YALE-NEW HAVEN HOSPITAL	TITLE: **Folate Serum and RBC - Access 2**		**DEPT OF LAB MEDICINE** Immunology, Flow Cytometry, and Molecular Diagnostics Laboratories
			DOCUMENT #: IMM 182
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WRITTEN BY: Susan Goettlich, (ASCP)	**EFFECTIVE DATE:** April 9, 2012	**REVISION DATE:** N/A	**SUPERCEDES:** Folate Access Serum and RBC Doc# IMM 155

I. INTRODUCTION:

Folate is an essential vitamin 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. In nature folate is present in a variety of forms where it functions biochemically as a coenzyme for the transfer of single carbon units (1). It is vital to normal cell growth and DNA synthesis. A folate deficiency can lead to megaloblastic anemia and ultimately to severe neurological problems (1).

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). 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 B12 are linked by the reaction pathway methionine synthesis. A deficiency in either leads to a disruption of this pathway and to similar clinical symptoms (1). Another consequence of this common metabolic pathway is that a B12 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.

As of April 9, 2012, YNHH has switched to the restandardized Beckman Coulter Access Folate assay. The Access Folate assay calibrators, previously standardized to USP reference material, have been restandardized by measuring for accuracy to World Health Organization (WHO) International Standard 03/178. The WHO Technical Consultation on folate and B12 deficiencies has determined that deficient folate concentrations are considered to be less than 4 ng/mL.

II. PRINCIPLES OF THE PROCEDURE:

The Access Folate assay is a competitive binding receptor assay. For the assay of folate in serum, 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. 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 Lumi-Phos* 530 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.

III. REAGENTS:

A. Access Folate Reagent Pack
Cat. No. A98032: 100 determinations, 2 packs, 50 tests/pack

1. Store upright and refrigerate at 2 to 10°C.
2. Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
3. Stable until the expiration date stated on the label when stored at 2 to 10°C.
4. Stable at 2 to 10°C for 14 days after initial use.
5. Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range.
6. If the reagent pack is damaged (i.e., broken elastomer), discard the pack.
7. All antisera are polyclonal unless otherwise indicated.
8. Reagent 1a: Mouse monoclonal anti-folate binding protein, paramagnetic particles coated with goat anti-mouse IgG buffer, human serum albumin(HSA) and 0.1% ProClin**300.
9. Reagent 1b: 1.0M Ascorbate, 0.05N HCl, pH 5.5.

10. Reagent 1c: Milk folate binding protein(bovine) in buffer, HAS and 0.1% ProClin 300.

11. Reagent 1d: Folic Acid alkaline phosphatase(bovine) conjugate in buffer,
12. HAS and 0.1% ProClin 300.

13. Reagent 1e: 0.6M K3PO4.

B. Quality Control (QC) materials:

Biorad Anemia Control

Lyphochek levels 1,2,3. Lyphochek Whole BloodControls(Biorad)

Access Substrate, Cat. No. 81906

Access Wash Buffer II, Cat. No. A16792

Access Red Blood Cell Folate Lysing Agent, Cat. No. A14206-store at 2-8 degrees C. (Reconstitute with 100ml CLRW-let sit for 45 minutes, mix by inversion 10 times, stable for 2 weeks at 2-8 degrees centigrade. Bottle contents-150 mg of ascorbic acid. Allow reagent to come to room temperature before use.

C. Contrad (Beckman Coulter)

D. Citronox (Beckman Coulter)

N.B. Please note that all test components, reagents and non reagents are all ordered separately.

IV. WARNINGS AND PRECAUTIONS:

A. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up (8).

B. Xi. Irritant: 0.5N NaOH
R 36/38: Irritating to eyes and skin.
S 26, 37/39: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable gloves and eye/face protection.

C. Xi. Irritant: 0.25% ProClin 300.
R 43: May cause sensitization by skin contact.
S28-37: After contact with skin, was immediately with plenty of soap and water. Wear suitable gloves.

V. CALIBRATORS:

Quantitative assay calibration is the process by which samples with known analyte concentrations (i.e. assay calibrators) are tested like patient samples to measure the response. The mathematical relationship between the measured responses and the known analyte concentrations establishes the calibration curve. The mathematical relationship or calibration curve is used to convert RLU (relative light units) measurements of patient samples to specific quantitative analyte concentrations.

A. Access Folate Calibrators: 4.0 mL/vial(Cat# A98033)

- Provided ready to use
- Store at -20°C. Thaw only once.
- Mix contents by gently inverting before use. Avoid bubble formation
- Stable until the expiration date stated on the vial labels when stored at -20°C
- **Stable for three months when stored at 2 to 10°C**
  Provided at zero and approximately 1.2, 3.1, 6.2, 12.4 and 24.8 ng/mL.

B. When to Calibrate

- Every 28 days
- New reagent lots
- After major maintenance
- QC failures or Westgard rule violations

VI. ADDITIONAL SUPPLIES:

A. Extra long filter pipet tips(0-200ul)-USA Scientific
B. 12x75 plastic tubes(Sarstedt)
C. Caps for 12x75 tubes(Cardinal)
D. Reaction Vessels(Beckman Coulter)
E. Rainin P200 Microliter Pipetman
F. Rainin P1000 Microliter Pipetman
G. Eppendorf Repeater Pipet
H. Tips for P200 Microliter Pipet(Rainin)
I. Tips for P1000 Microliter Pipet(Rainin)
J. 12.ml Eppendorf tips(Cardinal)
K. Cryovials-2ml size(Cardinal)
L. 2 and 3 ml Volumetric Pipets(Cardinal)
M. 100ml Graduate Cylinder(Cardinal)

VII. SPECIMEN REQUIREMENTS:

A. Serum Folate

The test should be performed on serum only (from red top tube). Separate serum by centrifugation, 3000 rpm for 15 minutes. If not tested immediately, serum aliquots are stored refrigerated at 2 - 8°C over night for up to 24 hours. If testing can not be performed within 24 hours, store specimens at -20°C. Specimens may be kept at frozen at -20°C for 6 months. Sera may be frozen and thawed only once. Do not perform the test

on hemolyzed or lipemic serum. Lipemic serum should be spun at 10,000 rpm for 20 minutes to remove contaminating lipids.

Standard Aliquot volume = 300 uL
Minimum Aliquot volume = 200 uL
Hemolyzed: Reject
Grossly Lipemic: Spin at 10,000 rpm for 20 min.
Stability: 24 hours at 2-8°C or 6 months at -20°C

B. RBC Folate

One EDTA lavender top tube is used for testing. DO NOT SPIN LAVENDER TOP TUBE. Lavender top tube must be lysed within three days of draw time. A hematocrit must be performed on the sample prior to analysis.

Hemolysate Procedure:

1. Gently invert the whole blood sample several times to insure that it is well mixed.
2. Combine 50 µL of the whole blood with 1 mL of the lysing agent. Gently invert the mixture several times and allow it to stand at room temperature for a minimum of 90 minutes.
3. After 90 minutes, hemolysates are frozen at -20°C until testing and are stable for 30 days.
4. Once thawed, hemolysates must be tested within 1.5 hours.

Minimum volume = 1 mL
Stability: EDTA tube- 3 days
Hemolysates frozen – 30 days and thawed 1.5 hours.
Reject: Clotted Specimens

VIII. CONTROL PREPARATION:

A. Biorad Lyphochek Controls, 3 levels, are rehydrated with 5ml of CLRW and allowed to sit for 15 minutes. The controls are mixed thoroughly and aliquoted in 0.3ml amounts in 2ml cryovials. Controls are then stored frozen at -20°C for up to 20 days.

B. Biorad Anemia Control is rehydrated with 3ml of CLRW and allowed to sit for 15 minutes. The control is mixed thoroughly and aliquoted in 0.25ml amounts in 2ml cryovials . It is frozen at -20°C and is stable for up to 20 days.

C. Biorad Whole Blood Lyphochek Controls, 3 levels. Whole Blood controls are rehydrated with 2ml of CLRW and allowed to sit for 15 minutes. The controls are aliquoted in 0.15ml amounts in cryovials

and frozen at -20°C for up to 20 days. The controls are thawed and lysed like the patient samples and run for RBC folate on the Access.

IX. TEST PROCEDURE:

A. Remove serum samples and RBC lysing reagent from the refrigerator. Lysing reagent must come to room temperature prior to use.

B. Remove Lyphochek controls-levels 1-3, Anemia Control, and Whole Blood Controls-levels 1-3 from the freezer. Thaw all controls and mix thoroughly prior to using.

C. If a calibration is needed, remove Calibrators from freezer-allow to thaw and mix completely
before use. See Calibrators section under reagents for long term storage conditions. Calibrations are required every 28 days or when a reagent pack lot number is changed or when major maintenance has been done on the instrument.

D. Check the Instrument Supplies Screen to verify that there are enough tests on the reagent pack on the instrument. There should be a minimum of 50 tests left on the instrument(serum and whole blood).

E. To add a reagent pack, select F1-Load Reagent Pack from the reagent supplies screen. Take a Folate reagent pack from the refrigerator and invert till the pack is mixed thoroughly. Follow the prompts on the access 2 screen about loading of the reagent pack.

F. To set up a Calibration Run-Select Sample Manager from the instrument menu screen. Enter the number of the rack that the calibration will be performed on. Use a rack that has a barcode on it for test cups. Place six cups on the rack. Load all 6 calibrators into the test cups-use a minimum of 300ul per calibrator, as all calibrators are run in duplicate.

G. To load the rack on the instrument-select F3 test request from the Sample Manager Screen. Next select F6-Request Calibration. another window will open. Select the Folate calibrator lot number in use from this screen. Then select OK. This will order up a request for calibration to be done on the six cups on the rack. Next exit this screen by selecting the Back button and select the Sample Manager Screen. Next select F1-Load a Rack, follow the onscreen prompts for loading the rack.

H. Next from the Sample Manager Screen-select F3-Test Requests. In the test requests screen, select F5-Request QC-select the Anemia, and Lyphocheks levels 1-3. From the test menu insert, select FOLW for all 4 QC's. Next place 4 test cups in to the selected rack. Be sure to use a barcoded test cup rack! Select the Back button to exit the test

requests screen. Select the Sample Manager screen and select F1-Load a Rack. Load the rack and press RUN on the upper left hand corner of the screen. Minimum Sample Volume=0.3ml.

I. While the calibration is running, log into the Soft Computer and access the Instruments Menu program. Select the Access workstation and build a load list for the running of the days samples-see Soft Immunology Manual (Doc# IMM 120) for more information of building loadlists.

J. Next using a repeating Eppenorf pipettor, add 1 ml of lyse to three tubes marked WB1, WB2, WB3-these are the three lyse tubes for the Whole Blood controls, Levels 1-3 (Prepare 2 sets of controls). Mix each control, and add 50ul of each control using the extra long P200 filter tips. Cap each control and invert 2-3 times. Do not Vortex! Next put the tubes into thecabinet, to protect the tubes from light. Incubate the tubes for 90 minutes. At the end of 90 minutes, invert each tube 3-4 times to remix them. Next the samples should be immediately programmed and run on the instrument. Following the same procedure as in Step 8, instead of selecting the Lyphochek controls, under the request QC(F5) screen, select the three whole blood controls. Next select the RBCW test for each of the three controls. Load the rack as previously described in step 8. Minimum Sample Volume=0.5ml.

K. Acceptance of Calibration-after the calibration has been performed, a calibration curve will print. If the calibration has passed, a note will appear in the upper right hand corner of the Calibration report. Also all 4 QCs for serum folate and all three QCs must be in range before any patients can be run. This is a must since all samples are autoverified in Soft as they are generated by the instrument.
Note: Both serum folates and RBC folates are run off of the same Calibration Curve!

L. Next patient samples for serum Folate can be loaded if all the controls are in range. Under Sample Manager Screen: Enter a rack number under in the Rack ID box. Be sure to use the racks bar-coded for sample tubes(12x75mm). Next select F1-Load a Rack button. Follow on the onscreen prompts for loading of the sample rack. Then press the RUN button on the top left corner of the screen. The rack will then be read by the Access 2 and test request for serum folate will be assigned to each sample by the Soft computer interface. Be sure to always down load your Worklist prior to starting the day's run!

M. Once the serums are done being aspirated, the rack can be removed off of the Access 2 and another rack can be loaded in its place. To unload a rack, select the rack to be unloaded by clicking on it, next select F6-Get Selected Rack button and follow the onscreen prompts for removing the rack. Warning-Do not clear the rack until the word "DONE" appears on the rack and the aspiration and completion times

have disappeared. The unloaded rack will appear on the right hand side of the screen in the Off Board section. To load more sample racks-follow the instructions in step 12. Be sure to always press RUN, after loading or unloading any racks. Otherwise the instrument will remain in a Paused mode and no samples will be run! A maximum of 6 racks can be loaded onto the Access 2 at one time.

N. Once the Whole Blood Controls are done and in range for the RBC folates, the patient whole blood lysates can be thawed and run. The lysates should be removed from the -20◦C freezer and thawed at room temperature protected from light. Once the lysates are thawed, they should be inverted 4-6 times, and then be placed on a rack(use rack for barcoded tubes). DO NOT VORTEX SAMPLES! To load the rack, follow the instructions in step 12. Do not load the lysates on the instrument when there are 3 or more racks already loaded, waiting to be aspirated. The instrument will not assay the whole blood lysates until after all the other tests loaded ahead of these racks have been aspirated. Whole Blood RBC lysates should be tested within 3 hours of thawing-this includes any dilutions needing to be run.

X. QUALITY CONTROL-ACCEPTANCE OF RUNS:

A. Control Ranges
All control ranges are established using a minimum of 30 data points and are set at +/- 3 standard deviations. All new lots of controls are pretested to obtain the 30 data points necessary to establish an in-house range. On rare occasions the manufactures range may be used until an in-house range can be established.

B. New Reagent Lots
All new reagent lots are verified by running a minimum of 5 patient samples. The 5 samples will have been tested on a previously validated reagent lot and should span the reportable range of the assay.

C. Serum Folate
All 4 levels of quality control must be in range before any patient results can be reported. If any one of the controls is out of range repeat twice using two new aliquots of QC. If both replicates are not within range, calibration is required. The 10x and 2-2S Westgard rules are also followed. See the Quality Control procedure (Doc# IMM 174) for more detailed instruction.

D. RBC Folate
All 3 levels of quality control must be in range before any patient results can be reported. If any one of the controls is out of range repeat the second set of lysed controls. If still not in range calibration may be required. The 10x and 2-2S Westgard rules are also followed. See the Quality Control procedure (Doc# IMM 174) for more detailed instruction

XI. RESULTS:

Serum Folate

Serum Folate results are sent via Access2 interface to the Soft LIS system. Results less than 24.0ng/mL are autoverified by Soft. Results greater than the upper limit of detection are reported as greater than 24.0.

The following comment will be added to each result for approximately 1 year.

"As of April 9, 2012, a new Folate assay, now calibrated to a World Health Organization standard, has been introduced. The new reference range for serum folate is ≥ 5.9 ng/mL. It is estimated that patient sample results with the new assay will shift upward by 30-45% compared to the old assay. The 'WHO Technical Consultation on Folate and Vitamin B12 Deficiencies' has determined that serum folate concentrations less than 4 ng/mL are considered deficient, based on the values for which plasma metabolites such as homocysteine become elevated (Food and Nutrition Bulletin, vol. 29, no. 2 (supplement), p. S238, 2008)."

RBC Folate Results:

The following comment will be added to each result for approximately 1 year.

"As of April 9, 2012, a new Folate assay, now calibrated to a World Health Organization standard, has been introduced. The new reference range for RBC folate is ≥ 366 ng/mL. It is estimated that patient sample results with the new assay will shift upward by 30-45% compared to the old assay. The 'WHO Technical Consultation on Folate and Vitamin B12 Deficiencies' has determined that RBC folate concentrations < 340 ng/mL are considered deficient, based on the values for which plasma metabolites such as homocysteine become elevated (Food and Nutrition Bulletin, vol. 29, no. 2 (supplement), p. S238, 2008)."

Use the following procedure to calculate RBC folate results if the softcomputer is down.

A. Multiply the RBC folate hemolysate result by 21 to correct for the 1:21 dilution that was made during preparation of the hemolysate.

B. 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%

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

RBC Folate(ng/ml)=184 ng/ml packed RBC

The above calculation is done automatically in the soft computer. If any RBC folate value is >24.8 ng/ml. Make a 1:2 dilution of the lysate using the Red Blood Cell Lysing Reagent and rerun the sample at the 1:2 dilution. The result will be automatically multiplied by the Access computer and the RBC folate calculated by the Soft computer system. Do not dilute the sample > 1:2! If the sample is >49.6 at a 1:2 dilution, manually calculate the RBC folate result using 49.6 as the raw RBC folate value and enter the final result as a greater than number in the Soft Computer. Greater than results will not be autoverified in the Soft computer. All RBC folate final results are reported out in whole numbers.

Serum Results are automatically posted in the in the Soft Computer. All results >24.8 ng/ml will flag and must be manually posted in the Soft computer Access instrument menu. All results less than 1.0 ng/ml are resulted as <1.0 ng/ml by the Soft computer.
All results in AMR are resulted out to one decimal point.

XII. AMR/ARR:

The manufactures Analytical Measuring Range (AMR) for both Serum and RBC Folate is approximately 1.0 – 24.8 ng/mL. and varies based on the lot of calibrator. Due to this variability, all Folate results greater than 24.0 will not be reported.

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XIII. REFERENCE RANGE:

Serum Folate: >/= 5.9 ng/mL

RBC Folate: >/= 366 ng/mL

XIV. LIMITATIONS OF THE PROCEDURE:

A. 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.Such interfering antibodies may cause erroneous results. Carefully

evaluate the results of patients suspected of having these antibodies.

B. 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.

XV. VALIDATION

A. Correlation

A regression analysis (EP Evaluator) was performed using the results from the USP reference material calibration (current assay) as the reference method and the results from the restandardized calibration (new assay) as the test method. Results greater than or less than the reportable range were not included. The acceptability limit for the Correlation Coefficient (R) was set at > 0.9000.

Serum Folate
# of samples: 67 (60 included)
Corr Coef (R): 0.9873
Slope: 1.330
RBC Folate
# of samples: 61 (61 included)
Corr Coef (R): 0.9905
Slope: 1.500

B. Reference ranges

The reference ranges suggested by the manufacturer (1) were verified by running samples from the YNHH normal population. The acceptability limit was set at <10% below the reference range stated.

Serum Folate: Reference Range =/>5.9 ng/mL
23 samples were tested, only 1 (4%) sample was less than 5.9 ng/mL
RBC Folate: Reference Range =/>366 ng/mL
24 samples were tested, only 1 (2%) sample was less than 366 ng/mL

XVII. REFERENCES:

1. Folate Assay Procedure (CLSI document). Beckman Coulter, Brea, CA 92821, 2011

XVIII. APPENDIX

182		Folate Serum and RBC - Access2
182-A		Folate Serum and RBC - Access2 Quiz
182-B		Folate Serum and RBC - Access2 Training Checklist

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April 9, 2012

Signature Approval for Annual Review
Folate Serum and RBC – Access2
Document #: IMM 182

Name (Print)	Title	Signature	Date of Review	Revision Page and Section # (Use Procedure Review Log to document staff review)	Issue Date for Training if Applicable	Effective Date for Use
TEODORICO LEE	LAB MANAGER	Teodorico Lee	4/9/12	New procedure. Replaces Folate Access Serum and RBC. Beckman has updated assay to WHO standardization.		4/9/12
BRIAN SMITH	LAB DIRECTOR	[illegible]	4/10/12			4/9/12

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Staff Procedure Review Log

Name: Folate Serum and RBC - Access2

Document # IMM 182

[X] New [ ] Revision

[ ] Annual Review

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