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

A. 510(k) Number:

k161646

B. Purpose for Submission:

New Device

C. Measurand:

Folate

D. Type of Test:

Quantitative, competitive enzymatic method.

E. Applicant:

Diazyme Laboratories

F. Proprietary and Established Names:

Diazyme Folate Assay, Diazyme Folate Calibrator Set, Diazyme Folate Control Set

G. Regulatory Information:

Product code	Regulation Name	Classification	Regulation Section	Panel
CGN	Diazyme Folate Assay	II	21 CFR 862.1295	Chemistry 75
JIT	Diazyme Folate Calibrator	II	21 CFR 862.1150	Chemistry 75
JJX	Diazyme Folate Control	I, reserved	21 CFR 862.1660	Chemistry 75

H. Intended Use:

1. Intended use(s):

   See in the indications of use below.

2. Indication(s) for use:

The Diazyme Folate Assay is a homogeneous enzyme method intended for use in the quantitative analysis of folate in human serum. Folic acid measurements are used in the diagnosis and treatment of anemias. For in-vitro diagnostic use only.

The Diazyme Folate Calibrator Set is intended for use in the calibration of the Diazyme Folate Assay. For in vitro diagnostic use only.

The Diazyme Folate Control Set is intended for use as quality controls for the Diazyme Folate Assay. For in vitro diagnostic use only.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

Roche Hitachi 917 analyzer.

I. Device Description:

The Diazyme Folate Assay contains four reagents and the ingredients are shown below:

Reagent	Concentrations
REAGENT 1 (R1a) 1x20mL	Nitro-phenyl-ß-galactoside (NPG) substrate: 2.9 mM
REAGENT 1 (R1b) 1X0.5 mL	Enzyme donor-folate conjugate: 0.1mM
REAGENT 2 (R2) 1X15mL	Folate binding protein: 0.01mM, Phosphate Salt:195mM
REAGENT 2 (R3) 1X10mL	Enzyme acceptor: 0.1mM, Tris-HCL:50mM, NaCl: 195mM.

Materials required but sold separately:

- The Diazyme Folate Calibrator Set: five-level set supplied in liquid form (5 x1mL). Calibrator sets contains human serum and additives, sodium azide <0.1% with five different concentrations (0, 1.8, 5.00, 12.0 and 25.0 ng/mL). Concentration of the calibrators is lot specific and the folate concentrations are provided in ng/mL.

- The Diazyme Folate Control Set: two-level set that is supplied in liquid form (2 x 1 mL). Control sets contains human serum and additives, sodium azide <0.1% with concentration values of 4 ng/mL for level 1 and 11 ng/mL for level 2. Concentration of the controls is lot specific and the folate concentrations are provided in ng/mL

Each donor unit of serum used in the preparation of this Calibrator Set and Control Set was tested by FDA-approved methods and found negative for the Human

Immunodeficiency Virus Antibody (HIV I/II Ab), Hepatitis B Surface Antigen (HBsAg), and Hepatitis C Virus Antibody (HCV).

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Roche Elecys Folate III assay
   Roche Elecsys Folate III CalSet
   Roche Elecsys Preci-Control Anemia

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

   k141426 (for assay)
   k082340 (for calibrator and control)

3. Comparison with predicate:

   Reagent:

Similarities and Differences		
Item	Predicate Device Roche Elecsys Folate III Assay (k141426)	Candidate Device Diazyme Folate Assay (k161646)
Intended use	For the in vitro quantitative determination of folate in human serum. Folic acid measurements are used for the diagnosis and treatment of anemias.	Same
Specimen Type	Human serum	Same
Principle	Quantitative immunoassay	Quantitative, homogeneous enzymatic method
Assay range	Assay Range: 2.0 – 20.0 ng/mL	Same
Reagent Stability	Unopened: stable at 2-8^{0}C up to the stated expiration date Opened 2-8^{0}C – 8 weeks Onboard stability 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	Unopened stable until expiration date on vial at 2-8^{o}C On-board 4 days at 2-8^{o}C. Shelf Life of 12 months at 2-8^{o}C

Similarities and Differences		
Item	Predicate Device Roche Elecsys Folate III Assay (k141426)	Candidate Device Diazyme Folate Assay (k161646)
Detection limits	LoB = 0.64 ng/mL LoD = 1.5 ng/mL LoQ = 2.0 ng/mL	LoB = 0.27 ng/mL LoD = 0.91 ng/mL LoQ = 2.0 ng/mL

Calibrator

Similarities/Differences		
Item	Predicate Device Roche Elecsys Folate III Cal Set (k082340)	Candidate Device Diazyme Folate Calibrator Set (k161646)
Intended use	Intended for use in the calibration of the folate assay. For in vitro diagnostic use only.	Same
Matrix	Lyophilized	Liquid
Levels	2 levels Level 1= <0.4 ng/mL Level 2= 17.0 ng/mL	5 level Level 1 = 0 ng/mL Level 2 =1.80 ng/mL Level 3 = 5.00 ng/mL Level 4 = 12.00 ng/mL Level 5 = 25.00 ng/mL
Handling	Reconstitute with 1 mL of water before use	Liquid ready to use
Stability	Unopened stable until expiration date on the vial. Open reconstituted calibrator at 2-8^{o}C for 3 days. Frozen 20-25^{o} C stable for 3 months. On-board stability 20-25^{o}C single time use only.	Unopened vial is stable up to the expiration date on the vial. Open vial stability is up to 30 days at 2-8^{o}C.

Control

Similarities/Differences		
Item	Predicate Device Roche Elecsys Preci-control Anemia (k082340)	Candidate device Diazyme Folate Control Set (k161646)
Intended use	Control Set is intended for use as quality controls for the folate assay. For *i*n vitro diagnostic use only	Same
Matrix	Lyophilized	Liquid
Levels	2 level	2 level
Target values	Level 1= 3.9 ng/mL Level 2 = 12 ng/mL	Level 1 mean value = 4.4 ng/mL Level 2 mean value =11.2 ng/mL
Handling	Reconstitute by adding 3mL of water.	Liquid ready to use
Stability	Frozen stable for 31 days 2-8^{o}C stable for 72 hrs. Onboard 20-25^{o}C up to 5 hrs.	Unopened vial stable until expiration date on the vial at 2-8^{o}C Open vial is stable for 30 days at 2-8^{o}C.

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

CLSI EP6-A - Evaluation of Linearity of Quantitative Analytical methods: Approved Guideline.

CLSI EP5-A2 - Evaluation of Precision of Clinical Chemistry Devices: Approved Guideline-Second Edition.

CLSI EP7-A2 - Interference Testing in Clinical Chemistry : Approved Guideline-Second Edition.

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

CLSI EP9-A2 - Method Comparison and Bias Estimation using Patient Sample: Approved Guideline-Second Edition

CLSI CA28-A3 - Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory: Approved Guideline-Third Edition.

L. Test Principle

The test is based on the principle of α-complementation of the enzyme β-galactosidase and competition between an enzyme donor-folate conjugate, a folate binding protein and the folate content of a serum sample. Samples with higher folate concentrations produce higher β-galactosidase activities and *vice versa*. A nitro-phenyl-ß-galactoside derivative (NPG) is used as the enzyme substrate. The reaction's product has maximum absorbance at 415 nm. The folate concentration of a sample is proportional to the measured β-galactosidase activity.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

In the precision study, three lots of the reagents were used, and for each lot of the reagent, the same set of seven levels of human serum containing folate of 3.4 ng/mL, 4.9 ng/ml, 5.9 ng/mL, 8.9 ng/mL, 12.3 ng/mL, 15.1 ng/mL, and 16.8 ng/mL respectively were tested. These samples were obtained from a commercial vendor. The samples were tested in duplicates per run, 2 runs per day for 20 working days according to CLSI EP5-A2 protocol. All 3 lots of reagents yielded similar results. The precision study summary is presented in the table below showing the results for one representative lot.

Precision Summary of one representative lot:

	Mean ng/mL (N=80)	Within Run		Between Run		Between Day		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
Serum 1	3.4	0.2	6.4	0.1	1.7	0.1	2.7	0.2	7.2
Serum 2	4.8	0.2	4.6	0.2	4.5	0.2	3.8	0.4	7.4
Serum 3	5.8	0.3	4.9	0.2	3.3	0.3	4.6	0.4	7.5
Serum 4	8.9	0.4	4.1	0.4	4.0	0.2	2.8	0.6	6.4
Serum 5	12.3	0.5	3.9	0.5	4.3	0.0	0.0	0.7	5.8
Serum 6	15.1	0.6	3.8	0.5	3.5	0.2	1.5	0.8	5.4
Serum 7	16.8	0.8	4.6	0.5	2.8	0.7	4.2	1.1	6.8
Con 1	4.4	0.2	3.7	0.2	4.3	0.2	4.8	0.3	7.4
Con 2	11.2	0.5	4.5	0.4	3.9	0.4	3.6	0.8	7.0

b. Linearity/assay reportable range:

i.) Linearity study followed CLSI EP6-A guideline was performed.
The linearity study was performed on the Hitachi 917. The linearity set was created as follows: a human serum sample was spiked with folate stock solution to a final concentration of 21 ng/mL. This high pool sample was diluted with human serum in different ratios to produce 11 dilutions covering the intended assay range. All diluted

samples were tested in triplicates with Diazyme Folate Assay. Sample range tested from 1.78 to 20.43 ng/mL and generated the following regression equation.
The linear regression is: Y = 1.003x-0.1881, with an $r^{2 =}$ 0.996

Sponsor claimed that the Diazyme folate assay has a measuring range from 2.0 to 20.0 ng/mL.

ii) Sample dilution study:

Additionally sample dilution study was performed to determine the levels of folate that exceed 20 ng/mL. Eight serum samples with folate concentrations >20 ng/mL, and 3 samples with folate concentration above 30ng/mL was tested. The folate concentration was first determined by the predicate device. Then the samples were 1:1 diluted manually with Diazyme folate calibrator 1 (which is the zero folate value) and tested with Diazyme Folate Assay. Results were then multiplied by a factor of 2. All results in the dilution study are within 10% of the expected value. Based on the results obtained from the Diazyme folate assay dilution studies, the sponsor recommend in the labeling that samples with serum folate greater than 20 ng/mL may be 1:1 diluted with the provided calibrator 1 and re-assayed.

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

Traceability:
The calibrators for the Diazyme Folate Assay are traceable to the predicate device, Roche Diagnostics, Elecsys Folate III (k141426).

Folate was purchased from a commercial vendor and was used in the preparation of both calibrators and controls. Folate stock solutions were prepared according to the vendor's certificate of analysis. 5 levels of master calibrators were prepared by spiking folate into pooled human serum, and value assigned by an internal procedure.

Calibrators and value assignment
The Diazyme Folate Calibrator set were prepared by spiking folate into pooled human serum except calibrator level 1 which was prepared in a buffer base. Non-spiked level 1 was automatically assigned a value of 0 ng/mL. The value point of the other four levels of the calibrators is set across the reportable range. The highest calibrator covers the upper measuring range. The four spiked calibrator levels (2-5) were initially assigned by running them as samples using master calibrators and released folate reagents. The production calibrators were verified and adjusted according to an internal procedure. Below is the target value of one lot of calibrators.

Level 1 = 0 ng/mL
Level 2 = 1.80 ng/mL
Level 3 = 5.00 ng/mL
Level 4 = 12.00 ng/mL
Level 5 = 25.00 ng/mL

Controls and value assignment:

The Diazyme Folate Control set were made from folate stock solution obtained from a commercial vendor, prepared by spiking folate into pooled human serum. Two folate target concentrations were prepared. For the first level control the manufacturing target is approximately 4 ng/mL. For the second level control the manufacturing target is approximately 11 ng/mL. Lot specific values will differ from lot to lot.

Value assignment of the folate controls by replicate analysis: The Diazyme folate Assay and calibrators are used in replicate analysis to determine the mean value of the newly prepared controls. Final value is the mean of replicate values and the expected range is calculated as ± 25% from the mean value for the respective controls. The final mean values and control ranges of one lot of controls are provided below.
Level 1 = 4.34 ng/mL, range = 3.26-5.43 ng/mL
Level 2 = 11.33 ng/mL, range = 8.50-14.17 ng/mL

Stability:

The Diazyme calibrator and control stability:

Accelerated study was performed at 37^{o} for 7 days to simulate real time storage at 2-8°C for one year. The study results support a shelf-life of 12 months for both the control and the calibrator or longer at 2-8°C. Real time study is ongoing. Protocols and acceptance criteria were reviewed and found to be acceptable. Unopened vials for calibrators and controls are stable to the expiration date on the label when stored at 2 to 8^{o}C.

Open vial stability for controls and calibrators is 1 month. Protocols and acceptance criteria were reviewed and found to be acceptable.

d. Detection limit:

Limit of Blank (LoB):

To calculate the LoB of the Diazyme Folate Assay, calibrator 1 was run as a sample with 60 replicates on the Hitachi 917.

LoB was determined as the 95th percentile of the measurement of the blank samples.

Limit of Detection (LoD):

Five serum samples obtained commercially were diluted with calibrator l and tested with Folate reagent on Hitachi 917 with 12 replicates each. The five low samples prepared were tested in 3 runs, 4 replicates per run.

The LoD was calculated to be = 0.91 ng/mL.

Limit of Quantitation (LoQ):

The LoQ of the Diazyme Folate Assay was determined by following CLSI EP17-A2 Approved Guideline. Five serum samples obtained from commercially were diluted with calibrator 1 to concentrations of 1 to 20 times of assumed LOB. The diluted serum samples were tested with three lots of the Diazyme Folate reagent on the Hitachi 917. Each sample was assayed with the Diazyme Folate assay in 40 replicates. For each sample, the 40 replicates were obtained from at least 5 independent runs.

Data was processed using the EP evaluator software to determine the LoQ value. The lowest concentration for which CV≤20% is the LoQ, which was determined to be 2.0 ng/mL.

The Diazyme folate assay has a measuring range of 2.0 to 10.0 ng/mL.

e. *Analytical specificity:*

Endogenous substances and therapeutic drugs:

To determine the level of interference from the substances present in serum, the Diazyme Folate Assay was used to test three serum samples, which contain "low", "medium", and "high" folate concentrations following CLSI EP7-A2. Three serum pools containing low, medium, and high folate were prepared by mixing human serum to create low value sample, and spiking folate for high value sample. Serum pools purchased from a commercial vendor. To ensure a suitable degree of precision, each serum sample spiked with interference substances was tested in triplicates on the Hitachi 917.

The common interfering endogenous substances of ascorbic acid, bilirubin, conjugated bilirubin, hemoglobin, and triglycerides showed no significant interference (< ± 10%) up to the concentrations summarized in the table below:

Interferent	Concentration showing no significant interference in (mg/dL)
Ascorbic Acid	44
Bilirubin	15
Bilirubin Conjugated	7.5
Hemoglobin	200
Triglycerides	1000

Sponsor has the following limitation in the package insert:
"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."

"Unconjugated bilirubin at 20 mg/dL causes an increase in folate by more than 11.7 %"

"Conjugated bilirubin at 10 mg/dL causes an increase in folate by more than 20.1 %."

The common therapeutic substances showed no significant interference (< ± 10%) up to the concentrations summarized below

Interference substance	Concentration showing no significant interference in mg/L
Acetylsalicylic Acid	1000
Metronidazole	200
Theophylline	10
Phenylbutazone	40
Acetaminophen	200
Cefoxitin	660
Acetylcystein	566
Rifampicin	60
Ibuprofen	50
Ampicillin-Na	1000
Cyclosporine	5
Doxycyclin	50
Levodopa	20
Methyldopa	20

Cross –reactivity:
The cross-reactivity studies were performed using a similar protocol as the interference studies, below is the result summary:

Interference Substance	Maximum Concentration tested (ng/mL)	Highest % cross-reactivity
Amethopterin	150	9.32
Aminopterin	75	3.94
Folinic acid	25	7.84

Sponsor has the following limitations in their labeling:

"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. Amethopterin, Aminopterin, and Folinic acid exhibit cross reactivity of 9.3%, 3.9%, and 7.8% respectively with the assay."
"Serum from patients with renal impairment or failure (including dialysis patients) may exhibit varying degress of falsely depressed folate values."

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

Diazyme Folate Assay was evaluated on the Hitachi 917 using 141 native individual serum samples containing folate covering the claimed measuring range of the assay with comparison to a predicate device, Roche Elecsys Folate III Assay (k141426), using CLSI EP9-A2 as a guideline. The individual samples used for this study were from certified commercial sources. All the Diazyme folate values were obtained from single measurements.

The comparison results are tabulated in the following table:
Regression analysis:

N=141	Deming regression
Slope	0.955
95% CI	0.927-0.984
Intercept	0.125
95% CI	-0.231-0.481
Correlation Coefficient	0.9841
R^2	0.9684
Sample Range (Diazyme)	2.07-19.85 ng/mL

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:

To establish the reference interval for the Diazyme Folate Assay, 213 healthy adults with 110 females and 103 male ages from 18 to 65 were tested in singlet using the Diazyme Folate Assay on the Hitachi 917 according to CLSI C28-A3 guideline. The individual serum samples were all native obtained from commercial sources. Additionally all folate samples with values higher than 20ng/mL were 1:1 diluted with calibrator 1 and re-assayed. The results were then multiplied by a factor of 2.

The established reference range interval is from 5.54 to 28.27 ng/mL (2.5th – 97.5th percentile) with a median of 11.60 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.