Alinity i Folate-10

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BIENNIAL REVIEW:

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SUPERSEDES: Procedure titled _____

WARNING:

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 which employ mouse monoclonal antibodies. These specimens should not be assayed with the Alinity i Folate assay. Refer to the LIMITATIONS OF THE PROCEDURE section in this package insert.

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 folate patients with renal impairment or failure, it is recommended that low Alinity i Folate values be confirmed by an alternate folate method.

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 Alinity i Folate assay for patients using these drugs.

INTENDED USE

The Alinity i Folate assay is a chemiluminescent microparticle folate binding protein assay for the quantitative determination of folate in human serum and plasma on the Alinity i analyzer.

Folate measurements are used in the diagnosis and treatment of megaloblastic anemia.

SUMMARY AND EXPLANATION OF THE TEST

Folates are a class of vitamin compounds related to pteroylglutamic acid (PGA), which serve as cofactors in the enzymatic transfer of single carbon units in a variety of metabolic pathways.*1*, *2* Folate mediated one-carbon metabolism represents one of the most important biochemical reactions that occur in cells. Folates are necessary for nucleic acid and mitochondrial protein synthesis, amino acid metabolism, and other cellular processes that involve single carbon transfers. Folates can serve as carbon donors or acceptors. Since different metabolic pathways require carbon groups with different levels of oxidation, cells contain numerous enzymes that change the oxidation state of carbon groups carried by folates*2* resulting in different metabolically active forms of folate. The predominant form of circulating folate is 5-methyltetrahydrofolic acid (5-mTHF). A methyl group is transferred from 5-mTHF to cobalamin in the pathway that links metabolism of folic acid and vitamin B12.*3*

Folate deficiency can be caused by low dietary intake, malabsorption due to gastrointestinal diseases, inadequate utilization due to enzyme deficiencies or folate antagonist therapy, drugs such as alcohol and oral contraceptives, and excessive folate demand, such as during pregnancy.*4* Because deficiencies of both vitamin B12 and folate can lead to megaloblastic (macrocytic) anemia, appropriate treatment requires differential diagnosis of the deficiency; thus, both vitamin B12 and folate values are needed. Low serum folate levels reflect the first stage of negative folate balance, and precede tissue depletion.*5*

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

This assay is a two-step immunoassay for the quantitative determination of folate in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology.

Two pre-treatment steps mediate the release of folate from endogenous folate binding protein.

Sample and Pre-Treatment Reagent 2 (Dithiothreitol or DTT) are combined. Pre-Treatment Reagent 1 (potassium hydroxide or KOH) is added to an aliquot of sample/Pre-Treatment Reagent 2. An aliquot of the pre-treated sample, Folate Binding Protein (FBP) coated paramagnetic microparticles, and assay specific diluent are combined and incubated. The folate present in the sample binds to the FBP coated microparticles. The mixture is washed. Pteroic acid acridinium-labeled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.

The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is an inverse relationship between the amount of folate in the sample and the RLUs detected by the system optics.
For additional information on system and assay technology**, refer to the Alinity ci-series Operations Manual, Section 3.**

REAGENTS

Kit Contents

Alinity i Folate Reagent Kit 08P14

NOTE: This product is composed of 6 components, which are packaged as a 2 cartridge reagent set. Both cartridges are required to perform the assay.

Volumes (mL) listed in the table below indicate the volume per cartridge set.

REF	08P1421	08P1431
Tests per cartridge set	100	600
Number of cartridge sets per kit	2	2
Tests per kit	200	1200
MICROPARTICLES	6.6 mL	32.1 mL
CONJUGATE	29.0 mL	33.8 mL
ASSAY SPECIFIC DILUENT	5.7 mL	30.5 mL
PRE-TREATMENT REAGENT 1	48.1 mL	48.1 mL
PRE-TREATMENT REAGENT 2	6.6 mL	32.5 mL
SPECIMEN DILUENT	5.9 mL	31.6 mL

MICROPARTICLES 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 Pteroic Acid (PTA) - acridinium labeled conjugate in MES buffer with protein stabilizer (porcine). Minimum concentration: 4 ng/mL. Preservatives: antimicrobial agents.

ASSAY SPECIFIC DILUENT Borate buffer. Preservatives: sodium azide and antimicrobial agents.

PRE-TREATMENT REAGENT 1 Potassium hydroxide.

REF	08P1421	08P1431
PRE-TREATMENT REAGENT 2	Dithiothreitol (DTT) in acetic acid buffer with EDTA.	
SPECIMEN DILUENT	TRIS buffer with protein stabilizer (human serum albumin). Preservative: sodium azide.	

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use
- Rx ONLY

Safety Precautions

CAUTION: This product contains human-sourced and/or potentially infectious components. Refer to the **REAGENTS** section of this package insert. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human-sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[6, 7, 8, 9]

The human-sourced material used in the Microparticles and Specimen Diluent is nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HCV, and anti-HIV-1/HIV-2.

The following warnings and precautions apply to: ASSAY SPECIFIC DILUENT	
	
DANGER	Contains disodium tetraborate, anhydrous and sodium azide.
H360	May damage fertility or the unborn child.
EUH032	Contact with acids liberates very toxic gas.
Prevention	
P201	Obtain special instructions before use.
P280	Wear protective gloves / protective clothing / eye protection.
Response	

P308+P313	IF exposed or concerned: Get medical advice / attention.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

The following warnings and precautions apply to: PRE-TREATMENT REAGENT 1	
	
DANGER	Contains potassium hydroxide.
H314	Causes severe skin burns and eye damage.
H290	May be corrosive to metals.
Prevention	
P234	Keep only in original container.
P260	Do not breathe mist / vapors / spray.
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P301+P330+P331	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P303+P361+P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water / shower.
P310	Immediately call a POISON CENTER or doctor / physician.
P390	Absorb spillage to prevent material damage.
Disposal	

P501	Dispose of contents / container in accordance with local regulations.

The following warnings and precautions apply to: MICROPARTICLES/ SPECIMEN DILUENT	
Contains sodium azide.	
EUH032	Contact with acids liberates very toxic gas.
P501	Dispose of contents / container in accordance with local regulations.

Safety Data Sheets are available at www.abbottdiagnostics.com or folder.

For a detailed discussion of safety precautions during system operation, **refer to the Alinity ci-series Operations Manual, Section 8.**

Reagent Handling

- Reagents are shipped on wet ice.

Upon receipt, gently invert the unopened reagent kit by rotating it over and back for a full 180 degrees, 5 times with green label stripe facing up and then 5 times with green label stripe facing down. This ensures that liquid covers all sides of the bottles within the cartridges. During reagent shipment, microparticles can settle on the reagent septum.

  - Place a check in the square on the reagent kit to indicate to others that the inversions have been completed.
- After mixing, place reagent cartridges in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- If a reagent cartridge is dropped, place in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results.

For a detailed discussion of reagent handling precautions during system operation, refer to the Alinity ci-series Operations Manual, Section 7.

Reagent Storage

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2 to 8°C	Until expiration date	Store in upright position. If cartridge does not remain upright, gently invert the cartridge 10 times and place in an upright position for 1 hour

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
			before use.
Onboard	System Temperature	30 days	
Opened	2 to 8°C	Until expiration date	Store in upright position. If cartridge does not remain upright during storage, discard the cartridge. Do not reuse original reagent caps or replacement caps due to the risk of contamination and the potential to compromise reagent performance.

Reagents may be stored on or off the system. If removed from the system, store reagents with new replacement caps in an upright position at 2 to 8°C. For reagents stored off the system, it is recommended that they be stored in their original trays or boxes to ensure they remain upright.

For information on unloading reagents, **refer to the Alinity ci-series Operations Manual, Section 5.**

Indications of Reagent Deterioration

Deterioration of the reagents may be indicated when:

- a calibration error occurs
- a control value is out of the specified range

Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary.

For troubleshooting information, **refer to the Alinity ci-series Operations Manual, Section 10**.

INSTRUMENT PROCEDURE

The Alinity i Folate assay file must be installed on the Alinity i analyzer prior to performing the assay.

For detailed information on assay file installation and viewing and editing assay parameters, **refer to the Alinity ci-series Operations Manual, Section 2.**

For information on printing assay parameters, **refer to the Alinity ci-series Operations Manual, Section 5.**

For a detailed description of system procedures, **refer to the Alinity ci-series Operations**

Manual.

Alternate Result Units

Edit assay parameter "Result Units" to select an alternate unit.

Conversion formula:

(Concentration in Default result unit) x (Conversion factor) = (Concentration in Alternate result unit)

Default Result Unit	Conversion Factor	Alternate Result Unit
ng/mL	2.265	nmol/L

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The specimen types listed below were verified for use with this assay.

Other specimen types and collection tube types have not been verified with this assay.

Specimen Types	Collection Tubes
Serum	Serum Serum separator
Plasma	Lithium heparin plasma Lithium heparin plasma separator

- **Do not use human plasma collected in dipotassium or tripotassium EDTA tubes for Folate.**
- Performance has not been established for the use of cadaveric specimens or the use of bodily fluids other than human serum and plasma.
- Human serum or plasma specimens to be tested for folate should be protected from light.*10*, *11*
- Serum or plasma specimens should be collected from fasting individuals. Recent food intake may appreciably increase the folate concentration.*11*
- The instrument does not provide the capability to verify specimen types. It is the responsibility of the operator to verify that the correct specimen types are used in the assay.

Specimen Conditions

Do not use:

- heat-inactivated specimens
- pooled specimens
- hemolyzed specimens. Serum or plasma specimens that are hemolyzed will give falsely elevated folate levels.
- specimens with obvious microbial contamination

- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.

  Serum or plasma specimens containing red blood cells may give falsely elevated folate levels.

- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.
- Specimens should be free of bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross-contamination.

To ensure consistency in results, recentrifuge specimens prior to testing if

- they contain fibrin, red blood cells, or other particulate matter.

NOTE: If fibrin, red blood cells, or other particulate matter are observed, mix by low speed vortex or by inverting 10 times prior to recentrifugation.

Prepare frozen specimens as follows:

- Frozen specimens must be completely thawed before mixing.
- Mix thawed specimens thoroughly by low speed vortex or by inverting 10 times.
- Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous.
- If specimens are not mixed thoroughly, inconsistent results may be obtained.
- Recentrifuge specimens.

Recentrifugation of Specimens

- Transfer specimens to a centrifuge tube and centrifuge.
- Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.

Specimen Storage

- Remove serum from clot or separator gel as soon as possible after complete clot formation.
- Remove plasma from red blood cells as soon as possible upon receipt.[11]
- If testing is not performed immediately after removal of serum from the clot or separator gel or plasma removal from red blood cells refer to the specimen storage table below.

Specimen Type	Temperature	Maximum Storage Time	Special Instructions
Serum/Plasma	2 to 8°C	7 days	Specimens should be protected from light.
	-10°C or colder	30 days	Specimens should be protected from light.

Avoid more than 3 freeze/thaw cycles.

Specimen Shipping

Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.

Do not exceed the storage limitations listed above.

PROCEDURE

Materials Provided

08P14 Alinity i Folate Reagent Kit

Materials Required but not Provided

- Alinity i Folate assay file
- 08P1402 Alinity i Folate Calibrators
- 08P1412 Alinity i Folate Controls or other commercially available controls
- 08P1460 Alinity i Folate Manual Diluent
- Alinity Pre-Trigger Solution
- Alinity Trigger Solution
- Alinity i-series Concentrated Wash Buffer

For information on materials required for operation of the instrument, **refer to the Alinity ci-series Operations Manual, Section 1.**

For information on materials required for maintenance procedures, **refer to the Alinity ci-series Operations Manual, Section 9.**

Assay Procedure

For a detailed description of how to run an assay, **refer to the Alinity ci-series Operations Manual, Section 5.**

- If using primary or aliquot tubes, **refer to the Alinity ci-series Operations Manual, Section 4 to ensure sufficient specimen is present.**
- To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.
- Order tests.

Select the appropriate assay protocol.

- If running a serum or plasma specimen/control, select Folate (assay number 66, "UNDILUTED").
- If running an automated dilution on a serum or plasma specimen, select the 1:2 protocol of Folate (assay number 66, "1:2").

Maximum number of replicates sampled from the same sample cup: 10

Priority:

- Sample volume for first test: 85 µL
- Sample volume for each additional test from same sample cup: 35 µL

≤ 3 hours on the reagent and sample manager:

- Sample volume for first test: 150 µL
- Sample volume for each additional test from same sample cup: 35 µL

> 3 hours on the reagent and sample manager:

- Replace with a fresh aliquot of sample.

- Refer to the Alinity i Folate calibrator package insert and/or Alinity i Folate control package insert for preparation and usage.
- For general operating procedures, refer to the Alinity ci-series Operations Manual, Section 5.
- For optimal performance, it is important to perform routine maintenance as described in the Alinity ci-series Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Sample Dilution Procedures

Samples with a folate serum or plasma value exceeding 20.0 ng/mL (45.3 nmol/L) are flagged with the code "> 20.0 ng/mL" (">45.3 nmol/mL") and may be diluted with either the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol

The system performs a **1:2** dilution of the sample and automatically calculates the

concentration by multiplying the result by the dilution factor.

Manual Dilution Procedure

Suggested dilution: 1:2

It is recommended that dilutions not exceed 1:4.

For a 1:2 dilution, add 100 µL of the sample to 100 µL of Alinity i Folate Manual Diluent.

For a 1:4 dilution, add 100 µL of the sample to 300 µL of Alinity i Folate Manual Diluent.

The operator must enter the dilution factor in the Specimen or Control tab of the Create Order screen. The system will use this dilution factor to automatically calculate the concentration of the sample and report the result.

The result should be ≥ 3.5 ng/mL (≥ 7.9 nmol/L) before the dilution factor is applied.

If the operator does not enter the dilution factor, the result must be manually multiplied by the appropriate dilution factor before reporting the result. If a diluted sample result is less than 3.5 ng/mL (7.9 nmol/L), do not report the result. Rerun using an appropriate dilution.

For detailed information on ordering dilutions, refer to the Alinity ci-series Operations Manual, Section 5.

Calibration

For instructions on performing a calibration, **refer to the Alinity ci-series Operations Manual, Section 5.**

Each assay control must be tested to evaluate the assay calibration.

Once a calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:

- A reagent kit with a new lot number is used.

Daily quality control results are outside of statistically-based quality control limits used to monitor and control system performance, as described in the Quality Control Procedures section of this package insert.

- If **statistically-based quality control limits are not available**, then the calibration should **not exceed a 30-day** limit for recalibration frequency.

This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed.

Quality Control Procedures

The recommended control requirement for the Alinity i Folate assay is that a single sample of each control level be tested once every day testing performed.

Additional controls may be tested in accordance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control policy.

To establish statistically-based control limits, each laboratory should establish its own concentration target and ranges for new control lots at each clinically relevant control level. This can be accomplished by assaying a **minimum of 20 replicates over several (3-5) days**

and using the reported results to establish the expected average (target) and variability about this average (range) for the laboratory. Sources of variation that should be included in this study in order to be representative of future system performance include:

- Multiple stored calibrations
- Multiple reagent lots
- Multiple calibrator lots
- Multiple processing modules (if applicable)
- Data points collected at different times of the day

Refer to published guidelines for information or general control recommendation, for example Clinical and Laboratory Standards Institute (CLSI) Document C24-A3 or other published guidelines, for general quality control recommendations.[12]

- If quality control results do not meet the acceptance criteria defined by laboratory QC procedure, sample results may be suspect. Follow the established quality control procedures to troubleshoot. Recalibration may be necessary. For troubleshooting information, **refer to the Alinity ci-series Operations Manual, Section 10.**
- Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

Commercial controls should be used according to the guidelines and recommendations of the control manufacturer. Concentration ranges provided in the control package insert should be used only for guidance.

For any control material in use, the laboratory should ensure that the matrix of the control material is suitable for use in the assay per the assay package insert.

Quality Control Guidance

Refer to "Basic QC Practices" by James O Westgard, Ph.D. for guidance on laboratory quality control practices.[13]

Verification of Assay Claims

For protocols to verify package insert claims, refer to Verification of Assay Claims in the Alinity ci-series Operations Manual.

RESULTS

Calculation

The Alinity i Folate assay utilizes a 4 Parameter Logistic Curve fit data reduction method (4PLC, Y-weighted) to generate a calibration and results.

For information on alternate result units, refer to the INSTRUMENT PROCEDURE, Alternate Result Units section of this package insert.

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the Alinity ci-series Operations Manual, Section 5.

Measuring Interval

Measuring interval is defined as the range of values in ng/mL (nmol/L) which meets the limits of acceptable performance for linearity, imprecision, and bias.
The measuring interval of the Alinity i Folate assay is **2.2 to 20.0 ng/mL** (5.0 to 45.3 nmol/L).

LIMITATIONS OF THE PROCEDURE

- Accumulation of denatured protein from the pre-treatment step in the sample probe may impact results of other assays on the Alinity i analyzer. Alinity i maintenance procedure *2500 Daily Maintenance* must be run to eliminate this effect.
- 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 such as Alinity i Folate that employ mouse monoclonal antibodies. Additional information may be required for diagnosis.*14*, *15*
- 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. Additional information may be required for diagnosis.*16*
- Serum and plasma specimens from patients with renal impairment or failure (including dialysis patients) may exhibit varying degrees of falsely depressed folate values.*17* Therefore, to evaluate patients with renal impairment or failure who also exhibit low folate levels, it is recommended that low Alinity i Folate values be confirmed by an alternate folate method.
- 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.*18* Do not use the Alinity i Folate assay for patients using these drugs.
- Results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical impressions.
- If the folate level is inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- Serum or plasma containing red blood cells may give falsely elevated folate levels. These samples should be centrifuged prior to use. Serum or plasma samples that are hemolyzed will give falsely elevated folate levels.
- Samples to be tested for folate should be protected from light. Light accelerates the degradation of folate.

EXPECTED VALUES

This study was performed on the ARCHITECT i System.

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

Manufacturers provided reference range adopted, effort made to verify locally.

A study was performed based on guidance from Clinical and Laboratory Standards Institute (CLSI) document C28-A3.[19] The nutritional status of the specimen donors was unknown. All specimens tested were from fasting, apparently healthy males and non-pregnant females greater than 18 years old from a US population. Serum samples were tested using the ARCHITECT Folate assay. Data from this study are summarized in the following table.

	N	Expected Values (ng/mL)
Serum[a]	137	**7.0 - 31.4**

[a] Central 95% distribution determined using a non-parametric analysis

Folate Deficients/Indeterminates

- Folate deficiency is typically associated with serum levels less than 3.5 ng/mL.[20]
- Occasionally, the diagnosis of folate deficiency cannot be based solely on serum folate levels and further testing may be required.[20], [21], [22]

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

The Alinity i analyzer and the ARCHITECT i System utilize the same reagents and sample/reagent ratios.

Unless otherwise specified, all studies were performed on the Alinity i analyzer.

Precision

Within-Laboratory Precision

A study was performed based on guidance from CLSI EP05-A2.[23] Testing was conducted using 1 lot of the Alinity i Folate Reagent Kit, 1 lot of the Alinity i Folate Calibrators, and 1 lot of the Alinity i Folate Controls and 1 instrument. Three controls and 3 serum panels were assayed in a minimum of 2 replicates at 2 separate times per day on 20 different days.

			Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
Sample	**n**	**Mean (ng/mL)**	**SD**	**%CV**	**SD**	**%CV**
Low Control	120	3.8	0.22	5.8	0.31	8.1
Medium Control	120	7.8	0.28	3.6	0.38	4.8
High Control	120	15.5	0.36	2.3	0.46	3.0

Sample	n	Mean (ng/mL)	Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
			SD	%CV	SD	%CV
Panel S1	120	3.7	0.23	6.1	0.29	7.9
Panel S2	120	11.1	0.36	3.2	0.41	3.7
Panel S3	120	17.9	0.40	2.2	0.49	2.7

Sample	n	Mean (nmol/L)	Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
			SD	%CV	SD	%CV
Low Control	120	8.6	0.50	5.9	0.70	8.1
Medium Control	120	17.6	0.63	3.6	0.85	4.8
High Control	120	35.1	0.82	2.3	1.05	3.0
Panel S1	120	8.4	0.52	6.1	0.66	7.9
Panel S2	120	25.1	0.82	3.2	0.92	3.7
Panel S3	120	40.6	0.90	2.2	1.10	2.7

[a]Includes within-run, between-run, and between-day variability.

Accuracy to World Health Organization (WHO) Standard

This study was performed on the ARCHITECT i System.

The ARCHITECT Folate assay was evaluated for bias relative to the Folate WHO **International Standard 03/178. A minimum of 38 replicates of the WHO Standard was tested on each of 2 instruments. A different reagent lot was used on each instrument and one calibrator lot was used for both instruments.**

The Folate assay results **were accurate within ± 10%** to the 1st International Reference Standard (I.S.) for Serum Folate (03/178). Data from this study are summarized in the following table.

n	Median (ng/mL)	Target (ng/mL)	Diff.[a] (ng/mL)	Two-Sided 95%CL[b] (ng/mL)	%Diff.[a]	Two-Sided 95%CL[b] %Diff.[a]
38	5.3	5.3	-0.1	-0.1, 0.0	-1.0	-2.0, 0.1

[a] Diff. = Difference, [b] CL = Confidence Limit

Lower Limits of Measurement

A study was performed based on guidance from CLSI EP17-A2.[24] Testing was conducted using 3 lots of the Alinity i Folate Reagent Kit on each of 2 instruments over a minimum of 3 days. The maximum observed Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) values are summarized below.

	ng/mL	nmol/L
LoB[a]	0.9	2.0
LoD[b]	1.4	3.2
LoQ[c]	**2.2**	5.0

[a]The LoB represents the 95th percentile from n ≥ 60 replicates of zero-analyte samples.

[b]The LoD represents the lowest concentration at which the analyte can be detected with 95% probability based on n ≥ 60 replicates of low-analyte level samples.

[c] The LoQ was determined from n ≥ 60 replicates of low-analyte level samples and is defined as the lowest concentration at which a total allowable error of 39% was met.

Linearity

A study was performed based on guidance from CLSI EP06-A.[25]

This assay is linear across the measuring interval of **2.2 to 20.0 ng/mL** (5.0 to 45.3 nmol/L).

Cross-Reactants

This study was performed on the ARCHITECT i System.

A study was performed with the ARCHITECT Folate assay based on guidance from the CLSI document EP07-A2.[26] The cross reactants listed in the table below were added to processed human serum containing endogenous folate. Therapeutic levels of these drugs can greatly exceed the levels tested in this study and are expected to interfere with the ARCHITECT Folate assay.[18] Aliquots of human serum at two different folate concentrations were supplemented with potential cross-reactants and tested for folate. Data from this study are summarized in the following table.

	Reference		Test				
Cross-Reactant	n	Mean/ Median ng/Ml	n	Mean/ Median ng/mL	Diff.[a] ng/mL	%Diff.[b]	%Cross-Reactivity[c]
Aminopterin ≥ 500 ng/mL	40	2.6	40	8.3	5.7	219.2	1.1
	40	7.4	39	13.0	5.6	75.7	1.1
Folinic Acid ≥	40	2.9	40	3.4	0.5	17.2	0.5

Cross-Reactant	Reference n	Reference Mean/ Median ng/Ml	Test n	Test Mean/ Median ng/mL	Diff.[a] ng/mL	%Diff.[b]	%Cross-Reactivity[c]
100 ng/mL	40	7.9	44	7.3	-0.6	-7.4	-0.6
Methotrexate ≥ 100 ng/mL	45	2.7	40	4.8	2.1	77.8	2.1
	40	7.6	40	8.9	1.4	18.2	1.4

[a] Difference = test mean [or median] conc. - reference mean [or median] conc.

[b] % Difference = Difference / reference mean [or median] conc. x 100

[c] % Cross-Reactivity = Difference / interferent conc. x 100

Interference

This study was performed on the ARCHITECT i System.

Potentially Interfering Endogenous Substances

Potential interference in the ARCHITECT Folate assay from bilirubin, (conjugated and unconjugated), triglycerides, and protein was demonstrated in a study based on guidance from CLSI document EP07-A2.*26* Hemoglobin was not tested due to the high folate content in red blood cells. Refer to the LIMITATIONS OF THE PROCEDURE section. Observed differences were less than 12% between the reference and the test sample. Data from this study are summarized in the following table.

Interferent	Reference n	Reference Mean/Median ng/mL	Test n	Test Mean/Median ng/mL	Diff.[a] ng/mL	%Diff.[b]
Bilirubin (unconjugated)	40	2.1	40	2.0	-0.1	-4.0
≤ 20 mg/dL	40	7.9	40	7.6	-0.3	-3.8
Bilirubin (conjugated) ≤ 20 mg/dL	40	1.8	40	1.7	-0.1	-5.6
	40	7.5	40	7.0	-0.5	-6.7
Protein ≤ 12 g/dL	40	2.6	40	2.9	0.3	11.5
	40	8.8	40	9.1	0.3	2.8
Triglycerides	40	2.1	40	2.2	0.1	4.8
≤ 3000 mg/dL	40	7.9	39	8.0	0.1	1.8

[a] Difference = test mean [or median] - reference mean [or median]

[b] % Difference = Difference / reference mean [or median] x 100

Tube Type Matrix Comparison

This study was performed on the ARCHITECT i System.

The following tube types are acceptable for use with the ARCHITECT Folate assay:

- Plastic: Serum, Serum Separator Tube (SST), Lithium Heparin Plasma Tube, and Lithium Heparin Plasma Separator Tube (PST)

Regression analysis was performed using the Deming regression method to compare each evaluation tube type to the control tube type (serum plastic). Data from this study are summarized in the following table.

Evaluation Tube Type	n	Control Tube (serum plastic) Range (ng/mL)	Evaluation Tube Range (ng/mL)	r[a]	Intercept (ng/mL)	Slope
Serum Separator	27	6.1 - 19.3	6.4-19.2	0.997	0.31	0.98
Lithium Heparin	25		6.7-19.8	0.985	1.07	1.00
Plasma Separator[b]	27		6.3-19.7	0.988	0.79	1.00

[a] r = Correlation Coefficient

[b] Lithium Heparin Plasma Separator Tube

Method Comparison

A study was performed based on guidance from CLSI EP09-A3 using the Passing-Bablok regression method.[27]

		Units	n	Correlation Coefficient	Intercept	Slope	Concentration Range
Alinity i Folate vs ARCHITECT Folate	Serum	ng/mL	158	0.96	0.21	0.97	2.0 - 17.2
	Serum	nmol/L	158	0.96	0.51	0.97	4.4 - 39.0

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