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

A. 510(k) Number:

k092740

B. Purpose for Submission:

New Device

C. Measurand:

Folate

D. Type of Test:

Quantitative Immunoassay

E. Applicant:

Abbott Laboratories

F. Proprietary and Established Names:

ARCHITECT Folate

ARCHITECT Folate Calibrators

ARCHITECT Folate Controls

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
Folic Acid (CGN)	Class II	21 CFR 862.1295	75 Chemistry(CH)
Calibrator (JIT)	Class II	21 CFR 862.1150	75 Chemistry(CH)
Controls (JJX)	Class I, reserved	21 CFR 862.1160	75 Chemistry(CH)

H. Intended Use:

1. Intended use(s):

   See indications for use statements below.

2. Indication(s) for use:

   The ARCHITECT Folate assay is a chemiluminescent microparticle folate binding protein assay for the quantitative determination of folate in human serum and plasma on the ARCHITECT *i* system. Folate measurements are used in the diagnosis and treatment of megablastic anemia.

   The ARCHITECT Folate Calibrators are for the calibration of ARCHITECT *i* system when used for the quantitative determination of folate in human serum and plasma.

   The ARCHITECT Folate Controls are for the verification of the accuracy and precision of the ARCHITECT *i* system when used for the quantitative determination of folate in human serum and plasma.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Architect *i* 2000SR analyzer

I. Device Description:

Each ARCHITECT Folate Reagent Kit contains 1 bottle each of the following:

- Microparticles - 1 Bottle (6.6 mL per 100-test bottle/27.0mL per 500-test bottle). Anti-Folate Binding Protein (mouse, monoclonal) coupled to microparticles affinity-bound with Folate Binding Protein (bovine), in TRIS buffer with protein stabilizers (human serum albumin and caprine). Minimum concentration: 0.08% solids. Preservatives: sodium azide and antimicrobial agents.
- Conjugate – 1 Bottle (29.0 mL per 100-test bottle/29.0 mL per 500-test bottle) Pteroic Acid (PTA) - acridinium labeled conjugate in MES buffer with protein stabilizer (porcine). Minimum concentration: 4 ng/mL. Preservative: antimicrobial agents.
- Pre-Treatment Reagent 1 – 1 Bottle (50.2mL per 100-test bottle/50.2mL per 500-test bottle) Folate Pre-Treatment Reagent1 containing potassium hydroxide.
- Pre-Treatment Reagent 2 – 1 Bottle (6.6 mL per 100-test bottle/27.0 mL per 500-test bottle) Folate Pre-Treatment Reagent 2 containing dithiothreitol (DTT) in acetic acid

buffer with EDTA.

- Specimen Diluent: 1 Bottle (5.5 mL per 100-test bottle/25.9mL per 500-test bottle) Folate Specimen Diluent containing TRIS buffer with protein stabilizer (human serum albumin). Preservative: sodium azide.

Each ARCHITECT Folate Calibrator Kit contains 6 bottles of ARCHITECT Folate Calibrators A-F (2 mL each). Calibrator A contains TRIS buffer. Calibrators B-F contains pteroylglutamic acid (PGA) in TRIS buffer. All calibrators contain protein stabilizer (human serum albumin). Preservative: sodium azide.

Each ARCHITECT Folate Control Kit contains 3 bottles of ARCHITECT Folate Controls: Low, Medium, and High Control (8 mL each). The controls contain pteroylglutamic acid (PGA) in TRIS buffer with protein stabilizer (human serum albumin). Preservative: sodium azide.

All human source materials has been tested and found to be nonreactive for HbsAg, HIV-Ag or HIV-RNA, anti-HIV-1/2, and anti-HCV.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Abbott AxSYM Folate

2. Predicate K number(s):

   k972232

3. Comparison with predicate:

Item	ARCHITECT Folate- Candidate device	AxSYM Folate – Predicate device (k972232)
Similarities and Difference		
Intended Use	The ARCHITECT Folate assay is a Chemiluminescent Microparticle Folate Binding Protein assay for the quantitative determination of folate in human serum and plasma on the ARCHITECT *i* System.	The AxSYM Folate is an ion capture assay for the quantitative determination of folate in human serum, plasma, or red blood cells on the AxSYM System.
Methodology	Chemiluminescent Microparticle Immunoassay (CMIA)	Ion capture
Assay Protocol	Competitive	Competitive
Measuring Range	1.6 ng/mL – 20.0 ng/mL	0.9 ng/mL – 20 ng/mL

Reference range	Serum folate: 7.0 to 31.4 ng/mL;	Serum folate: 7.2-15.4 ng/mL;
Specimen Type	Serum (including serum separator tubes) or Plasma (collected in lithium heparin or lithium heparin separator tubes).	Serum (including serum separator tubes) or Plasma (collected in tripotassium EDTA, potassium oxalate or sodium citrate tubes). Whole blood (collected in tripotassium EDTA tubes).
Calibrators	6 levels (0, 1.5, 3.0, 5.0, 10.0, 20.0 ng/mL)	Same
Controls	3 levels (low, medium, and high)	Same
Traceability	WHO 13/178 standard reference materials	Internal reference standards for folate using folic acid (pteroylglutamic acid, PGA)

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

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

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

CLSI EP7-A: Interference Testing in Clinical Chemistry; Approved Guideline

CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline

CLSI EP9-A: Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline

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

L. Test Principle:

The ARCHITECT Folate assay is a two-step competitive assay for the quantitative determination of folate in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex. Two pre-treatment steps mediate the release of folate from endogenous folate binding protein. In Pre-Treatment Step 1, sample and Pre-

Treatment Reagent 2 (Dithiothreitol or DTT) are aspirated and dispensed into a reaction vessel (RV). In Pre-Treatment Step 2, an aliquot of sample/ Pre-Treatment Reagent 2 mixture is aspirated and dispensed into a second RV. Pre-Treatment Reagent1 (potassium hydroxide or KOH) is then added. An aliquot of the pre-treated sample is transferred into a third RV, followed by the addition of Folate Binding Protein (FBP) coated paramagnetic microparticles and assay specific diluent. Folate present in the sample binds to the FBP coated microparticles. After washing, pteroic acid-acridinium labeled conjugate is added and binds to unoccupied sites on the FBP-coated microparticles. Pre-Trigger and Trigger Solutions are then added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative light units (RLUs). An inverse relationship exists between the amount of folate in the sample and the RLUs detected by the ARCHITECT *i* optical system.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

All performance characteristics were done on the Architect *i* 2000 SR analyzer.

a. Precision/Reproducibility:

Precision studies were evaluated using CLSI EP5-A2 as a guideline. The sponsor conducted within-run, between-run, between-day, and total precision with three serum controls and three serum panels. All samples were tested in replicates of 3, in two runs per day, for a minimum of 20 days. Two lots of reagents were used and results are shown below.

			Within-run		Between-run		Between-day		Total	
Sample Level	**Reagent lot**	**Mean (ng/mL)**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Control (L)	1	3.8	0.14	3.8	0.08	2.1	0.08	2.2	0.18	4.9
	2	3.8	0.12	3.2	0.08	2.2	0.11	2.9	0.18	4.9
Control (M)	1	7.5	0.14	1.9	0.09	1.2	0.03	0.5	0.17	2.3
	2	7.4	0.17	2.3	0.07	1.0	0.11	1.5	0.22	2.9
Control (H)	1	15.1	0.26	1.7	0.16	1.1	0.00	0.0	0.31	2.1
	2	15.3	0.25	1.6	0.10	0.6	0.13	0.9	0.30	1.9
Serum (L)	1	3.5	0.12	3.5	0.06	1.7	0.03	0.7	0.14	3.9
	2	3.6	0.14	3.9	0.00	0.0	0.09	2.6	0.17	4.7

Sample Level	Reagent lot	Mean (ng/mL)	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Serum (M)	1	10.8	0.18	1.7	0.34	3.2	0.13	1.2	0.41	3.8
	2	11.2	0.21	1.9	0.38	3.4	0.07	0.7	0.44	4.0
Serum (H)	1	16.8	0.27	1.6	0.45	2.7	0.00	0.0	0.53	3.1
	2	17.0	0.24	1.4	0.55	3.2	0.09	0.5	0.61	3.6

b. Linearity/assay reportable range:

Linearity studies were evaluated using the CLSI EP6-A as a guideline. A low serum pool sample and a high serum pool sample were mixed to create inter-dilutions of 11 mixed serum samples for the linearity study. All samples were tested in duplicates on the Architect i 2000SR 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 claimed that the assay is linear from 1.6 to 20.0 ng/mL.

Extended dilution study:
If serum folate is greater than 20 ng/mL, the analyzer can perform an auto-dilution of 1:2. To demonstrate the recovery of the auto-dilution capability of the analyzer, a dilution recover study was performed to evaluate the auto-dilution capability. 18 serum samples with folate values ranging from 20 to 40 ng/mL were automatically diluted by 1:2 and results were compared with the manual dilution of 1:2 and 1:4. All samples were tested in triplicate. The percent recovery of all the samples ranged from 98.5% to 119.6% when auto-dilution of 1:2 was compared to manual-dilution of 1:2. Based on the data, the sponsor concluded that when results are greater than 20 ng/mL, the user can either perform a manual 1:2 or a 1:4 dilution or use the analyzer to automatically dilute the sample by 1:2 to get the final results.

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

Traceability:

ARCHITECT Folate assay is traceable to the WHO serum folate International Standard 03/178.

Value assignment:

Architect folate assay calibrators and controls are manufactured using pteroylglutamic acid (PGA or folic acid) and the concentration values are assigned against internal reference standards. The internal reference standards are assigned against the WHO serum folate International standard 03/178. The concentration values of the working reference calibrators and controls were assigned by testing multiple times on three instruments using two lots of reagents. The sponsor's protocol and acceptance criteria was reviewed and found to be acceptable.

Stability:

ARCHITECT Folate assay utilize a six-level calibrators, calibrators A to F with assigned target values. Calibrators must be stored in the carton box to protect them from light. Stability of the calibrators is determined by the real-time stability study and is still on-going. The sponsor determined that the calibrators have a shelf-life stability of 10 months when stored at $\leq$-10^{o}C and an open-vial stability of 3 months when stored at $\leq$-10^{o}C. Calibrators must be discarded after 3 freeze-thaw cycle.

ARCHITECT Folate controls contain 3 levels of control materials, low, medium, and high. Controls must be stored in the carton box to protect them from light. Stability of the calibrators is determined by accelerated life testing and the real-time stability study is still on-going. The sponsor determined that the controls has a shelf-life stability of 18 months when stored at 2-8^{o}C and an open-vial stability of 3 months when stored at 2-8^{o}C.

The sponsor's protocol and acceptance criteria was reviewed and found to be acceptable.

Recommendations in the labeling for preparation of calibrator and control materials include the following: calibrators and controls must be completely thawed before use at room temperature for at least 45 minutes and mixed gently before use.

d. Detection limit:

The sponsor determined the detection limits according to the CLSI EP 17-A guideline. Limit of detection is defined as the lowest amount of analyte that can be detected. Limit of Quantitation is defined as the lowest concentration at which the analyte is reliably detected and at which the uncertainty of the observed test result is less than or equal to the goal set. The LoB, LoD, and

LoQ were determined by assaying one blank sample 80 times and five low folate samples in replicates of 80 on two instruments over 3 days. All the samples were assayed on the ARCHITECT i 2000SR analyzer. The sponsor determined that the LoB is 0.3 ng/mL, LoD is 0.6 ng/mL and LoQ is 2.0 ng/dL at the observed total error of 20%.

The ARCHITECT folate assay has a linearity range of 1.6 to 20.0 ng/mL.

e. *Analytical specificity:*
Interference studies were evaluated according to the CLSI EP7-A2 guideline. Two different levels of folate samples were spiked with one level of potential interferants. The test (spiked) samples and reference (unspiked) samples were assayed in replicates of 38 on the ARCHITECT i 2000SR analyzer. The differences between the spiked and unspiked samples were calculated and the sponsor defined no significant interference as differences of <10% with folate levels >3.5 ng/mL and ≤ 0.4 ng/mL for folate levels ≤ 3.5 ng/mL. Based on the data, the sponsor claims no significant interference for the substances and concentrations listed in the table below:

Substance	Concentration	Folate mean of the unnspiked sample	Folate mean of the spiked sample	Difference (ng/mL)	Difference (%)
Bilirubin (unconjugated)	20 mg/dL	2.1	2.0	-0.1	-4.0
		7.9	7.6	0.3	-3.8
Bilirubin (conjugated)	20 mg/dL	1.8	1.7	-0.1	-5.6
		7.5	7.0	-0.5	-6.7
Protein	12 g/dL	2.6	2.9	0.3	11.5
		8.8	9.1	0.3	2.8
Triglycerides	3000 mg/dL	2.1	2.2	0.1	4.8
		7.9	8.0	0.1	1.8

It is well-known that hemolysis falsely elevated the serum folate results; therefore, the sponsor has the following limitation in their package insert: "Do not use hemolyzed specimens. Serum or plasma specimens that are hemolyzed will give falsely elevated folate levels."

Cross-reactivity: Aminopterin, Folinic acid, and Methotrexate are chemotherapeutic agents whose structures are similar to folate and will cross-react with the folate assay and patients who are using these drugs should not have their folate tested with Architect folate assay. Therefore, the sponsor has the following limitation in their package insert:

"Methotrexate, aminopterin, and folinic acid (Leucovorin) are

chemotherapeutic agents whose molecular structures are similar to folate. These agents cross react with folate binding protein in folate assays. Do not use the Architect folate assay for patients using these drugs".

Cross-reactivity studies were performed and the results are listed below.

Interferent	Folate value (ng/dL)	Concentration tested	% cross-reactivity
Aminopterin	2.6	≥ 500 ng/mL	1.1
Aminopterin	7.4	≥ 500 ng/mL	1.1
Folinic acid	2.9	≥ 100 ng/mL	0.5
Folinic acid	7.6	≥ 100 ng/mL	-0.6
Methotrexate	2.7	≥ 100 ng/mL	2.1
Methotrexate	7.6	≥ 100 ng/mL	1.4

Additionally, the sponsor has the following statements in the limitation section of their labeling:

"Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits that employ mouse monoclonal antibodies."

"Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed."

"Serum and plasma specimens from patients with renal impairment or failure (including dialysis patients) may exhibit varying degrees of falsely depressed folate values. Therefore, to evaluate patients with renal impairment or failure who also exhibit low folate levels, it is recommended that low ARCHITECT folate values be confirmed by an alternate folate method."

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

A comparison study was performed between the Architect folate assay (candidate method) and the AxSYM folate assay (predicate method)

according to the CLSI EP9-A2 guideline. 137 serum samples were assayed on the Architect i 2000SR analyzer (Y) and the AxSYM analyzer (X). The serum folate Deming regression equation is Y = 1.06X – 2.77 (95% CI for slope is 0.98, 1.15 and 95% CI for intercept is -3.86, -1.67) with a correlation coefficient (R) of 0.90. The results ranged from 1.6 to 19.9 ng/mL.

b. Matrix comparison:

i.) Twenty-seven paired serum samples with 5 different types of blood collection tubes (plain glass serum, plain plastic serum, plastic serum with separator, lithium heparin plasma, and lithium heparin plasma with gel separator) spanning the folate range from 6.3 to 19.8 ng/mL were assayed on the Architect i2000SR analyzer. Summary of the Deming regression analysis is shown in the table below:

	Slope with CI	Intercept with CI	R	N
Serum glass vs. serum plastic	0.95 (0.89,1.02)	0.95 (0.01, 1.90)	0.988	27
Serum separator plastic vs. serum plastic	0.98 (0.95,1.00)	0.31 (-0.03, 0.64)	0.997	27
Lithium heparin plasma vs. serum plastic	1.00 (0.92, 1.08)	1.07 (-0.03, 2.17)	0.985	25
Lithium heparin separator plasma vs. serum plastic	1.00 (0.93, 1.06)	0.79 (-0.21, 1.79)	0.988	27

The sponsor concluded that plain glass tube, serum separator tube, lithium heparin tubes with or without gel separator are acceptable blood collection tube types 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 evaluated according to the CLSI C28-A3 guideline. 138 serum samples were collected from apparently healthy adult subjects, fasting male and fasting non-pregnant female >18 years old, in the U.S. The expected values were determined from the central 95% distribution using a non-parametric analysis. The expected values of the serum folate are 7.0 to 31.4 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.