BECKMAN COULTER

ACCESS
Immunoassay Systems

Instructions For Use

Access Folate

REF A98032

FOR PROFESSIONAL USE ONLY

Rx Only

ANNUAL REVIEW

Reviewed by	Date	Reviewed by	Date

PRINCIPLE

INTENDED USE

The Access Folate assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of folic acid levels in human serum and plasma (heparin) or red blood cells using the Access Immunoassay Systems.

Folate levels in serum and plasma (heparin) or red blood cells are used to assess folate status. The serum folate levels is an indicator of recent folate intake. A low RBC folate value can indicate a prolonged folate deficiency.

SUMMARY AND EXPLANATION

Folate is an essential vitamin vital to normal cell growth and DNA synthesis. It is present in a wide variety of foods such as dark, leafy vegetables, citrus fruits, yeast, beans, eggs, and milk. It is absorbed by the small intestine and stored in the liver. A folate deficiency can lead to megaloblastic anemia and ultimately to severe neurological problems.[1,2,3]

Folate deficiency can be caused by insufficient dietary intake, malabsorption or excessive folate utilization. Excessive utilization occurs very commonly during pregnancy. Alcoholism, hepatitis, or other liver-damaging diseases can also cause excessive folate utilization.[1,2,3] Folate levels in both serum and red blood cells are used to assess folate status. The serum folate level is an indicator of recent folate intake. Red blood cell (RBC) folate is the best indicator of long term folate stores. A low RBC folate value can indicate a prolonged folate deficiency.

Folate and vitamin B_{12} are linked by the reaction pathway for methionine synthesis. A deficiency in either leads to a disruption of this pathway and to similar clinical symptoms.[1,3] Another consequence of this common metabolic pathway is that a B_{12} deficiency disrupts the uptake of folate into red blood cells. This leads to a low RBC folate value even with adequate folate intake. For the above reasons, it is often necessary to measure both vitamins in a clinical workup. The treatment depends on which vitamin is deficient.

METHODOLOGY

The Access Folate assay is a competitive binding receptor assay. For the assay of folate in serum or plasma (heparin), no pre-treatment is required. For the assay of folate in red blood cells, a whole blood sample is first treated off-line with a lysing agent composed of ascorbic acid. This pre-treatment hemolyzes the red blood cells and converts the folate polyglutamic acid forms present in red cells to the monoglutamic acid form predominant in serum.[4] The sample from the pre-treatment of whole blood is defined as a hemolysate.

A serum, plasma (heparin), or hemolysate sample is treated to release folate from endogenous binding proteins. Folate binding protein, mouse anti-folate binding protein, folic acid-alkaline phosphatase conjugate, and goat anti-mouse capture antibody coupled to paramagnetic particles are added to the reaction vessel. Folate in the sample competes with the folic acid-alkaline phosphatase conjugate for binding sites on a limited amount of folate binding protein. Resulting complexes bind to the solid phase via mouse anti-folate binding protein.

After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is inversely proportional to the concentration of folate in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

SPECIMEN

SPECIMEN COLLECTION AND PREPARATION

Serum or Plasma (Heparin) Folate

1. Serum and plasma (heparin) folate from fasting individuals are the recommended samples.
2. Observe the following recommendations for handling, processing, and storing blood samples:[5]
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation.
   - Keep tubes stoppered at all times.
   - Physically separate serum or plasma from contact with cells as soon as possible.
   - If the assay will not be completed immediately, refrigerate the samples at 2 to 8°C.
   - If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C or colder.[6]
   - Beckman Coulter, Inc. recommends that frozen specimens can be stored up to six months before testing.
   - Thaw samples only once.
   - Studies have shown that degradation of folate due to fluorescent light up to one week after blood collection is small and unlikely to affect accuracy of results.[7]
3. Use the following guidelines when preparing specimens:
   - Ensure residual fibrin and cellular matter have been removed prior to analysis.
   - Follow blood collection tube manufacturer's recommendations for centrifugation.
4. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot-to-lot.
5. Do not use hemolyzed samples. The folate level in the red cells is much greater than that of the serum or plasma (heparin), leading to spuriously high results.

Red Blood Cell Folate

1. Collect whole blood specimens in tubes containing EDTA or heparin. Determine and record the hematocrit for use in the calculations. The specimen may be stored at 2 to 8°C for up to 4 hours before preparing the hemolysate.

2. Reconstitute the vial of Access Red Blood Cell Folate Lysing Agent (Cat. No. A14206) with 100 mL of deionized water. Allow to stand until completely dissolved, a minimum of 40 minutes. Swirl gently to mix before use. The solution may be stored at 2 to 8°C for up to 2 weeks. Allow the solution to come to room temperature before each use.
3. Gently invert the whole blood sample several times to insure that it is well mixed and combine 50 µL of the whole blood with 1 mL of the lysing agent.
4. Gently invert the mixture several times and allow it to stand at room temperature for a minimum of 90 minutes. After 90 minutes, either test the hemolysate within 1.5 hours or freeze it at -70°C or colder for storage or shipment. The hemolysate may be stored at -70°C or colder for up to 30 days. Alternatively, the hemolysate may be stored at -20°C or colder for up to 30 days; however, there may be some reduction (typically less than 10%) in the RBC folate hemolysate result.
5. To assay frozen hemolysates, thaw and allow sample to come to room temperature. Mix the sample by inverting the tube several times. Assay the sample within 1.5 hours.

REAGENTS

PRODUCT INFORMATION

Access Folate Reagent Pack

Cat. No. A98032: 100 determinations, 2 packs, 50 tests/pack

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Stable at 2 to 10°C for 14 days after initial use.
- Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range.
- If the reagent pack is damaged (i.e., broken elastomer), discard the pack.
- All antisera are polyclonal unless otherwise indicated.

R1a:	Mouse monoclonal anti-folate binding protein, paramagnetic particles coated with goat anti-mouse IgG, buffer, human serum albumin (HSA) and 0.1% ProClin* 300.
R1b:	1.0M Ascorbate, 0.05N HCl, pH 5.5.
R1c:	Milk folate binding protein (bovine) in buffer, HSA and 0.1% ProClin 300.
R1d:	Folic acid alkaline phosphatase (bovine) conjugate in buffer, HSA and 0.1% ProClin 300.
R1e:	0.6M K_3PO_4.

*ProClin™ is a trademark of The Dow Chemical Company (“Dow”) or an affiliated company of Dow.

WARNING AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant

for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.

- Human source material used in the preparation of the reagent has been tested and found negative or non-reactive for Hepatitis B, Hepatitis C (HCV), and Human Immunodeficiency Virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.[8]
- For hazards presented by the product refer to the following sections: REACTIVE INGREDIENTS and GHS HAZARD CLASSIFICATION.

REACTIVE INGREDIENTS

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

Folate PMP (Compartment R1a)	DANGER	
		
		
	H317	May cause an allergic skin reaction.
	H360	May damage fertility or the unborn child.
	P201	Obtain special instructions before use.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P308+P313	IF exposed or concerned: Get medical advice/attention.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		Sodium Borate Decahydrate 1 - 2%
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
Folate Binding Protein (Compartment R1c)	DANGER	
		

		
	H317	May cause an allergic skin reaction.
	H360	May damage fertility or the unborn child.
	P201	Obtain special instructions before use.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P308+P313	IF exposed or concerned: Get medical advice/attention.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		Sodium Borate Decahydrate 1 - 2%
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
Folate Conjugate (Compartment R1d)	EUH208	May produce an allergic reaction.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
Folate Phosphate (Compartment R1e)	DANGER	
		
	H314	Causes severe skin burns and eye damage.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P301+P330+P331	IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
	P303+P361+P353	IF ON SKIN (or hair): Rinse skin with water.
	P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P310	Immediately call a POISON CENTER or doctor/physician.
		Potassium Phosphate, Tribasic 10 - 15%

SDS	Safety Data Sheet is available at techdocs.beckmancoulter.com

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

1. Access Folate Calibrators
   Provided at zero and approximately 1.2, 3.1, 6.2, 12.4, and 24.8 ng/mL (2.8, 7.0, 14.0, 28.1, and 56.2 nmol/L).

Cat. No. A98033

2. Quality Control (QC) materials: commercial control material.
3. Access Substrate
   Cat. No. 81906
4. Access Wash Buffer II, Cat. No. A16792
   UniCel DxI Wash Buffer II, Cat. No. A16793
   UniCel DxI Access Immunoassay Systems Wash Buffer II, Cat. No A79784
   (Diluent pack for use with the UniCel DxI system onboard dilution feature.)
5. Access Red Blood Cell Folate Lysing Agent
   Cat. No. A14206
6. Access Folate Calibrator S0
   Cat. No. A99250

EQUIPMENT AND MATERIALS

R1	Access Folate Reagent Packs

CALIBRATION

CALIBRATION INFORMATION

An active calibration curve is required for all tests. For the Access Folate assay, calibration is required every 28 days. Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

QUALITY CONTROL

Quality control materials simulate the characteristics of patient samples and are essential for monitoring the system performance of immunochemical assays. Because samples can be processed at any time in a "random access" format rather than a "batch" format, quality control materials should be included in each 24-hour time period.[9] Include commercially available quality control materials that cover at least two levels of analyte. More frequent use of controls or the use of additional controls is left to the discretion of the user, based on good laboratory practices or laboratory accreditation requirements and applicable laws. Follow manufacturer's instructions for reconstitution and storage. Each laboratory should establish mean values and acceptable ranges to assure proper performance. Quality control results that do not fall within acceptable ranges may indicate invalid test results. Examine all test results generated since obtaining the last acceptable quality control test point for this analyte. Refer to the appropriate system manuals and/or Help system for information about reviewing quality control results.

TESTING PROCEDURE(S)

PROCEDURAL COMMENTS

1. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.
2. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.
3. Use fifty-five (55) µL of sample for each determination in addition to the sample container and system dead volumes. Use one hundred fifty-five (155) µL of sample in addition to the sample container and system dead volumes for

each determination run with the DxI system onboard dilution feature. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.

4. The system default unit of measure for sample results is ng/mL. To change sample reporting units to the International System of Units (SI units), nmol/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply ng/mL by multiplication factor 2.266.[10]

PROCEDURE

Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.

Use **FOLW** as the test name for serum or plasma (heparin) samples and **RBCW** as the test name for red blood cell hemolysates. The same reagent pack is used for both sample types.

RESULTS INTERPRETATION

Patient test results are determined automatically by the system software. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Patient test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

Use the following procedure to calculate RBC folate results:[6]

1. Multiply the RBC folate hemolysate result by 21 to correct for the 1:21 dilution that was made during preparation of the hemolysate.
2. Divide this result by the patient's hematocrit.

$$\text{RBC Folate (ng/mL)} = \frac{\text{hemolysate folate x 21}}{\text{(hematocrit/100)}}$$

Example:

Hemolysate folate value = 3.5 ng/mL
Hematocrit = 40%

$$\text{RBC Folate (ng/mL)} = \frac{\text{3.5 ng/mL x 21}}{\text{(40/100)}} = \frac{73.5}{0.4}$$

$$\text{RBC Folate (ng/mL)} = \text{184 ng/mL packed RBC}$$

The previous calculation assumes that the serum or plasma (heparin) folate value is low relative to the RBC folate value. Occasionally, this is not the case. Use the following calculation when the serum or plasma (heparin) folate is elevated and the RBC folate is low:

$$\text{RBC Folate (ng/mL)} = \frac{\text{(hemolysate folate x 21) - [serum folate x (1 - hematocrit/100)]}}{\text{(hematocrit/100)}}$$

Example:

Hemolysate folate value = 3.5 ng/mL
Serum or plasma (heparin) folate value = 18 ng/mL
Hematocrit = 40%

$$\text{RBC Folate (ng/mL)} = \frac{\text{(3.5 x 21) - [18 x (1 - 40/100)]}}{\text{(40/100)}} = \frac{62.7}{0.4}$$

$$\text{RBC Folate (ng/mL)} = \text{157 ng/mL packed RBC}$$

REPORTING RESULTS

EXPECTED RESULTS

1. Each laboratory should establish its own reference ranges to assure proper representation of specific populations. Reference intervals from several populations have shown increased folate levels as compared to historical data due to folic acid fortification of foods.[11,12,13,14] Two reference interval studies were conducted, one from a population where folic acid fortified foods are commonly available and one from a population where folic acid fortified foods are not commonly available. The reference intervals resulting from these studies are provided below. The folate and RBC folate expected values should only be considered as guidelines.
2. **Serum Folate:** Sera from 171 normal subjects from the United States were assayed to establish expected ranges. The normal values ranged from 2.3 to greater than 24.8 ng/mL (5.2 to > 56.2 nmol/L). The two-sided, non-parametric 90% reference range of this study is:

Units	Reference Range
ng/mL	5.9 to > 24.8
nmol/L	13.4 to > 56.2

The WHO Technical Consultation on folate and vitamin B_{12} deficiencies has determined that deficient folate concentrations are considered to be less than 4 ng/mL (10 nmol/L).[15]

3. **Red Blood Cell Folate:** Whole blood samples from 144 normal subjects from the United States were assayed to establish an expected range. The normal values ranged from 215 to greater than 1,356 ng/mL (487 to > 3,073 nmol/L) packed red blood cells (RBCs). The two-sided, non-parametric 90% reference range of this study is:

Units	Reference Range
ng/mL	366 to > 1,356
nmol/L	829 to > 3,073

Note: The folate and RBC folate expected values should only be considered as guidelines. Data was obtained on normal subjects in the United States and may not apply to countries/populations where folic acid fortification of foods does not occur.

4. **Serum Folate:** Sera from 311 normal subjects from the United Kingdom (a population where foods are not commonly fortified with folic acid) were assayed to establish expected ranges. The normal values ranged from 1.6 to greater than 24.8 ng/mL (3.6 to > 56.2 nmol/L). The two-sided, non-parametric 90% reference range of this study is:

Units	Reference Range
ng/mL	3.1 to 19.9
nmol/L	7.0 to 45.1

The WHO Technical Consultation on folate and vitamin B_{12} deficiencies has determined that deficient folate concentrations are considered to be less than 4 ng/mL (10 nmol/L).[15]

5. **Red Blood Cell Folate:** Whole blood samples from 190 normal subjects from the United Kingdom (a population where foods are not commonly fortified with folic acid) were assayed to establish an expected range. The normal

values ranged from 105 to greater than 903 ng/mL (238 to > 2,046 nmol/L) packed red blood cells (RBCs). The two-sided, non-parametric 90% reference range of this study is:

Units	Reference Range
ng/mL	140 to 836
nmol/L	317 to 1,894

Note: The folate and RBC folate expected values should only be considered as guidelines. Data was obtained on normal subjects in the United Kingdom where folic acid fortification of foods does not occur.

PROCEDURAL NOTES

LIMITATIONS

1. **Serum or plasma (heparin) Folate**
   Samples can be accurately measured within the analytical range of the lower limit of detection and the highest calibrator value (approximately 1.0-24.8 ng/mL [2.27-56.2 nmol/L]).
   - If a sample contains less than the lower limit of detection for the assay, report the results as less than that value (i.e., < 1.0 ng/mL [< 2.27 nmol/L]). When the DxI system onboard dilution feature is used, the system will report results as less than 21 ng/mL (47.6 nmol/L).
   - If a sample contains more than the stated value of the highest Access Folate Calibrator (S5), report the result as greater than that value (i.e., > 24.8 ng/mL [> 56.2 nmol/L]). Alternatively, dilute one volume of sample with one volume of Access Folate Calibrator S0 (zero), which is also available as Access Folate Calibrator S0 Cat. No. A99250 or dilute one volume of sample with one volume of Access Wash Buffer II. Refer to the appropriate system manuals and/or Help system for instructions on entering a sample dilution in a test request. The system reports the results adjusted for the dilution.
   - The DxI system onboard dilution feature automates the dilution process, using one volume of sample with one volume of UniCel DxI Access Immunoassay Systems Wash Buffer II, allowing samples to be quantitated up to approximately 49.6 ng/mL (112.4 nmol/L). The system reports the results adjusted for the dilution.
2. **Red Blood Cell Folate**
   If a red blood cell hemolysate reads > 24.8 ng/mL, calculate the minimum value using the equation above. Report the result as greater than this minimum value. Alternatively, dilute one volume of red blood cell hemolysate with one volume Access Red Blood Cell Folate Lysing Agent (Cat. No. A14206). After assaying the diluted hemolysate, multiply the calculated value by the dilution factor two or refer to the appropriate system manuals and/or Help system for detailed instructions on processing pre-diluted samples. Use the corrected value to recalculate ng/mL packed RBC using the equation above.
3. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples.[16,17]

   Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
4. The Access Folate results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests, and other appropriate information.

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS

METHODS COMPARISON

Representative data for methods comparison are provided for illustration only. Performance obtained in individual laboratories may vary.

A comparison of 158 serum folate values using the Access Folate assay (Cat. No. A14208) and the restandardized Access Folate assay (Cat. No. A98032) on the Access 2 Immunoassay System gave the following statistical data using Deming calculations:

n	Cat. No. A14208 Mean (ng/mL) (Range)	Cat. No. A98032 Mean (ng/mL) (Range)	Intercept (ng/mL)	Slope	Correlation Coefficient (r)
158	10.03 (0.59-19.05)	13.18 (0.61-25.30)	-0.38	1.35	0.99

A comparison of 40 paired serum and plasma (Lithium Heparin) using the restandardized Access Folate assay gives the following statistical data using Deming calculations:

n	Range of Observations Serum (ng/mL)	Intercept (ng/mL)	Slope	Correlation Coefficient (r)
40	5.37-23.11	0.064	1.04	1.00

A comparison of 200 red blood cell folate values using the Access Folate assay (Cat. No. A14208) and the restandardized Access Folate assay (Cat. No. A98032) on the Access 2 Immunoassay System gave the following statistical data using Deming calculations:

n	Cat. No. A14208 Mean (ng/mL Packed RBC) (Range)	Cat. No. A98032 Mean (ng/mL Packed RBC) (Range)	Intercept (ng/mL Packed RBC)	Slope	Correlation Coefficient (r)
200	291.55 (85.97-883.99)	357.94 (110.70-1,122.45)	1.36	1.22	0.99

A comparison of 57 paired EDTA and Lithium Heparin whole blood samples using the restandardized Access Folate assay gives the following statistical data using Deming calculations:

n	Range of Observations† Lithium Heparin (ng/mL)	Intercept† (ng/mL)	Slope	Correlation Coefficient (r)
57	8.41-23.73	-0.75	1.08	0.96

†These are uncorrected for dilution factor and hematocrit.

DILUTION RECOVERY (LINEARITY)

Representative data for dilution recovery (linearity) are provided for illustration only. Performance obtained in individual laboratories may vary.

Multiple dilutions of two serum samples containing various folic acid levels with Access Folate Calibrator S0 (zero) resulted in the following data:

Sample 1 (%)	Expected Concentration (ng/mL)	Determined Concentration (ng/mL)	Recovery (%)
Neat	N/A	21.1	N/A
80	16.9	16.3	96
50	10.6	11.3	106
25	5.4	6.4	120
17	3.6	4.4	122
10	2.2	2.6	118
		Mean % Recovery	**112**

Sample 2 (%)	Expected Concentration (ng/mL)	Determined Concentration (ng/mL)	Recovery (%)
Neat	N/A	22.3	N/A
80	17.8	20.1	113
50	11.2	10.4	93
25	5.7	5.5	98
17	3.8	4.0	106
10	2.3	2.4	104
		Mean % Recovery	**103**

IMPRECISION

Representative data for imprecision are provided for illustration only. Performance obtained in individual laboratories may vary.

The serum folate assay exhibits total imprecision of less than or equal to 15% at concentrations greater than 2.0 ng/mL with a standard deviation (SD) less than or equal to 0.3 ng/mL at concentrations less than or equal to 2.0 ng/mL.

This assay exhibits within run imprecision of less than or equal to 9% at concentrations greater than 2.0 ng/mL with a SD less than or equal to 0.18 ng/mL at concentrations less than or equal to 2.0 ng/mL.

One study, using commercially available and in-house prepared human-based control material generating a total of 40 assays, 2 replicates per assay, over 20 days provided the following data, calculated based on CLSI EP5-A2 guidelines:[18]

Sample	Grand Mean (n=80) (ng/mL)	Total SD (ng/mL)	Within Run (%CV)	Between Run (%CV)	Total Imprecision (%CV)
Low	2.22	0.13	3.6	4.7	6.0
Medium	8.31	0.33	2.0	3.4	4.0

Sample	Grand Mean (n=80) (ng/mL)	Total SD (ng/mL)	Within Run (%CV)	Between Run (%CV)	Total Imprecision (%CV)
High	10.65	0.81	3.0	7.0	7.6
QC 1	3.30	0.19	2.4	5.2	5.7
QC 2	8.37	0.48	2.3	5.2	5.7
QC 3	14.27	1.41	2.6	9.6	9.9

The red blood cell folate assay exhibits total imprecision of less than or equal to 15% at concentrations greater than 2.0 ng/mL with a standard deviation (SD) less than or equal to 0.3 ng/mL at concentrations less than or equal to 2.0 ng/mL.

This assay exhibits within run imprecision of less than or equal to 9% at concentrations greater than 2.0 ng/mL with a SD less than or equal to 0.18 ng/mL at concentrations less than or equal to 2.0 ng/mL.

One study, using commercially available and in-house prepared human-based control material generating a total of 40 assays, 2 replicates per assay, over 20 days provided the following data, calculated based on CLSI EP5-A2 guidelines:[18]

Sample	Grand Mean (n=80) (ng/mL)†	Total SD (ng/mL)	Within Run (%CV)	Between Run (%CV)	Total Imprecision (%CV)
Low	5.14	0.08	1.1	1.0	1.5
Medium	10.10	0.22	1.4	1.6	2.1
High	12.77	0.62	4.3	2.3	4.8
QC 1	2.48	0.15	3.5	4.7	5.9
QC 2	8.77	0.34	1.4	3.6	3.8
QC 3	14.06	0.59	2.2	3.6	4.2

† These are uncorrected for dilution factor and hematocrit.

ANALYTICAL SPECIFICITY / INTERFERENCES

Representative data for analytical specificity/interferences are provided for illustration only. Performance obtained in individual laboratories may vary.

Samples containing up to 10 mg/dL (171 µmol/L) bilirubin, 300 IU/mL rheumatoid factor, and lipemic samples containing up to 1,800 mg/dL (20.32 mmol/L) triglycerides do not affect the concentration of folate assayed.

In addition, samples with 5 g/dL (50 g/L) paraprotein (as human serum albumin) added to the endogenous albumin in the samples do not affect the concentration of folate assayed. The following table describes the cross-reactivity of the assay with substances that are similar in structure to folate. The analytes were spiked into serum samples. Values for cross-reactivity were calculated as described in CLSI EP7-A2.[19]

Substance	Analyte Added	Cross-reactivity (%)
Aminopterin	500 ng/mL	0.3
Phenytoin	100 µg/mL	< 0.1
Methotrexate	100 ng/mL	4.0
Folinic Acid (Leucovorin)	100 ng/mL	0.3

Representative data for Limit of Blank, Limit of Detection and Limit of Quantitation are provided for illustration only. Performance obtained in individual laboratories may vary.

Limit of Blank (Analytical Sensitivity)

The Access Folate assay has a Limit of Blank (LoB) of < 0.80 ng/mL (< 1.81 nmol/L). One study determined the LoB for Access Folate to be 0.53 ng/mL (1.20 nmol/L). LoB was tested using a protocol based on CLSI EP17-A.[20] A total number of 468 replicates of a zero analyte sample (Access Folate Calibrator S0) were measured in 12 runs using multiple reagent and calibrator lots on a DxI Immunoassay System.

Limit of Detection

The Access Folate assay has a Limit of Detection (LoD) of < 1.0 ng/mL (< 2.27 nmol/L). One study determined the LoD for Access Folate to be 0.85 ng/mL (1.93 nmol/L). LoD was tested using a protocol based on CLSI EP17-A.[20] Nine replicates from 5 low-level samples were measured using multiple reagent and calibrator lots in 12 runs on a DxI Immunoassay System.

Limit of Quantitation (Functional Sensitivity)

The Access Folate assay has a Limit of Quantitation (LoQ) of < 2.0 ng/mL (< 4.53 nmol/L). One study determined the LoQ at 20% CV to be 1.24 ng/mL (2.81 nmol/L). LoQ was tested using a protocol based on CLSI EP17-A.[20] Nine replicates of 7 samples were measured using multiple reagent pack and calibrator lots in 22 runs on a DxI Immunoassay System.

ADDITIONAL INFORMATION

REVISION HISTORY

Revision L

IFU updated to add Dutch, Finnish, Macedonian, Traditional Chinese, and Estonian

SYMBOLS KEY

Glossary of Symbols is available at techdocs.beckmancoulter.com (document number C02724)

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