



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

**I Background Information:**

**A 510(k) Number**

K233060

**B Applicant**

Roche Diagnostics

**C Proprietary and Established Names**

Elecsys Folate III

**D Regulatory Information**

<b>Product Code(s)</b>	<b>Classification</b>	<b>Regulation Section</b>	<b>Panel</b>
CGN	Class II	21 CFR 862.1295 - Folic Acid Test System	CH - Clinical Chemistry

**II Submission/Device Overview:**

**A Purpose for Submission:**

Modified device

**B Measurand:**

Folate

**C Type of Test:**

Quantitative; electrochemiluminescence immunoassay

### **III Intended Use/Indications for Use:**

#### **A Intended Use(s):**

See Indications for Use below.

#### **B Indication(s) for Use:**

Binding assay for the in vitro quantitative determination of folate in erythrocytes (red blood cells, RBC). Folate measurements are used in the diagnosis and treatment of anemia. The electrochemiluminescence immunoassay, "ECLIA" is intended for use on cobas e immunoassay analyzers.

#### **C Special Conditions for Use Statement(s):**

Rx - For Prescription Use Only

For in vitro diagnostic use

#### **D Special Instrument Requirements:**

Cobas e 801 analyzer

### **IV Device/System Characteristics:**

#### **A Device Description:**

The Elecsys Folate III (RBC Application) is a modified version of the predicate assay to increase tolerance to biotin interference. The Elecsys Folate III (RBC Application) reagent pack includes the following reagents:

- PT1 Pretreatment reagent 1
- PT2 Pretreatment reagent 2
- M Streptavidin-coated microparticles
- R1 Folate-binding protein~Ru (bpy)
- R2 Folate~biotin

Other materials required to run the assay, but not included in the kit include: CalSet Folate, PreciControl Varia and Folate RBC Hemolyzing Reagent.

#### **B Principle of Operation:**

Elecsys Folate III (RBC Application) is a competition principle assay with a total duration of 27 minutes. Whole blood treated with anticoagulants is mixed with ascorbic acid solution and incubated at 20-25 °C for lysis of the erythrocytes to take place, with liberation and stabilization of the intracellular folate. Fifteen (15 µL) of resulting hemolysate sample is incubated with the folate pretreatment reagents 1 and 2. Bound folate is released from endogenous folate binding proteins. The pretreated sample is then incubated with the ruthenium-labeled folate binding protein forming a folate complex, the amount of which is dependent upon the analyte

concentration in the sample. After addition of streptavidin-coated microparticles and folate labeled with biotin, 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 becomes bound to the solid phase via interaction of biotin and streptavidin. 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 II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the cobas link.

**V Substantial Equivalence Information:**

**A Predicate Device Name(s):**

Elecsys Folate RBC

**B Predicate 510(k) Number(s):**

K103716

**C Comparison with Predicate(s):**

<b>Device &amp; Predicate Device(s):</b>	<u>K233060</u>	<u>K103716</u>
Device Trade Name	Elecsys Folate III	Elecsys Folate RBC
<b>General Device Characteristic Similarities</b>		
Intended Use/Indications For Use	Quantitative determination of folate. Measurements are used in the diagnosis and treatment of anemia.	Same
Assay Technology	Electro-chemiluminescence immunoassay	Same
Analytical Measuring Interval	120 - 620 ng/mL	Same
Sample Matrix	Hemolysate	Same
<b>General Device Characteristic Differences</b>		
Biotin tolerance	≤ 1200 ng/mL	≤ 21 ng/mL

**VI Standards/Guidance Documents Referenced:**

CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition

CLSI EP06 2<sup>nd</sup> Edition: Evaluation of the Linearity of Quantitative Measurement Procedures - 2nd Edition

CLSI EP07-Ed3: Interference Testing in Clinical Chemistry - 3rd Edition

CLSI EP09c: Method Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Third Edition

CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition

## VII Performance Characteristics (if/when applicable):

### A Analytical Performance:

#### 1. Precision/Reproducibility:

Precision studies were conducted for both 21 days and 5 days with the Elecsys Folate III (RBC Application) to evaluate repeatability (within-run precision) and intermediate precision (within-laboratory precision) according to the CLSI guideline EP05-A3.

#### **Repeatability and Intermediate Precision (21 Days)**

Precision was evaluated on one cobas e 801 analyzer with one reagent lot. Five human hemolysate samples prepared from whole blood samples collected in K3-EDTA tubes and each of the three levels of control were tested in two replicates per run, two runs per day for 21 days (n=84 measurements per sample). Repeatability and intermediate imprecision were calculated in accordance with CLSI EP05-A3. Results are summarized in the table below.

#### Summary of 21-Day Precision Results

Sample	Mean (ng/mL)	Repeatability (within-run)		Intermediate Precision (within-lab)	
		SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)
Sample 1	152	5.73	3.8	6.17	4.1
Sample 2	206	6.14	3.0	7.17	3.5
Sample 3	252	6.70	2.7	8.28	3.3
Sample 4	363	8.01	2.2	10.0	2.8
Sample 5	605	10.7	1.8	14.6	2.4
Control 1	222	6.88	3.1	8.14	3.7
Control 2	391	11.1	2.8	11.5	2.9
Control 3	500	10.8	2.2	13.1	2.6

#### **Lot-to-Lot Reproducibility (5 days)**

A multi-site-multi-lot reproducibility study was performed at three sites using three reagent lots (1 site used all three lots and the other 2 sites used 1 lot each). Seven human hemolysate

samples prepared from whole blood samples collected in K3-EDTA tubes and three control levels were tested. Samples were tested in five replicates per day for five days on one cobas e 801 analyzer per site, per lot for a total N=125 measurements per sample. Repeatability and intermediate imprecision were calculated in accordance with CLSI EP05-A3 and results are summarized below.

Lot-to-Lot Reproducibility - All Lots Combined

			Reproducibility	
Sample	N	Mean	SD (ng/mL)	CV (%)
Hemolysate 1	125	128	10.5	8.20
Hemolysate 2	125	149	10.6	7.12
Hemolysate 3	125	201	10.2	5.08
Hemolysate 4	125	247	10.7	4.35
Hemolysate 5	125	356	14.1	3.95
Hemolysate 6	125	601	20.4	3.41
Hemolysate 7	125	605	28.3	4.67
Control 1	125	212	10.7	5.06
Control 2	125	382	15.0	3.93
Control 3	125	490	17.5	3.56

Between-Lot and Between-Day Summary

Sample	N	Mean	Between-site		Between-lot		Between-day	
			SD	CV (%)	SD	CV (%)	SD	CV (%)
Hemolysate 1	125	128	6.69	5.23	4.36	3.41	5.13	4.02
Hemolysate 2	125	149	6.71	4.52	3.43	2.31	5.44	3.66
Hemolysate 3	125	201	2.61	1.30	3.57	1.77	6.13	3.05
Hemolysate 4	125	247	3.62	1.47	2.80	1.13	6.32	2.56
Hemolysate 5	125	356	8.93	2.51	1.34	0.38	4.99	1.40
Hemolysate 6	125	601	8.06	1.34	15.1	2.52	8.45	1.41
Hemolysate 7	125	605	16.8	2.77	19.5	3.23	7.37	1.22
Control 1	125	212	5.41	2.55	0	0	5.19	2.44
Control 2	125	382	7.82	2.05	2.87	0.75	8.33	2.18
Control 3	125	490	12.2	2.48	2.14	0.44	7.53	1.54

2. Linearity:

Linearity of the Elecsys Folate III (RBC Application) was assessed using human hemolysate samples prepared from whole blood samples collected in K3-EDTA tubes on the cobas e 801 analyzer. The studies were performed in accordance with CLSI EP06-Ed2 using three series comprised of 9-10 levels of samples ranging from ~103 to 656 ng/mL. Samples were tested using one lot of reagent, run in 4 replicates within a single run. The linearity data was analyzed using a weighted linear regression model according to CLSI EP06-Ed2. The deviation from linearity did not exceed 5% within the claimed measuring interval.

The results support the Elecsys Folate III (RBC Application)'s measuring range of 120 - 620 ng/mL.

**Dilution Study**

The sponsor provided information to support that samples with folate concentrations above the measuring range can be diluted with Elecsys Folate RBC Hemolyzing Reagent. For the Elecsys Folate III (RBC Application), the recommended manual dilution is 1:2. The concentration of the diluted sample must be  $\geq 265$  ng/mL.

3. Analytical Specificity/Interference:

**Interference - Endogenous substances**

An interference study was performed using eight endogenous substances (biotin, lipemia, bilirubin, rheumatic factor, albumin, human IgG, human IgM, and human IgA) for potential interference with the Elecsys Folate III (RBC Application) on the cobas e 801 analyzer. For each potentially interfering substance, three human hemolysate samples prepared from whole blood samples collected in K3-EDTA tubes containing low, mid, and high concentrations of 142 ng/mL, 359 ng/mL, 488 ng/mL were tested with 5 replicates per sample. Recovery (absolute deviation or % recovery) was calculated for each sample compared to the reference (control sample with no potential interferent added). Results are summarized below.

<b>Interferent</b>	<b>Highest concentration tested with no significant interference observed</b>
Biotin	1200 ng/mL
Lipemia (Intralipid)	1500 mg/dL
Bilirubin	29 mg/dL
Rheumatoid Factor	1000 IU/mL
Serum Albumin	7.0 g/dL
Human IgG	1.6 g/dL
Human IgM	1 g/dL
Human IgA	0.4 g/dL

**Interference - Exogenous substances**

An interference study was conducted to evaluate 17 drugs and human erythropoietin for potential interference with the Elecsys Folate III (RBC Application). Two human hemolysate samples prepared from whole blood samples collected in K3-EDTA tubes with folate concentrations of ~150 ng/mL and ~430 ng/mL were used for the testing. Recovery was calculated as percent recovery compared to the reference sample (control sample with no potential interferent added). Results are summarized below.

<b>Drug</b>	<b>Highest concentration tested with no significant interference observed</b>
Acetyl cysteine	150 mg/L
Acetylsalicylic Acid	30 mg/L

Ampicillin-Na	75 mg/L
Ascorbic acid	52.5 mg/L
Cefoxitin	750 mg/L
Doxycycline	18 mg/L
Heparin	3300 IU/L
Levodopa	7.5 mg/L
Methyldopa	22.5 mg/L
Metronidazole	123 mg/L
Rifampicin	48 mg/L
Acetaminophen	156 mg/L
Cyclosporine	1.8 mg/L
Ibuprofen	219 mg/L
Theophylline	60 mg/L
Phenylbutazone	321 mg/L
Itraconazole	30 mg/L
Erythropoietin	2000 u/mL

### Cross-reactivity

A cross-reactivity study was performed in which potential cross-reactants were added at defined concentrations to one hemolysate sample prepared from a whole blood sample collected in K3-EDTA tubes. Samples were measured on the cobas e 801 analyzer and cross-reactivity was calculated and summarized in the table below.

Cross reactant	Highest Con. Tested (ng/mL)	Highest % Cross-reactivity
Amethopterin	750	1.7
Aminopterin	750	2.0
Folinic acid	750	2.6

#### 4. Assay Reportable Range:

See section VII.A.2 Linearity.

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

**Traceability:** The Elecsys Folate III (RBC Application) is traceable to a commercially available assay.

#### **Stability**

The Elecsys Folate III (RBC Application) reagent kit can be stored on-board the analyzers for up to 112 days (16 weeks). A new calibration of the kit kept on-board is recommended every 28 days.

6. Detection Limit:

Determination of the Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantification (LoQ) for the Elecsys Folate III (RBC Application) was determined in accordance with CLSI EP17-A2 guideline. For the LoQ study, the highest CV% reported across all lots in the study was 10.4%. Results are summarized below.

<b>Matrix</b>	<b>LoB (ng/mL)</b>	<b>LoD (ng/mL)</b>	<b>LoQ (ng/mL)</b>
Hemolysate	45	70	120

7. Assay Cut-Off:

Not applicable.

**B Comparison Studies:**

1. Method Comparison with Predicate Device:

A method comparison study was performed comparing the performance of the Elecsys Folate III (RBC Application) (candidate device, Y) versus the predicate device, Elecsys Folate RBC (non-biotin remediated, X) on one cobas e 801 analyzer. The study was conducted in accordance with CLSI Guideline EP09c-A3.

A total of 119 hemolysate samples prepared from native whole blood samples collected in K3-EDTA tubes (including 99 native samples, 10 spiked samples and 10 diluted samples) that span the measuring range were tested with one replicate per sample. Samples were measured with one lot of reagents. Results are summarized in the tables below (Passing-Bablok).

Sample N=119	<b>Passing/Bablok</b>
Sample concentration range 132 - 618 ng/mL	
Slope	1.04
Intercept	14.6
Linear Correlation coefficient r	0.991

2. Matrix Comparison:

The effect of anticoagulants on the measurement of the analyte with the Elecsys Folate III (RBC Application) was assessed by comparing values obtained from native whole blood samples drawn into Na-heparin and K3-EDTA tubes. At least 40 sample pairs were tested in singleton with one reagent lot on one cobas e 801 analyzer. Passing/Bablok regression analysis was performed. Results are summarized below.

K3-EDTA vs. Na-Heparin	Passing/Bablok
N	53
Range (ng/mL)	135-601 ng/mL
Slope (95% CL)	1.07
Correlation coefficient r	0.992

**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):

Not applicable

**D Clinical Cut-Off:**

Not applicable

**E Expected Values/Reference Range:**

Previously established in K082340.

**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.