 (lyopylized, stored at -20°C) and in addition three human serum samples (stored at -80°C).

Data for the time-points at 0, 7, 10, 13, 19 and 25 months tested in duplicate will be available. The average on-test recovery value will be calculated as percent recovery compared to the reference value:

- assigned value for PreciControl Varia 3, Level 0, 1 and 2
- value measured at T=0 for the human serum samples

The acceptance criterion is recovery of 80 -120 % for PC Varia and the human serum samples.

4.13. Sample Stability

To test sample stability, three studies were completed. Because these studies are not analyzer dependent, and the assay is a global product currently available rest of world (ROW), the studies were executed on the **cobas e** 601. Study results can be applied to the **cobas e** 411 since sample stability is independent of the analyzer. For each study, the samples used were all single donors (native, spiked and diluted) and the following acceptance criteria was applied.

Acceptance criteria for recovery compared to the reference value:

- ≤ 200 pg/mL: ± 28 pg/mL
- > 200 pg/mL: ± 14 %

4.13.1. Study 1. Sample stability at 2-8°C

Five samples for each sample type (Serum, K2-EDTA-, K3-EDTA-, Li-Heparin- and Na-Heparin plasma) were aliquoted and measured after storage at 2-8°C for 49 hours. Measurements were performed with three-fold determination and recovery was calculated as percent of the reference value.

4.13.2. Study 2. Sample stability at Room Temperature (15-25°C)

Five samples for each sample type (Serum, K2-EDTA-, K3-EDTA-, Li-Heparin- and Na-Heparin plasma) were aliquoted and measured after storage at 15-25°C for 3 hours. The aliquot for the reference value was stored at -80°C and measured at the same time point.

Measurements were performed with three-fold determination and recovery was calculated as percent of the reference value.

4.13.3. Study 3. Sample stability -15 to -25°C

Five samples for each sample type (Serum, K2-EDTA-, K3-EDTA-, Li-Heparin- and Na-Heparin plasma) were aliquoted and measured after storage at -15 to -25°C for 57 days. The aliquot for the reference value was stored at -80°C and measured at the same time point. Measurements were performed with three-fold determination and recovery was calculated as percent of the reference value.

4.14. Calibrator studies

The Elecsys Vitamin B12 II CalSet was evaluated for value assignment, reconstitution and stability.

4.14.1. Value assignment

Value assignment testing was conducted and passed pre-defined acceptance criteria. The target values for the two levels of the Vitamin B12 II CalSet kit are chosen to obtain the best fit with the Master Calibration Curve, together with the Rodbard curve parameters encoded in the reagent barcode. For each Elecsys Vitamin B12 II CalSet lot manufactured, the calibrators are run in duplicate on at least three (3) **cobas e** 411 analyzers and at least three (3) **cobas e** 601/MODULAR ANALYTICS E170 analyzers with all Vitamin B12 II reagent lots available. The assigned value of each calibrator is defined as the mean value obtained over at least six (6) runs on at least three (3) analyzers) of the respective calibrator.

Measurement values for PreciControl Varia (Levels 0, 1 & 2), a multi-analyte control recommended for use to monitor accuracy and precision of specified analytes, are read from the calibration curves generated. The pre-defined acceptance criteria for PreciControl Varia have to be met to release the Assigned Values for Vitamin B12 II CalSet.

4.14.2. Reconstitution

Reconstitution time for the lyophilized Vitamin B12 II CalSet was tested. Two sets of Vitamin B12 II CalSet were reconstituted, one for 15 minutes and the other for 30 minutes. Signal recovery after 30 minutes reconstitution was compared to the signal value after 15 minutes.

Vitamin B12 II CalSet was evaluated in duplicate on the cobas e 411 analyzer as a reference.

The acceptance criterion was 90 to 110 % signal recovery of the reference material value.

4.14.3. Stability

Three studies were performed in order to verify the stability claims for the Vitamin B12 II CalSet. Stability studies after reconstitution and an accelerated stability study were completed on the **cobas e** 411.

4.14.3.1. Study 1- Stability at -20ºC (after reconstitution)

The on-test and reference materials were tested in duplicate on the **cobas e** 411. The on-test material was reconstituted and stored in closed vials for 85 days at -20°C. The on-test signal recovery was calculated as percent of the reference value.

The acceptance criterion was 90 to 110 % signal recovery of the reference material value.

4.14.3.2. Study 2- Stability at 2 to 8°C after reconstitution

The on-test and reference materials were tested in duplicate on the **cobas e** 411. The on-test material was reconstituted and stored in closed vials for 73 hours at 2-8°C. The on-test signal recovery was calculated as percent of the reference value.

The acceptance criterion was 90 to 110 % signal recovery of the reference material value.

4.14.3.3. Study 3- Onboard stability at 20-25ºC after reconstitution

The on-test and reference materials were tested in duplicate on the **cobas e** 411. The on-test material was reconstituted and stored in open vials for 6 hours at 20-25°C. The on-test signal recovery was calculated as percent of the reference value.

The acceptance criterion was 90 to 110 % signal recovery of the reference material value.

4.14.3.4. Real-time Stability

Since there was no change in formulation and target values to the Vitamin B12 II CalSet the real-time stability data (30 months at 2-8°C) of the current Vitamin B12 CalSet II is applicable.

5. CLINICAL PERFORMANCE EVALUATION

Clinical samples were collected at four sites in the United States in order to establish the reference range values for the Elecsys Vitamin B12 II assay. Reference ranges for apparently healthy males and apparently healthy females were determined using the median value and the 2.5^{th} - 97.5^{th} percentiles (pg/mL) as lower and upper limit of normal, respectively. The evaluation was done at one site in Germany (Roche Diagnostics GmbH, R&D department, Penzberg) with one reagent lot (MP) using one **cobas e** 411 analyzer. Samples were all native human serum samples measured in singleton, over 2 runs for 2 days.

6. CONCLUSIONS

The information provided in this Premarket Notification [510(k)] will support a determination of substantial equivalence for the Elecsys Vitamin B12 II Test System for the measurement of vitamin B12 in serum and plasma samples The data supports a safe, effective device which performs as well as or better than the predicate device.