

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

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

k141426

**B. Purpose for Submission:**

Modification of traceability to a previously cleared assay (k082340)

**C. Measurand:**

Folate

**D. Type of Test:**

Quantitative immunoassay

**E. Applicant:**

Roche Diagnostics, Inc.

**F. Proprietary and Established Names:**

Elecsys Folate III

**G. Regulatory Information:**

1. Regulation section:

862.1295; Folic acid test system

2. Classification:

II

3. Product code:

CGN

4. Panel:

75; Clinical chemistry

## **H. Intended Use:**

1. Intended use(s):

Binding assay for the in vitro quantitative determination of folate in human serum. The binding assay is intended for use on Elecsys and cobas e immunoassay analyzers. Folic acid measurements are used in the diagnosis and treatment of anemias.

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 g/L

Streptavidin-coated microparticles with preservative

R1: Ruthenium labeled folate binding protein, stabilizers and preservatives

R2: Biotinylated folate, stabilizers and preservatives

Elecsys Folate III calibrators are required for the calibration of the folate III assay. The Elecsys Folate III calibrators were previously cleared in k082340 and the calibrator materials have not been changed.

## **J. Substantial Equivalence Information:**

1. Predicate device name(s):

Elecsys Folate III assay

2. Predicate 510(k) number(s):  
k082340

3. Comparison with predicate:

<b>Similarities</b>		
<b>Item</b>	<b>Predicate (k082340)</b>	<b>Candidate Device</b>
Intended Use	Intended for the in vitro quantitative determination of folate in human serum. Measurements obtained by these devices are used in the diagnosis and treatment of anemias.	Same
Assay Protocol	The Elecsys Folate assay employs a competitive test principle using natural folate binding protein (FBP) specific for folate. Folate in the sample competes with the added folate (labeled with biotin) for the binding sites on FBP (labeled with ruthenium).	Same
Instrument Platform	Elecsys and cobas e immunoassay analyzers.	Same
Sample Volume	25 µL	Same
Sample Type	Human serum.	Same
Calibrator	Elecsys Folate III CalSet	Same
Controls	Elecsys PreciControl Varia	Same
Calibration Frequency	Once per reagent lot and <ul style="list-style-type: none"> <li>•After 1 month when using same reagent lot</li> <li>•After 7 days when using same reagent kit</li> <li>•As required per QC findings or pertinent regulations</li> </ul>	Same
Reagent Stability	Unopened: 2-8°C - Up to the stated expiration date Opened 2-8°C - 8 weeks On Analyzers – 2 weeks or 4 weeks when stored alternatively in the refrigerator and on the analyzer, with the total time on-board the analyzer not exceeding 10x8 hours	Same

<b>Differences</b>		
<b>Item</b>	<b>Predicate (k082340)</b>	<b>Candidate Device</b>
Traceability	Standardized against the Elecsys Folate II assay (k043318)	Standardized against WHO International Standard NIBSC code: 03/178 material
Measuring Range	1.5-20.0 ng/mL	2.0-20.0 ng/mL
Analytical Sensitivity	Limit of Blank (LoB): = 0.64 ng/mL Limit of Detection (LoD): = 1.5 ng/mL Limit of Quantitation (LoQ): = 2.0 ng/mL	Limit of Blank (LoB): = 0.64 ng/mL Limit of Detection (LoD): = 1.2 ng/mL Limit of Quantitation (LoQ): = 2.0 ng/mL

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

CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods

CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures

CLSI EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach

CLSI EP-09-A2-IR: Method Comparison and Bias Estimation Using Patient Samples

**L. Test Principle:**

The serum sample is first 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.

Results are determined using a calibration curve that is generated specifically on each instrument by a 2 point calibration and a master curve (5-point-calibration) provided with the reagent bar code.

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

1. Analytical performance:

a. *Precision/Reproducibility:*

Precision of the Elecsys Folate III reagent was evaluated according to the CLSI EP5-A2 guideline using one cobas e411 Immunoassay analyzer and one reagent lot. The protocol consisted of testing 2 replicates of each control (PC=PreCiControl Varia) and human sera (HS) from native single donors per run, 2 runs per day in duplicate for 21 days (n=84). Total and within-run precision was calculated according to EP5-A2. The results are summarized below.

Sample	Mean (ng/mL)	Within-run (Repeatability)		Total (Intermediate Precision)	
		SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)
Human serum 1	2.3	0.16	6.8	0.25	10.8
Human serum 2	3.9	0.20	5.1	0.32	8.1
Human serum 3	11.9	0.35	2.9	0.57	4.8
Human serum 4	13.4	0.30	2.2	0.57	4.3
Human serum 5	17.8	0.44	2.5	0.67	3.7
PC Varia 1	3.2	0.22	6.6	0.31	9.5
PC Varia 2	11.6	0.31	2.7	0.57	4.9

b. *Linearity/assay reportable range:*

The linearity of the Elecsys Folate III was evaluated on the cobas e411 Immunoassay Analyzer using CLSI EP6-A as a guideline. Serum linearity was determined by diluting a high analyte serum sample with low analyte serum sample pool. A total of 14 different concentrations of folate ranging from 1.8 ng/mL to 21.7 ng/mL were tested in triplicate within a single run. The linear equation generated is  $y=1.0969x - 1.2552$  with a regression coefficient ( $R^2$ ) of 0.997. A polynomial regression analysis was conducted and the 3<sup>rd</sup> order was found to be significant. The deviation of the 3<sup>rd</sup> order regression was compared against to the first order regression (see table below).

Sample Number	Linear regression (First order) in ng/mL	Predicted third order in ng/mL	Absolute difference in ng/mL	Relative difference in %
1	0.7248	0.9813	0.2565	N/A
2	1.3852	1.4759	0.0908	6.5515
3	2.7059	2.5535	-0.1524	-5.6329
4	4.0255	3.734	-0.2915	-7.2425
5	6.667	6.3524	-0.3245	-4.8674
6	9.3073	9.1666	-0.1406	-1.5111
7	11.9476	12.0751	0.1275	1.067
8	14.588	14.9353	0.3474	2.3812
9	15.9087	16.3065	0.3978	2.5007
10	18.549	18.8459	0.2969	1.6005

The results of the linearity study support the sponsor's claim that the assay is linear from 2 to 20 ng/mL.

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

Elecsys Folate III CalSet, CalCheck and the PreciControl Varia products were previously cleared in k082340 and k111506 (PreciControl Varia).

*d. Detection limit:*

The Limit of Blank (LOB), Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined in accordance with CLSI EP17-A guideline. A zero level (blank) sample buffered in human serum albumin was used for the LOB studies. The LOB was determined as the 95th percentile of measurements of blank samples. The Sponsor claims a LoB value of 0.60 ng/mL. For LOD determination, five low-level serum samples (native as well as diluted with Elecsys Diluent Universal) were used. The LOD was calculated as  $LOD = LOB + 1.653 \times SD \text{ total}$ . The Sponsor claims a LoD value of 1.2 ng/mL. For LOQ determination, a low level sample set of 10 human serum samples with folate concentrations ranging from 0.9 to 3.5 ng/mL were evaluated using 12 replicates/sample/lot across 3 reagent lots to calculate the concentration which corresponds to an inter-assay coefficient of variation (CV) of 20%. The Sponsor claims a LOQ value of 2.0 ng/mL. This assay has a measuring range of 2- 20 ng/mL for serum folate.

*e. Analytical specificity:*

The cross-reactivity of the Elecsys Folate III assay was determined using 3 serum samples, with approximate folate concentrations of 4, 9 and 18 ng/mL folate, from single native donors spiked with amethopterin, aminopterin and folonic acid. The spiked and non-spiked samples were tested in duplicate on the cobas e 411 Immunoassay analyzer.

Cross-reactivity studies were performed and a summary of the results are listed below:

<b>Interferent</b>	<b>Maximum Concentration Tested (ng/mL)</b>	<b>Highest % cross-reactivity</b>
Amethopterin	1500	2.5
Aminopterin	1500	4.4
Folonic Acid	1500	0.7

The labeling states the following regarding cross-reactive compounds: It is contraindicated to measure samples of patients receiving therapy with certain pharmaceuticals, e.g. methotrexate or leucovorin, because of the cross-reactivity of folate binding protein with these compounds.

The effects of endogenous interference by Biotin, Lipemia, Bilirubin, Rheumatoid Factor, Human IgG, Human IgA and Human IgM on the quantitation of Folate by the Elecsys Folate III assay were determined on the cobas e411 Immunoassay Analyzer. One aliquot of human serum samples containing low, mid and high folate concentrations (approximately 3, 9 and 19 ng/mL) was spiked with interfering samples. The second aliquot of serum samples was spiked with the same volume of sample 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. No significant interference was defined as  $\leq 0.4$  ng/mL for samples  $<4.0$  ng/mL and  $\leq 10\%$  for samples  $>4$  ng/mL. The following results were obtained:

<b>Endogenous Substance</b>	<b>Concentration Showing No Significant Interference</b>
Biotin	21 ng/mL
Intralipid (Lipemia)	1500 mg/dL
Bilirubin	29 mg/dL
Rheumatic Factor	1000 IU/mL
Human IgG	16 g/L
Human IgM	10 g/L
Human 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 samples are not suitable for use in this assay.

Sixteen commonly used pharmaceuticals were examined for potential effect on folate determination by the Folate III assay. Each drug, including erythropoietin, was spiked into 3 different human folate serum samples (approximate folate concentrations of 4, 11 and 18 ng/mL) and were tested in 13 replicates using the cobas e411 Immunoassay

Analyzer. The mean value was compared to the reference value (folate sample with no drug added) and the deviation from the reference value was calculated. No significant interference was defined interference as  $\leq 0.4$  ng/mL for samples  $<4.0$  ng/mL and  $\leq 10\%$  for samples  $>4$  ng/mL. The results showed no significant interference at the concentrations below:

<b>Drug Substance</b>	<b>Concentration Showing No Significant Interference</b>
Acetylcystein	566 mg/L
Ampicillin-Na	1000 mg/L
Ascorbic Acid	300 mg/L
Cyclosporine	5 mg/L
Cefoxitin	660 mg/L
Heparin	5000 U
Levodopa	20 mg/L
Methyldopa + 1.5	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	500 mg/L
Theophylline	100 mg/L
Erythropoietin	2000 U/mL

f. *Assay cut-off:*

Not applicable.

2. Comparison studies:

a. *Method comparison with predicate device:*

A method comparison study was performed with the Abbott Architect Folate assay (k092740), which is also traceable to WHO 03/178 reference material. One hundred and six native serum samples with folate values ranging from 2.08 to 19.6 ng/mL were tested on the cobas e 411. Results are shown below (Deming Regression):

$$y = 0.976x + 0.041; r = 0.984$$

*b. Matrix comparison:*

Not applicable.

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:

A reference range study was performed to update the reference range values for the current assay after standardization to the WHO International Standard NIBSC 03/178. Clinical samples previously collected to establish the reference range for the predicate device were used to establish the reference interval. The samples tested include 214 serum samples from apparently healthy adults, fasting males (N=110) and fasting non-pregnant females (N=104) between 21-59 years old. All samples were measured on the cobas e 411 over 3 runs for 2 days. The reference interval for the current assay is 4.8 – 24.2 ng/mL (2.5th - 97.5th percentile), with a median value of 11.8 ng/mL.

**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.