

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

A. 510(k) Number:

k091332

B. Purpose for Submission:

New device

C. Measurand:

RBC folate (red blood cell folate)

D. Type of Test:

Quantitative, Immunoassay

E. Applicant:

Tosoh Bioscience Inc.

F. Proprietary and Established Names:

AIA-PACK RBC Folate

G. Regulatory Information:

1. Regulation section:

862.1295

2. Classification:

Class II

3. Product code:

CGN

4. Panel:

75 (Chemistry)

H. Intended Use:

1. Intended use(s):

See Indications for use below.

2. Indication(s) for use:

Tosoh AIA-PACK RBC Folate is intended for in vitro diagnostic use only for the quantitative measurement of red blood cell folate (RBC folate) in whole blood (heparin or EDTA) samples on Tosoh AIA system analyzers. Measurement of RBC folate is used in the diagnosis and treatment of anemia.

3. Special conditions for use statement(s):

For Prescription use only.

4. Special instrument requirements:

TOSOH AIA-1800 analyzer

I. Device Description:

The AIA-RBC Folate kit consists of the following:

- 10 trays × 20 test cups (AIA-PACK RBC Folate test cup): Plastic test cups containing lyophilized twelve magnetic beads coated with anti-fluorescein isothiocyanate (FITC) mouse monoclonal antibody and fluorescein labeled bovine folate binding protein and 100 µL folate conjugated to bovine alkaline phosphatase with sodium azide as a preservative.
- AIA-PACK RBC folate calibrator set: The folate calibrator set contains 6 levels of buffered bovine serum albumin with different assigned concentration (0, 3.25, 6.5, 13.5, 25.0, and 44.0 ng/mL) of folate (described on each vial), and sodium azide as a preservative (lyophilized).
- AIA-PACK Folate sample dilution solution: The folate sample dilution solution contains a bovine protein matrix with no detectable concentration of folate, and sodium azide as a preservative.
- AIA-PACK Folate pretreatment solution: The folate pretreatment solution consists of Pretreatment-1, Pretreatment-2, and Pretreatment-3 reagents. Pretreatment-1 contains 1.5 mg/mL of dithiothreitol with sodium azide as a preservative. Pretreatment-2 contains 1.6% of sodium hydroxide. Pretreatment-3 contains buffer solution with

surfactant and sodium azide as a preservative.

- AIA-PACK Folate hemolyzing reagent set: The folate hemolyzing reagent set consists of Hemolyzing reagent-1 and reagent-2. Hemolyzing reagent-1 contains 1.0% ascorbic acid after reconstitution with 5.0 ml of distilled water. Hemolyzing reagent-2 contains buffer solution with surfactant and sodium azide as a preservative.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Bayer ADVIA Centaur® FOL Lite Reagent

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

k010050

3. Comparison with predicate:

Similarities and differences		
Item	Candidate device - Tosoh AIA-PACK RBC Folate	Predicate - Bayer ADVIA Centaur® FOL Lite Reagent K010050
Intended Use	For in vitro diagnostic use for the quantitative measurement of red blood cell folate (RBC Folate) in whole blood (heparin or EDTA) samples on TOSOH AIA system analyzer. Measurement of RBC folate is used in the diagnosis and treatment of anemia.	In Vitro Diagnostic Use for the quantitative determination of folate in serum or red blood cells.
Methodology	Competitive enzyme immunoassay using fluorescence detection	Competitive immunoassay using direct chemiluminescent technology
Sample Type	Whole blood EDTA or heparin	Serum, whole blood EDTA or heparin
Assay Components	AIA-PACK RBC FOLATE test cups, pretreatment set, hemolyzing reagent set, calibrator set, sample diluting solution	ADVIA Centaur Lite reagent pack, DTT/Releasing Agent, FOL Ascorbic acid/Ascorbic acid diluent, FOL diluent, FOL calibrator

Similarities and differences		
Item	Candidate device - Tosoh AIA-PACK RBC Folate	Predicate - Bayer ADVIA Centaur® FOL Lite Reagent K010050
Solid Phase	Lyophilized magnetic beads coated with anti-FITC mouse monoclonal antibody and fluorescein labeled bovine folate binding protein and preservatives	Purified avidin bonded to paramagnetic particles in buffer with human serum albumin and preservatives
Conjugate	Alkaline phosphatase	Acridinium ester
Calibrators	6-point calibration	2-point calibration
Folate assay low	0.62 ng/ml	0.35 ng/ml
Folate assay high	24 ng/ml	24 ng/ml
Calibration Frequency	90 Days	7 Days
Time to prepare hemolysate	30 minutes	90 minutes
Incubation	40 Minutes	5 Minutes + 2.5 Minutes
Specimen Vol (Hemolysate)	160 ul	150 ul
RBC folate reference range	148.3 – 531.1 ng/ml	280-791 ng/ml
Hemolyzing Reagent-1 (5mL) Stability	1 day after reconstitution	30 days after reconstitution
Hemolyzing Reagent -2 (65 mL) Stability	30 days after reconstitution	Not applicable
Hemolysate Stability	5 hours at room temperature	3 hours at room temperature
	60 days at -20°C	90 days at -20°C
Hemolysate Multiplication Factor	22	21
Final RBC folate calculations	Manual	Automatically by the instrument

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

1. CLSI Guideline, EP5-A2 *Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline Second edition*
2. CLSI Guideline, EP6-A *Evaluation of the Linearity of Quantitative Analytical Methods; Approved Guideline*
3. CLSI Guideline, EP7-A2 *Interference Testing in Clinical Chemistry; Approved Guideline Second edition*
4. CLSI Guideline, EP9-A2 *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline Second edition*
5. CLSI Guideline, EP17-A *Protocols for Demonstration, Verification, and Evaluation of*

Limits of Detection and Quantitation; Approved Guideline

6. CLSI Guideline, C28-A2 *How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline Second edition*

L. Test Principle:

The Tosoh AIA-PACK RBC folate is a competitive enzyme immunoassay which, after sample hemolysis and sample pretreatment, is performed entirely within the AIA-PACK. First, sample hemolysis is performed to achieve complete hemolysis of erythrocytes and deconjugation to monoglutamate in the whole blood sample using sample hemolyzing reagents (containing ascorbic acid). After sample hemolysis, sample pretreatment is performed to release folate from endogenous binding proteins in the hemolysate sample using sample pretreatment reagents (containing sodium hydroxide and dithiothreitol).

Folate present in the pretreated test sample competes with enzyme-labeled folate for a limited number of binding sites on a fluorescein labeled bovine folate binding protein which then binds to anti-FITC antibody immobilized on magnetic beads. The beads are washed to remove the unbound enzyme-labeled folate and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme labeled folate that binds to the beads is inversely proportional to the folate concentration in the test sample. A standard curve using a range of known standard concentrations is constructed and unknown folate concentrations are calculated using this curve.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

All the performance characteristics were performed using the TOSOH AIA-1800 analyzer.

- a. Precision/Reproducibility:*

Precision studies were evaluated using CLSI EP5-A2 as a guideline. The sponsor conducted within-run precision and total precision with one human whole blood sample (EDTA) and three commercially available whole blood controls. All samples were tested twice daily, in duplicate over 20 days (n=80). Results of the precision studies are shown below.

Sample	Folate Mean ng/mL	Within-run SD	Within-run %CV	Total imprecision SD	Total imprecision %CV
Whole blood control level 1	1.90	0.112	5.9	0.155	8.2
Whole blood control level 2	6.92	0.232	3.4	0.317	4.6
Whole blood control level 3	23.33	0.509	2.2	0.761	3.3
Whole blood sample	6.14	0.133	2.2	0.256	4.2

b. Linearity/assay reportable range:

Linearity studies were evaluated using the CLSI EP6-A as a guideline. A low and a high folate sample were prepared by adding a spiking solution containing folate to achieve the target folate concentrations (0.43 and 43 ng/mL). Inter-dilutions of the low and high samples were used to prepare nine levels of folate samples for the linearity study. All samples were tested in triplicate on the AIA-18000 analyzer. The recovered folate values were plotted against the expected values and an appropriate line fitted by standard linear regression was performed. The percent recovery of all the samples ranged from 97.5% to 104.8%. The linear equation generated is $Y=1.0062X + 0.0342$ with regression coefficient (R^2) of 0.999. The results of the study support the sponsor's claim that the assay is linear from 0.62 to 24.0 ng/mL.

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

Traceability: There is no reference material available for traceability. The AIA-PACK RBC folate calibrator set contains 6 levels of different concentrations of folate, which are prepared gravimetrically and compared to an internal standard material.

The folate calibrator has a shelf-life stability of 12 months when stored at 2-8°C and an open-vial stability of 1 day when stored at 2-8°C.

d. Detection limit:

The sponsor determined the Detection limits according to the CLSI EP 17-A guideline. The Limit of Detection (LoD) was determined by assaying one blank sample 60 times and six low samples in replicates of ten. The Limit of Quantitation (LoQ) was determined by assaying 10 low samples 10 times each with a target CV of 20%. The sponsor determined that the LoB for folate is 0.19 ng/mL, LoD is 0.43 ng/mL and the LoQ is 0.62 ng/mL.

The AIA-PACK folate assay has a linearity range of 0.62 to 24.0 ng/mL.

e. *Analytical specificity:*

Interference studies were evaluated according to the CLSI EP7-A2 guideline. Two controls and two whole blood samples (EDTA) were spiked with increasing amounts of the potential interferants. The spiked samples and unspiked samples were assayed in triplicate and the mean concentration was used to calculate the recovery. The sponsor defined non-interference as <10% deviation from the un-spiked samples. Based on the data, the sponsor claims no interference for the substances and concentrations listed in the table below:

Substance	Concentration
Ascorbic acid	20 mg/dL
Bilirubin (unconjugated):	18 mg/dL
Bilirubin (conjugated):	19 mg/dL
EDTA	10 mg/mL
Heparin	100 U/mL
Hemoglobin	470 mg/dL
Lipemia (triglyceride):	1600 mg/dL
Rheumatoid factor	500 IU/mL
Trisodium citrate	20 mg/mL

Cross-reactivity: Two drugs, Amethopterin and Leucovorin, were evaluated for potential cross-reactivity similar to the interference study protocol evaluated above. The sponsor defined no cross-reactivity as <2% deviation from the un-spiked samples. Based on the data, the sponsor claims no cross-reactivity for the substances and concentrations listed in the table below:

Substance	Concentration
Amethopterin	2,518 ng/mL
Leucovorin	5,231 ng/mL

f. *Assay cut-off:*

Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

A comparison study was performed between the AIA-PACK folate assay (candidate method) and the ADVIA Centaur® FOL Lite Reagent assay (predicate method) according to the CLSI EP9-A2 guideline. 101 whole blood (EDTA) samples were assayed on the AIA-1800 analyzer and the ADVIA Centaur analyzer. Hemolysis and pre-treatment of the samples were carried out according to each manufacturer's

instruction. Dilution correction and hemocrit factors were used with the folate results to calculate the final RBC folate results:

$$\text{RBC folate} = \frac{(\text{Hemolysate folate value (ng/mL)}) \times \text{dilution factor} \times 100}{\text{Hematocrit (\%)}}$$

The final calculated RBC folate results ranged from 185 to 1333 ng/mL. The resulting Deming regression equation is $Y = 1.00X - 26.60$ with a correlation coefficient (R) of 0.86.

b. Matrix comparison:

Fifty paired EDTA and heparin whole blood samples spanning the folate range were assayed on the TOSOH AIA- 1800 analyzer. The final calculated RBC folate results ranged from 151.6 to 1404.7 ng/mL. The resulting Deming regression equation is $Y = 0.955X - 9.144$ with a correlation coefficient (R) of 0.99.

The sponsor concluded that heparin is an acceptable anticoagulant for this assay.

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 according to the CLSI C28-A guideline. 122 EDTA whole blood samples were collected from apparently healthy subjects, both male and female, between the ages of 26 to 59 years old. A normal distribution result was generated from these samples and the central 95% interval was obtained to determine the reference range.

Reference range of the RBC folate is determined to be 148.3 to 531.1 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.