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

A. 510(k) Number:

k082340

B. Purpose for Submission:

New Device

C. Measurand:

Folate

D. Type of Test:

Quantitative immunoassay

E. Applicant:

Roche Diagnostics, Inc.

F. Proprietary and Established Names:

Roche Elecsys Folate III, Roche Elecsys RBC Folate Hemolysing Reagent, Roche Elecsys Folate III CalSet, Roche Elecsys Folate III CalCheck

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
CGN	II	862.1295	75
JIT	II	862.1150	75
JJY	I (reserved)	862.1660	75
JJX	I (reserved)	862.1660	75

H. Intended Use:

1. Intended use(s):

(1) Elecsys Folate III: Binding assay for the in vitro quantitative determination of folate in human serum and plasma. The binding assay is intended for use on Elecsys and cobas e immunoassay analyzers. Measurements obtained by these devices are used in the diagnosis and treatment of anemias. For in vitro diagnostic use.

(2) Elecsys RBC Folate Hemolyzing Reagent: Elecsys RBC Folate Hemolyzing Reagent is used together with the Elecsys Folate III assay for the quantitative determination of folate in erthrocytes (RBC (red blood cell) folate). Binding assay for the in vitro quantitative determination of folate in human serum and plasma. The binding assay is intended for use on Elecsys and cobas e immunoassay analyzers. Measurements obtained by these devices are used in the diagnosis and treatment of anemias.

(3) Elecsys Folate III CalSet: Elecsys Folate III CalSet is used for calibrating the quantitative Elecsys Folate III assay on the Elecsys and cobas e immunoassay analyzers.

(4) Elecsys Folate III CalCheck: Elecsys Folate III CalCheck is used for the verification of the calibration established by the Elecsys Folate III reagent on the indicated Elecsys and cobas e immunoassay analyzers.

(5) Elecsys PreciControl Anemia: Elecsys PreciControl Anemia is used for the quality control of specified Elecsys immunoassays on Elecsys and cobas e immunoassay analyzers.

2. Indication(s) for use:

   See indications for use above

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Roche Elecsys 2010/cobas e411

I. Device Description:

The Elecsys Folate III assay consists of the following:

Pretreatment reagent 1: Sodium 2-mercaptoethanesulfonate

Pretreatment reagent 2: Sodium hydroxide 25

Streptavidin-coated microparticles with preservative.

R1: Ruthenium labeled folate binding protein, stabilizers and preservatives

R2: Biotinylated folate, stabilizers and preservatives

The Elecsys RBC Folate Hemolyzing reagent consists of ascorbic acid.

The Folate III Calset consists of lyophilizedhuman serum with folic acid in two concentration ranges.

The Elecsys Folate III CalCheck consists of human serum with added folic acid at three concentration levels.

The Elecsys PreciControl Anemia consists of lyophilized human serum matrix in three concentration ranges. 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. The testing methods applied were FDA-approved or cleared in compliance with the European Directive 98/79/EC, Annex II, List A

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Elecsys Folate II Assay

   Elecsys RBC Folate Hemolyzing Reagent

   Elecsys Folate II CalSet II

   Elecsys Folate II CalCheck II

   Elecsys PreciControl Anemia

2. Predicate K number(s):

   k043318

   k051292

   k042490

   k043320

   k051517

3. Comparison with predicate:

Similarities		
Item	Device	Predicate (k043318)
Assay Protocol	Electrochemiluminescent Immunoassay	Electrochemiluminescent Immunoassay
Instrument Platform	Roche Elecsys 2010/ cobas e 411	Roche Elecsys 2010/ cobas e 411
Calibration frequency	Once per reagent lot and •After 1 month when using same reagent lot •After 7 days when using same reagent kit •As required per QC findings or pertinent regulations	Once per reagent lot and •After 1 month when using same reagent lot •After 7 days when using same reagent kit •As required per QC findings or pertinent regulations
CalSet and CalCheck Format	Lyophilized	Lyophilized
Precicontrol Matrix	Human serum	Human serum
Precicontrol Format	Lyophylized	Lyophilized

Differences		
Item	Device	Predicate (k043318)
Sample type	Serum/whole blood	Serum/whole blood
CalSet and CalCheck Matrix	Human serum preserved with 0.5% Bronidox L and buffered with 50mM HEPES	Human serum
Preci-control Anemia	Target values updated for Folate III assay	Target values updated for Folate II assay
Calibrator	Elecsys Folate III CalSet	Elecsys Folate II CalSet II
Control Traceability	Standardized against the Elecsys Folate II assay	Standardized against the Elecsys Folate assay
Measuring Range	1.5 - 20.0 ng/mL	0.600 - 20.00 ng/mL
Analytical Sensitivity	Limit of Blank = 0.640 ng/mL Limit of Detection = 1.5 ng/mL Limit of Quantitation = 2.0 ng/mL	Lower Detection Limit = 0.6 ng/mL

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

CLSI EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation

CLSI EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods

L. Test Principle:

Serum samples:
First, the serum sample is incubated with the folate pretreatment reagents to release bound folate from endogenous folate binding proteins. Then, the pretreated sample is incubated with the ruthenium labeled folate binding protein and a folate complex is formed, the amount of which is dependent upon the analyte concentration in the sample. Next, streptavidin-coated microparticles and folate labeled with biotin are added and the unbound sites of the ruthenium labeled folate binding protein become occupied, with formation of a ruthenium labeled folate binding protein-folate biotin complex. The entire complex is bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is then aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are washed away and application of a voltage to the electrode induces chemiluminescent emission which is measured by a photomultiplier. Results are determined via a calibration curve.

Whole blood samples:
Whole blood treated with anticoagulants (heparin or EDTA) is diluted with ascorbic acid solution and incubated. Lysis of the erythrocytes takes place releasing the intracellular folate. The hemolysate is then used as a "pre-diluted" sample (analogously to serum) for subsequent measurement in the Elecsys Folate III assay. The hematocrit value determined in whole blood and the dilution effect brought about by pretreatment of the sample is to be taken into account when calculating the erythrocyte folate concentration.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

   Elecsys Folate III Reagent Precision

   Precision of the Elecsys Folate III reagent was evaluated on the Elecsys 2010/cobas e411 Immunoassay analyzer according to CLSI EP5-A2 guideline. The protocol consisted of testing 2 replicates of each control (PC=PreciControl) or human serum sample pools (HS=human serum) per run, 2 runs per day for 21 days. Total and within run precision was calculated according to EP5-A2. The results are summarized below.

Within Run Precision Elecsys 2010/cobas e411

Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)	n
HS Pool 1	3.41	0.239	7.0	84
HS Pool 2	7.88	0.339	4.3	84
HS Pool 3	16.6	0.498	3.0	84
PC Anemia 2	7.33	0.415	5.7	84
PC Anemia 3	15.8	0.734	4.7	84

Elecsys RBC Folate Hemolyzing Reagent Precision

In addition to testing precision of the Elecsys Folate III reagent, precision studies were also performed on the hemolysate application, Elecsys RBC Folate. The same protocol as described above for the serum folate application was used to test human whole blood samples with the RBC folate reagent. The results are summarized below.

Precision Elecsys 2010/cobas e411

Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)	n
Whole blood 1	229	16.1	7.0	84
Whole blood 2	350	25.2	7.2	84
Whole blood 3	481	34.6	7.2	84

Total Precision Elecsys 2010/cobas e411

Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)	n
HS Pool 1	3.41	0.452	13.3	84
HS Pool 2	7.88	0.550	7.0	84
HS Pool 3	16.6	0.827	5.0	84
PC Anemia 2	7.33	0.467	6.4	84
PC Anemia 3	15.8	0.986	6.3	84

Within Run Precision Elecsys 2010/cobas e411

Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)	n
Whole blood 1	229	12.2	5.3	84
Whole blood 2	350	17.0	4.9	84
Whole blood 3	481	25.7	5.3	84

b. Linearity/assay reportable range:

The linearity of the Elecsys Folate III was evaluated on the Elecsys 2010/cobas e411 Immunoassay analyzer. Serum linearity was determined by diluting three high analyte level serum patient samples. Samples 1 & 2 were diluted with Elecsys Universal Diluent and Sample 3 was diluted with low analyte level serum. Mixtures of 10 different concentrations of folate were tested. The expected values were generated using the concentrations measured in the diluted and the undiluted patient sample and then applying the dilution factors. The percent recovery was determined by dividing the measured concentration with the expected concentration. The sample concentrations tested ranged from 20 ng/mL to 1.19 ng/mL. All of the results were within ≤ 0.75 ng/mL for results ≤ 5 ng/mL and ≤15% for results greater than 5 ng/mL. The results demonstrated that the assay was linear across the claimed measuring range of 1.5 to 20 ng/mL.

RBC linearity was determined by diluting one high level RBC hemolysate sample to concentrations evenly spaced across the measuring range. Eleven concentrations across the measuring range were tested. The linearity data were analyzed with regards to linear, quadratic and cubic polynomials. In a first step, a linearity check was performed with a first order (linear) regression and then with higher order models (quadratic and cubic). The result has shown that higher orders are not significant. The sample concentrations tested ranged from 0 ng/mL to 653 ng/mL. All of the results were within the sponsors acceptance criteria and demonstrated that the assay was linear across the claimed measuring range of 46.5 to 620 ng/mL. The analyzer calculates this value by automatically correcting for the 1:31 pre-dilution that occurs during the hemolysis step.

This assay has a measuring range of 1.5- 20 ng/dL for serum folate and 46.5-620 ng/dL for RBC folate.

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

The CalSet, CalCheck and the PreciControl Anemia products are assayed and compared to Roche master calibrators, which are traceable to an in-house standard, and target values and ranges are assigned. Values are assigned using multiple Elecsys® 2010/ cobas e® 411 analyzers. Several runs are performed on each analyzer platform with duplicate determinations for each sample. The target value is then calculated as the median of the determined values.

Stability testing protocols and acceptance criteria were described and found to be

acceptable. The CalSet, CalCheck, and PreciControl Anemia are stable until the expiration date printed on the vial when stored unopened at 2 – 8° C. Reconstituted CalSet vials are stable for 5 hours at 20 - 25°C, 3 days at 2 – 8°C, and 3 months at -20°C. Reconstituted CalCheck vials are stable for 4 hours at 20 - 25°C. PreciControl Anemia vials are stable for 8 hours at 20 - 25°C, 5 hours on the analyzers at 20 - 25°C, 3 days at 2 – 8°C, and 3 months at -20°C.

d. Detection limit:

The Limit of Blank (LOB) and Limit of Detection (LOD) were determined in accordance with CLSI EP17-A requirements. One human serum sample pool, free of analyte, was used in the LOB experiment. Five low-level human serum samples were used in the LOD experiment. Analyte levels were determined by testing on the Elecsys 2010/cobas e411 Immunoassay analyzer. Each of these samples was evaluated n=60 on multiple runs over 3 days on multiple analyzers and reagent lots. The Limit of Blank was determined as the 95th percentile of measurements of blank samples. In this case, after a top-down ranking of the 60 measured concentrations, the mean of the concentrations at position #57 and #58 = LoB = (0.632 + 0.634)/2 = 0.633 ng/mL. The LoB claim in the package insert is 0.640 ng/mL. The LOD was calculated as LOD = LOB + 1.6529 x SD total = 0.633 + 1.6529 x 0.2827 = 1.10 ng/mL. In the labeling, Roche claims an LoD of ≤ 1.5 ng/mL.

For calculating the limit of quantitation (LOQ), two human serum samples and three human serum sample pools (folate depleted) with concentrations ranging from 1.20 to 3.16 ng/mL were used to calculate the concentration which corresponds to an interassay coefficient of variation (CV) of 20%. The samples were tested once per day, for 10 days. The mean, standard deviation and coefficient of variation for each sample were calculated. As a result, by linear interpolation, a concentration of approximately 1.8 ng/mL generated a CV of 20 %. The sponsor claims a functional sensitivity for the device of 2 ng/mL.

The lower end to the RBC measuring range is defined as 46.5 ng/mL in the Package Insert. This concentration is derived from the serum/plasma Folate claimed LoD of 1.5 ng/mL (and dilution effect x31). Roche performed a functional sensitivity study. The study was performed using hemolysates on the Elecsys® 2010 / cobas e® 411 Immunoassay Analyzer. Eight hemolysates with concentrations ranging from 29.5 to 210 ng/mL were tested for 5 days, 2 runs per in singleton. The mean, standard deviation and coefficient of variation for each sample were calculated. As a result, a concentration of 54.8 ng/mL generated a CV of 15.8 %. The claimed functional sensitivity of 2 ng/mL for serum/plasma Folate III would correspond to a calculated value of 62 ng/mL for RBC Folate (based on the dilution effect x31).

This assay has a measuring range of 1.5- 20 ng/dL for serum folate and 46.5-

620 ng/dL for rbc folate.

e. Analytical specificity:

The effects of interference by Biotin, Lipemia, Bilirubin, Rheumatoid Factor, Human IgG and Human IgA on the quantitation of Folate by the Elecsys Folate III assay were determined on the Elecsys 2010/cobas e411 Immunoassay Analyzer. Human serum samples, spiked with varying levels of interferent, were divided into two aliquots after collection. One aliquot was spiked with the interferent while the other aliquot was spiked with the same volume diluent. The interferent spiked sample was then diluted into the unspiked aliquot in 10% increments. Percent recovery was calculated by comparing the measured folate concentration to the expected folate initial concentration. The sponsor defined significant interference as greater than +/- 0.5 ng/mL, absolute deviation for samples </= 5 ng/mL and greater than +/- 10% recovery for samples >5 ng/mL. The following results were obtained

Endogenous Substances	Concentration Showing No Interference
Bilirubin	33 mg/dL
Biotin	21 ng/mL
Intralipid	1500 mg/dL
IgG	16 g/L
IgA	4 g/L

The labeling states the following regarding hemoglobin interference: Hemolysis may significantly increase folate values due to high concentrations of folate in red blood cells. Therefore, hemolyzed serum samples are not suitable for use in this assay.

18 commonly used pharmaceuticals were examined for potential effect on folate determination by the Folate III test system. Each drug was added to folate serum samples in two defined concentrations and the resulting samples were tested in triplicate using the Elecsys 2010/cobas e411 Immunoassay Analyzer. The median value was compared to the reference value (folate sample with no drug added) and the deviation from the reference value was calculated. The sponsor defined interference as +/- 3SD of the reference value or +/- 10% if the 3SD range < +/- 10%. The results showed not significant interference at the concentrations below.

Drug Substances	Concentration Showing No Interference
Acetylcystein	1662 mg/L

Ampicillin-Na	1000 mg/ L
Ascorbic acid	60 mg/ L
Ca- Dobesilate	200 mg/L
Cyclosporine	20 mg/L
Cefoxitin	660 mg/L
Heparin	5000 U
Levodopa	20 mg/L
Methyldopa	20 mg/L
Metronidazole	200 mg/L
Phenylbutazone	400 mg/L
Doxycyclin	50 mg/L
Acetylsalicylic Acid	1000 mg/L
Rifampicin	60 mg/L
Acetaminophen	200 mg/L
Ibuprofen	700 mg/L
Theophyllin	100 mg/L
Human Erythropoietin	2000 U

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was performed by analyzing 101 serum samples ranging from 1.76 ng/mL to 20 ng/mL with the submitted Folate III assay and the previously cleared Folate II assay on the Elecsys 2010. Linear regression analysis was performed with the following results:

$y = 1.05x – 0.40$ $r = 0.963$

RBC Hemolysate:

A method comparison study was performed by analyzing 98 fresh whole blood samples ranging from 162 - 620 ng/mL (with values corrected for dilution factor (31)) with the submitted RBC Hemolysate Folate III assay and the previously cleared RBC Hemolysate Folate II assay on the Elecsys 2010/e411. Passing-Bablok and linear regression analysis was performed with the following results:

$y = 1.096x – 3.874$ $r = 0.921$

b. Matrix comparison:

A matrix comparison study between matched K3-EDTA and Na-heparin hemolysate (whole blood samples diluted with ascorbic acid) was performed with the Elecsys Folate III Immunoassay on the Elecsys 2010 / cobas e 411 Immunoassay Analyzer. The results of the study show that the two anticoagulants have comparable performance across themeasuring range. The samples tested had folate concentrations ranging from 101 - 588 ng/mL. Passing/Bablock regression analysis was performed with the following results:

$$Y = 1.00 - 1.16 \qquad r = 0.992$$

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 reference interval for serum samples tested on the Elecsys 2010/cobas e411 (with N=261) was 7.3 - 26.1 ng/mL (2.5th - 97.5th percentile) with a median value of 14.0 ng/mL. The sample donors tested had homocysteine concentrations <15 umol/L, were between 18 and 65 years old, were apparently healthy and were fasting. Exclusion criteria were: pregnant and lactating women, body mass index >30 and subjects who take vitamins on the day of sample collection.

The data obtained in this reference interval study verifies data reported by The American Journal of Clinical Nutrition. The American Journal of Clinical Nutrition data is provided in the labeling as an additional reference.

Elecsys RBC Folate Hemolyzing Reagent

The 262 Whole blood samples collected in this study were tested using the Elecsys RBC Folate Hemolyzing Reagent in combination with the Elecsys Folate III assay. As reported in the labeling, samples were tested on the Elecsys 2010/cobas e411 Immunoassay analyzer.

The reference interval for the Elecsys 2010/cobas e411 Immunoassay analyzer for whole blood was 499 - 1504 ng/mL (2.5th - 97.5th percentile) with a median value of 902 ng/mL. The sample donors tested had homocysteine concentrations <15 umol/L, were between 18 and 65 years old, were apparently healthy and were fasting. Exclusion criteria were: pregnant and lactating women, body mass index >30 and subjects who take vitamins on the day of sample collection.

For both studies, the Elecsys Folate III and the Elecsys RBC Folate, samples from the same 129 men and 133 women were tested.

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.