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

A. 510(k) Number:

k172201

B. Purpose for Submission:

New device

C. Measurand:

Folate

D. Type of Test:

Quantitative, competitive immunoassay

E. Applicant:

Siemens Healthcare Diagnostics Inc.

F. Proprietary and Established Names:

Atellica IM Folate Assay

G. Regulatory Information:

Product code	Classification	Regulation Section	Panel
CGN	II	21 CFR 862.1295 Folic acid test system	Clinical Chemistry 75

H. Intended Use:

1. Intended use(s):

   See in the indications of use below.

2. Indication(s) for use:

   The Atellica IM Folate assay is for in vitro diagnostic use in the quantitative determination of folate in serum or red blood cells using the Atellica IM Analyzer. Folic acid measurements are used in the diagnosis and treatment of anemias.

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   Atellica IM Analyzer

I. Device Description:

The Atellica IM Folate Assay contains the following reagents:

Lite Reagent: Folate labeled with acridinium ester (~9.8 ng/mL) in buffer; bovine serum albumin; sodium azide (0.1%) and preservatives.

Solid Phase Reagent: Buffered avidin (~20 µg/mL) covalently coupled to paramagnetic particles in buffer; human serum albumin and preservatives.

Folate Binding Protein: Purified folate binding protein (~1.0 µg/mL) covalently coupled to biotin in buffer; bovine serum albumin and preservatives.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   ADVIA Centaur Folate Assay

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

   k010050

3. Comparison with predicate:

Similarities and Differences		
Item	Atellica IM Folate Assay (Candidate Device) k172201	ADVIA Centaur Folate Assay (Predicate Device) k010050
Intended use	For in vitro diagnostic use in the quantitative determination of folate	Same
Specimen Type	Serum, red blood cells	Same
Principle	Chemiluminescence Competitive immunoassay	Same
Calibration	2 Point Siemens Folate Calibrators	Same

Similarities and Differences		
Item	Atellica IM Folate Assay (Candidate Device) k172201	ADVIA Centaur Folate Assay (Predicate Device) k010050
Assay range	0.56 – 24 ng/mL for serum 0.98 – 17.51 ng/mL for red blood cells	0.35 – 24 ng/mL
Instrument	Atellica IM Analyzer	ADVIA Centaur

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

CLSI EP06-A - Evaluation of Linearity of Quantitative Analytical methods: Approved Guideline.

CLSI EP05-A3 - Evaluation of Precision of Clinical Chemistry Devices: Approved Guideline-Third Edition.

CLSI EP07-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 EP28-A3 - Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory: Approved Guideline - Third Edition.

L. Test Principle

The Atellica IM Folate assay is a competitive immunoassay using direct chemiluminescent technology. The sample is pretreated to release the folate from endogenous binding proteins in the sample. Folate in the patient sample competes with acridinium-ester-labeled folate in the lite reagent for a limited amount of biotin-labeled folate binding protein. Biotin-labeled folate binding protein binds to avidin that is covalently coupled to paramagnetic particles in the solid phase. An inverse relationship exists between the amount of folate present in the patient sample and the amount of relative light units (RLUs) detected by the system.

The Atellica IM Folate assay reports system generated serum folate concentration and system-generated hemolysate folate value.

RBC folate (ng/mL) is calculated using the following formula:
RBC folate (ng/mL) = (Folate result for hemolysate (ng/mL) x 21 x 100)/hematocrit

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

A 20-day precision study was performed according to CLSI EP5-A3. Four human serum and 5 whole blood samples and 2 levels of controls were tested using 3 lots of reagents. The samples were tested in duplicates per run, 2 runs per day for 20 working days. 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.

Sample Type	N	Mean (ng/mL)	Repeatability		Within-Lab Precision	
			SD	%CV	SD	%CV
Serum A	80	1.42	0.05	3.5	0.08	5.6
Serum B	80	4.13	0.10	2.4	0.24	5.9
Serum C	80	6.19	0.18	2.9	0.36	5.9
Serum D	80	9.23	0.24	2.6	0.6	6.5
Serum Control 1	80	2.82	0.09	3.3	0.17	6.1
Serum Control 2	80	5.43	0.14	2.5	0.35	6.4
Whole Blood Sample A	80	94.89	4.56	4.8	6.55	6.9
Whole Blood Sample B	80	153.11	4.40	2.9	11.21	7.3
Whole Blood Sample C	80	362.58	9.82	2.7	19.99	5.5
Whole Blood Sample D	80	563.94	19.34	3.4	35.18	6.2
Whole Blood Sample E	80	899.24	45.66	5.1	62.92	7.0
Whole Blood Control 1	80	77.24	2.68	3.5	5.48	7.1
Whole Blood Control 2	80	257.52	6.51	2.5	16.84	6.5

b. Linearity/assay reportable range:

i. Linearity of the Atellica IM Folate assay for serum was performed according to CLSI EP06-A guideline. Nine samples with folate concentrations distributed throughout the assay range were prepared using human serum pools with high and low levels of folate. The concentration range tested was 0.46 - 24.44 ng/mL. Each sample was test in triplicate and the mean was used in the regression analysis.

The result of the linear regression is: $y = 0.9706 x – 0.2368$, with an $r^2 = 0.9956$

The results of the study supports that the assay is linear across the claimed measuring range of 0.56- 24 ng/mL for serum.

Linearity of Atellica IM Folate assay for red blood cells was demonstrated by comparing the test result of the candidate device to that obtained from a previously cleared red blood cell folate device. Red blood cell lysates with folate concentrations between the measuring interval of 0.98 to 17.51 ng/mL (11 samples) were tested. The following regression result was obtained: y = 0.917 x + 0.596, r^2 =0.9834.

The results of the study supports that the assay is linear across the claimed measuring range of 0.98 – 17.51 ng/mL for red blood cells (prior to calculation).

ii. Sample dilution study:
Studies were performed to determine the sample recovery after a 1:2 dilution. Five human serum samples with folate concentrations up to 32.42 ng/mL were diluted 1:2 with Atellica IM Fol DIL and assayed for recovery. The average percent differences for the diluted sample versus the expected concentration was within 10%.

In the labeling, the sponsor states that dilution is for serum only; hemolysates with concentrations above the claimed measuring interval should not be diluted.

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

Traceability:

The Atellica IM Folate assay is traceable to an internal standard manufactured using highly purified material (N 5 methyl tetrahydrofolate). Assigned values for calibrators are traceable to this standardization.

d. *Detection limit:*

LoB, LoD and LoQ of serum matrix was determined using 3 lots of Atellica IM Folate reagents.

For limit of blank (LoB), 5 blank serum samples (unique human serum sample pools that have been filtered, stripped and mixed with Siemens Folate BA Diluent) were tested on 2 analyzers in 2 replicates per run, 2 runs per day for 5 test days, yielding a total of 200 blank measurements per reagent lot. LoB was determined as the 95th percentile of the measurement of the blank samples.

For limit of detection (LoD), 6 contrived low level serum samples were analyzed on 2 analyzers in 2 replicates per run, 2 runs per day for 15 runs yielding a total of 60 measurements per reagent lot for each sample. LoD corresponds to the lowest concentration of folate that can be detected with a probability of 95%.

For limit of quantitation (LoQ), 8 serum samples with folate concentrations ranging from 0.27 to 1.25 ng/mL were assayed in 10 replicates, 2 runs per day per reagent lot, over a period of 5 days. LoQ was defined as the analyte concentration for which CV≤20% is met.

Lot specific LoB, LoD, and LoQ values were calculated, and the highest of the 3 lots are the claimed serum LoB, LoD and LoQ, respectively. A similar study was performed for whole blood samples collected using lithium heparin and K_2EDTA, respectively using 2 lots of reagents.

The results of the detection limit studies are summarized below.

	Serum	Whole Blood
LoB	0.19 ng/mL	0.00 ng/mL
LoD	0.38 ng/mL	0.21 ng/mL
LoQ	0.56 ng/mL	0.56 ng/mL

The claimed measuring range for serum is 0.56 - 24 ng/mL and for red blood cells (before applying conversion factors) is 0.98 – 17.57 ng/mL.

e. *Analytical specificity:*

Interference studies were performed according to CLSI EP07-A2 to evaluate the performance of the Atellica IM Folate assay in the presence of endogenous and other interfering substances. Two human serum sample pools with folate concentrations of approximately 4.0 and 12.0 ng/mL were spiked with potential interferents. The sponsor defines non-significant interference as less than 10% bias between the spiked sample and the control.
The following substances were tested up to the levels indicated and demonstrated no significant interference:

Interferent	**Highest Concentration tested that showed no significant interference**
Conjugated Bilirubin	20 mg/dL
Lipemia (intralipid)	2000 mg/dL
Unconjugated Bilirubin	20 mg/dL
Acetaminophen	200 mg/dL
Acetylsalicylic Acid	1000 mg/dL
Ibuprofen	50 mg/dL
Acetylcysteine	566 mg/dL
Ampicillin-Na	1000 mg/dL
Cefoxitin	660 mg/dL
Cyclosporine	5 mg/dL
Doxycyclin	50 mg/dL
Levodopa	20 mg/dL

Interferent	Highest Concentration tested that showed no significant interference
Methyldopa	20 mg/dL
Metronidazole	200 mg/dL
Phenylbutazone	40 mg/dL
Rifampicin	60 mg/dL
Theophylline	10 mg/dL
Ascorbic Acid	44 mg/dL

Biotin interference of the Atellica IM Folate assay was tested up to 1,200 ng/mL with both serum and whole blood (lithium heparin whole blood and K_2EDTA whole blood, respectively). The results of are shown below.

Biotin Interference (% Bias for Samples Containing Various Concentration of Biotin)								
Sample and Folate (ng/mL)	**Biotin Concentration (ng/mL)**							
	1	5	19	38	75	150	300	600
Serum 9.23	1.4%	-1.1%	6.9%	4.7%	20.2%	>AMR*	>AMR*	>AMR*
Serum 17.37	1.3%	1.3%	3.6%	8.3%	35.3%	>AMR*	>AMR*	>AMR*
Whole Blood 12.76	-7.0%	-8.4%	-8.8%	-5.7%	-3.5%	-1.1%	49.6%	68.0%
Whole Blood 18.47	0.1%	4.5%	-0.2%	4.4%	7.8%	12.7%	>AMR*	>AMR*

*Interference could not be calculated because the results of the biotin spiked samples were above the claimed analytical measuring range.

Cross reactivity was evaluated in the Atellica IM Folate immunoassay using a normal human serum sample and blank assay diluent for each test compound. An aliquot of each sample was spiked with the potential cross-reactant and a second aliquot of the base pool was spiked with just the diluent to serve as a control sample. Multiple replicates of the test and control samples were processed. Cross-reactivity was calculated as the % difference between the mean test and control sample results, with respect to the test compound concentration. Cross-reactivity results are presented below.

Cross-Reactant	Concentration tested (ng/mL)	Cross-Reactivity
Amethopterin	150	≤2%
Aminopterin	75	≤1%
Folinic Acid	25	≤4%

The sponsor states the following limitations in the labeling:

- Hemolysis significantly increases folate values in serum due to the high folate concentrations found in red blood cells.

- Methotrexate and leucovorin interfere with folate measurement because these drugs cross-react with folate binding proteins.
- Specimens that contain biotin at a concentration of 50 ng/mL (in serum) or 75 ng/mL (in whole blood) demonstrate less than or equal to 10% change in results. Biotin concentrations greater than these may lead to falsely elevated results for patient samples.
- Do not test samples from patients who take high doses of biotin. If biotin interference is suspected, follow your established internal procedures to investigate the interference or test with an alternate assay that is not affected by biotin interference.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Method comparison studies were performed by testing 105 human serum and 100 native lithium heparin whole blood samples with the candidate and the predicate device (ADVIA Centaur Folate). The results of the regression analysis are presented below.

Specimen Type	N	r	Regression Equation	Sample Range
Serum	105	0.99	y = 0.94 x - 0.01	0.64 - 22.78 ng/mL
RBC	100	0.93	y = 1.06 x -2.52	229.66 - 2264.56 ng/mL

b. Matrix comparison:

A matrix comparison study was performed by testing matched K2 EDTA whole blood and lithium heparin whole blood sets of samples using the Atellica IM Folate assay. One replicate was processed for each sample, and a total of 90 sample sets were tested using one reagent lot. The results are summarized in the following table:

N	Regression Equation	Sample Range
100	y = 1.02 x – 32.86	432.70 - 1103.69 ng/mL

The study data supports the sponsor's claim that the K2-EDTA and lithium heparin whole blood samples are acceptable samples types to use with the Atellica IM Folate 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:

   The sponsor cited the following reference range as determined on the ADVIA Centaur system using the same reagent formulations:

Category	N[a]	Median (ng/ml)	Range (ng/ml)	Median (nmol/l)	Range (nmol/l)
Serum folate					
Deficient[c]	65	1.54	< 0.56-3.37	3.49	< 1.27-7.63
lndeterminate[d]			3.38-5.38		7.64-12.19
Normal	305	12.51	> 5.38[b]	28.34	> 12.19
RBC folate					
Normal	286	425	280-791	963	634-1792

[a] Number of samples
[b] Inner 97.5% of the distribution of apparently healthy individuals.
[c] Diagnosed by bone and/or peripheral blood smear pathology and other criteria including:
- megaloblastic anemia
- folate-deficient diet
- malabsorption
- alcoholism
- Tropical Sprue
- abnormal blood parameters including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and hematocrit (HCT)

[d] Range between deficient and normal range.

The sponsor states in the labeling that:

"Laboratories should consider these expected values as guidelines only. The data were obtained on apparently healthy males and females from the United States. Because of population demographic factors, diet, and assay methods, each laboratory should determine its own expected values for the diagnostic evaluation of patient results."

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.