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RBC-Folate
ADVIA Centaur XPT

By Chemiluminescence
LIS CODE = RBCFLT

I. Principle

The ADVIA Centaur Folate assay is a competitive immunoassay using direct, chemiluminescent technology. Folate in the patient sample competes with acridinium ester-labeled folate in the Lite Reagent for a limited amount of folate binding protein, which is covalently coupled to paramagnetic particles in the Solid Phase. The assay uses sodium hydroxide and DTT to release the folate from the endogenous binding proteins in the sample. An inverse relationship exists between the amount of folate present in the patient sample and the amount of RLUs (relative light units) detected by the system.

II. Clinical Significance

Folates are compounds of pteroylglutamic acid (PGA) that function as coenzymes in metabolic reactions involving the transfer of single-carbon units from a donor to a recipient compound. Folate, with Vitamin B12, is essential for DNA synthesis, which is required for normal red blood cell maturation. Humans obtain folate from dietary sources including fruits, green and leafy vegetables, yeast, and organ meats. Folate is absorbed through the small intestine and stored in the liver.

Low folate intake, malabsorption as a result of gastrointestinal diseases, pregnancy and drugs such as phenytoin are causes of folate deficiency. Folate deficiency is also associated with chronic alcoholism. Folate and vitamin B12 deficiency impair DNA synthesis, causing macrocytic anemias. These anemias are characterized by abnormal maturation of red blood cell precursors in the bone marrow, the presence of megaloblasts, and decreased red blood cell survival.

Since both folate and vitamin B12 deficiency can cause macrocytic anemia, appropriate treatment depends on the differential diagnosis of the deficiency. Serum folate measurement provides an early index of folate status. However, folate is much more concentrated in red blood cells than in serum so the red blood cell folate measurement more closely reflects tissue stores. Red blood cell folate concentration is considered the most reliable indicator of folate status.

III. Specimen

Whole blood (EDTA) is the recommended sample type to measure red cell folate.
Caution: Folates are light sensitive. Minimize exposure to light during sample handling and storage.
Collect: collect one EDTA tube (heparin is also acceptable).

Transport: Whole blood EDTA at ambient or 2–8^{0}C (see stability).

Stability: **For RBC Folate**: If testing is not done within 24 hours for whole blood specimens, **determine the hematocrit**, then freeze the EDTA whole blood or prepare the hemolysate and freeze. Whole blood specimens may be stored frozen at –20^{0}C for up to 2 months, and hemolysates may be stored at –20^{0}C for up to 3 months. Freeze specimens only once and mix thoroughly after thawing.

The sample volume pipetted for the folate assay is 150 ul. Folate results are not diluted.

A. Preparing the Red Blood Cell Hemolysate

1. Add the entire contents of the ascorbic acid diluent to the lyophilized folate ascorbic acid. Let stand for 15 min. and mix by inverting occasionally. Stable for 30 days at 2-8C.
2. Invert the patient sample several times to mix.
3. Pipet 1.0 ml reconstituted ascorbic acid and 50 ul of sample into a pour-off tube.
4. Invert several times to mix; or vortex gently to mix. Avoid foaming. **Protect from light**.
5. Let the hemolysate stand at room temp. for a minimum of 90 min. Do not exceed 3 hours. **Do not mix the hemolysate again before placing the sample on the system**.
6. Freeze the hemolysate at or below at –20^{0}C if testing cannot be completed within 4 hours of the time you finish prepating the hemolysate. Hemolysates can be stored at at –20^{0}C for up to 3 months.
7. If the hemolysate is frozen, thaw and mix by inverting. Let stand for 30 minutes at room temperature before testing. Do not invert again. Test the hemolysate within 3 hours from thawing.

IV. Reagents

1. ADVIA Centaur Folate ReadyPack primary reagent pack
   a. Lite Reagent
      10.0 ml folate labeled with acridinium ester in buffer with bovine serum albumin, sodium azide and preservatives. Store at 2-8^{0}C until the expiration date on the pack label.
   b. Solid Phase
      20.0 ml purified avidin covalently coupled to paramagnetic particles in buffer with human serum albumin and preservatives. Store 2-8^{0}C until the expiration date on the label.
   c. Folate Binding Protein
      10.0 ml purified folate binding protein covalently coupled to biotin in buffer with bovine serum albumin and preservatives. Store at 2-8^{0}C until the expiration date on the pack label.

2. ADVIA Centaur Folate DTT /Releasing Agent ReadyPack ancillary reagent pack. Stable for 108 hours in an ancillary reagent pack.

Preparing DTT/Releasing Agent

a. Carefully transfer the contents of one vial of Releasing Agent into one vial of DTT.
b. Firmly screw the cap on the DTT vial and invert several times to mix.
c. Pour the contents into the ancillary pack provided.
d. Place a pack seal on the ancillary pack. Ensure that the seal is centered and press firmly. Stable 108 hours.

Careful preparation of DTT/Releasing Agent is required to obtain accurate and consistent results. The absolute amount of DTT delivered for each test can affect results. Prepare fresh DTT/Releasing Agent before calibration.

3. Folate calibrator vials.
   Low or high levels of N-5-methyl-tetrahyydrofolic acid in buffer with human serum albumin with sodium azide and preservatives. Store as follows:
   2-8^{0}C lyophilized-until the expiration date on the label or
   2-8^{0}C reconstituted for 7 days or
   -20^{0}C reconstituted for 28 days or
   onboard 8 hours
4. Folate ascorbic acid/folate ascorbic acid diluent vials.
   30 ml lyophilized ascorbic acid bovine serum albumin in buffer with sodium azide and preservatives. Store 2-8^{0}C until the expiration date on the vial or 30 days reconstituted.

A. Reconstituting the Folate Ascorbic Acid:

1. Add the entire contents of the Folate Ascorbic Acid Diluent to the lyophilized Folate Ascorbic Acid.
2. Let the reconstituted mixture stand at room temperature for 15 minutes and mix by inverting the bottle occasionally. Stable 30 days at 2-8^{0}C.

B. Precautions

1. Sodium Azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides.
2. Potential biohazard, human and/or other biological source material. Handle as if potentially infectious, according to established good laboratory practices.
3. Prewarming or bringing the reagents to room temperature before use is not required.
4. Septum caps are required on all reagents loaded on reagent carousel.
5. Do not use kit components beyond expiration date.

6. Safety data sheets (MSDS/SDS) available on www.siemens.com/diagnostics.

7.

C. Loading Reagents:

1. Ensure that the system has sufficient primary and ancillary reagent packs.
2. **CAUTION:** Mix all primary reagent packs by hand before loading them onto the system. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and re-suspended.
3. Load the Ready Pack reagent packs in the primary reagent area using the arrows as a placement guide. The system automatically mixes the primary reagent packs to maintain homogenous suspension of the reagents.

D. Onboard stability

1. The ADVIA Centaur folate assay has an onboard stability of 28 days.
2. Discard the primary reagent packs at the end of the onboard stability interval.
3. Do not use reagents beyond the expiration date.

V. Instrumentation/Equipment

The ADVIA CENTAUR system is an automated, immunoassay analyzer that offers optimal productivity and efficiency. No-pause reloading of reagents, samples, and supplies means that the system is always ready to process samples. All assays use direct chemiluminescent technology. Chemiluminescence is a chemical reaction that emits energy in the form of light. When used in combination with immunoassay technology, the light produced by the reaction indicates the amount of analyte in the sample. Direct chemiluminescent reactions directly measure the light energy without the use of added steps or amplifying molecules. The ADVIA Centaur assays use acridinium ester as the chemiluminescent label, since it does not require the addition of a catalyst or substrate.

The QC and patients specimens need to be programmed for RBC folate then when the sample start key is pressed, the barcode labels on the sample cups are read, sample is aspirated, reagent is dispensed, and the assay process begins. Particles are magnetically separated in the cuvette incubation ring. The addition of hydroxyl groups to complete the flash reaction is accomplished by the addition of Reagent 1 & 2; Acid and Base. The chemiluminescent reaction occurs in the luminometer. The photomultiplier tube measures the chemical light reaction that takes place.

There is one (1) main system operation key on the ACS:CENTAUR, the "**Sample Start button**". Pressing this key performs the following actions:

a. Homes the subsystems.
b. The system starts specimen sampling.
c. If the start button is pressed while the instrument is running, it stops sampling additional specimens, however it continues to process the specimens in the incubation ring.

Additional Equipment and Supplies

Reagent Water
Sample cups / tubes
Cuvettes
Sample tips
Reagent 1 (0.5% H2O2; 0.1N HNO3)
Reagent 2 (less than 0.25N. Sodium Hydroxide and surfactant)
ACS:CENTAUR Cleaning Solution
ACS:CENTAUR primary and ancillary reagents.

VI. Calibration

The ADVIA Centaur system uses a Master Curve and a two-point operator initiated calibration to calibrate assays. The Master Curve is established as part of the manufacturing process for each assay lot number.

The ADVIA Centaur folate assay requires a Master Curve calibration when using a new lot number of Lite Reagent, Solid Phase, and Folate Binding Protein. For each new lot number, use the barcode reader to enter the Master Curve values on the system. The Master Curve Card contains the Master Curve values. Refer to the system operating instructions for more information.

A two-point calibration must be performed at regular, assay specific intervals. Replicates for two calibrators of known value are processed. If the calibrators meet defined validity criteria, the system is adjusted. Refer to the Centaur Operating Procedures for calibration procedure.

A. Calibration Frequency

Two-point calibration of the folate assay is required:

1. every 7 days
2. when changing lot numbers of primary reagent packs
3. when replacing system components
4. when QC results are unacceptable

B. Calibration Material

The folate assay is calibrated with lot specific calibrators, Low and High. Actual concentrations may change with lot numbers, but approximate folate concentrations are 2.78 ng/ml for the Low Calibrator and 18.60 ng/ml for the High Calibrator.

Caution: The Folate Calibrators provided in this kit are matched to the Solid Phase and Lite Reagent. DO NOT mix Folate Calibrator lots with different lots of Solid Phase and Lite Reagent.

1. Reconstitute each vial with 3.0 ml reagent grade water using a volumetric pipet.
2. Let the calibrator stand for 15 – 20 minutes at room temperature.
3. Gently swirl and invert the vials until homogenous.
4. Each lot of calibrators contains a Calibrator Assigned Value card. Calibrator values may be entered by using the barcode wand or the keyboard.

5. Affix the Low and High Calibrator barcode labels to the appropriate calibrator sample cups so that the system recognizes the sample as a calibrator.
6. Refer to the Centaur Operating Procedures for scheduling and running a calibration.
7. **Prepare and load fresh DTT/Releasing Agent before calibration.**

C. Stability / Storage

1. Store at 2-8^{o}C
2. Lyophilized calibrator stable until expiration date on the vial.
3. Reconstituted calibrator stable 7 days.
4. On board stability 8 hours.

D. Precautions

1. Sodium Azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides.
2. Potential biohazard, human and/or other biological source material. Handle as if potentially infectious, according to established good laboratory practices.
3. Do not return any calibrators back into the vials after calibration because evaporation can occur, which may affect performance.
4. Dispose of any calibrator remaining in the sample cups after 4 hours.
5. Do not refill calibrator sample cups when the contents are depleted. If required, dispense fresh calibrators into new cups.

VII. Quality Control

A. Folate Control: BioRad Immunoassay Plus Levels 1, and 3. BioRad, ECS Division, Anaheim,CA

B. Preparation/handling: Stored frozen. Allow thawing completely at either room temperature or at 2-8 °C. Stable for 14 days once thawed and 4 days after opening when stored capped at 2-8 °C.

C. Red Cell Folate Control: BioRad Whole Blood Control Levels 1 and 2. Stored 2-8C. Reconstitute with 2.0 ml DI water. Let stand 20 min. Swirl to mix. The folate constituent is stable for 3 days at 2-8C. **The control must be prepared as a patient before analyzing**. Make a hemolysate as you would a patient (1 ml ascorbic acid + 50 ul control). Aliquots of the hemolysate are stored frozen in immuno's freezer. Hemolysates are stable for 30 days at - -20C. Protect from light.

D. Frequency: Both levels of BioRad Immunoassay Plus are performed by first shift after the calibration is complete and valid, once per week. Both levels of RBC Folate controls are performed by first shift once per day of patient testing.

E. Acceptability Criteria: Acceptability of QC is determined by the lab internal QC policy. Corrective action for out-of-control QC is outlined in the QC policy and actions taken must be documented in the LIS system. No patient results may be released until QC results are acceptable.

VIII. Procedure

A. Prepare the sample container for each sample, ensuring that a barcode label is affixed.
B. Use the appropriately coded sample racks for the type of sample tube to be used:
   1. Position 1 – aliquot tube (blue screw cap)
   2. Position 2 – primary sample tube
   3. Position 3 – Sample cup-Siemens
C. Load each sample tube into a rack, ensuring that the barcode is visible through the slot in the rack.
D. Place the rack(s) in the entry queue.
E. Press 'START' **only** if the system is not currently 'In Process'. The analyzer will read the barcode label and run the appropriate tests via the Cerner interface.
F. For the folate assay, the system automatically performs the following steps:
   1. dispenses 150 ul of sample into a cuvette.
   2. dispenses 50 ul of DTT/Releasing Agent
   3. dispenses 100 ul of Folate Binding Protein and 200 ul of Solid Phase and incubates 5 minutes at 37^0C.
   4. dispenses 100 ul of Lite Reagent and incubates for 2.5 minutes at 37^0C.
   5. separates, aspirates, and washes the cuvettes with reagent water.
   6. dispenses 300 ul each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction.
   7. reports results.

IX. Reporting Results

A. Calculations:

The computer will automatically calculate the RBC folate when the hematocrit result and the folate result from the hemolysate is entered into the system. The calculation is as follows.

$$\text{RBC folate (ng/ml)} = \frac{\text{(folate result for hemolysate, ng/ml) x 21 x 100}}{\text{Hematocrit}}$$

B. Reference Intervals

RBC Folate: 280 – 791 ng/ml

C. Reporting:

1. **Analytical measurement range (AMR):** 0.35 – 24.00 ng/ml.

   Manufacturer's stated sensitivity and assay range: 0.35 – 24 ng/ml

2. **Clinical reportable range (CRR)** for RBC folate: 100 - 2000 ng/ml

3. If RBC hemolysate folate is >24 ng/ml, calculate using 24. The final result should then be resulted as greater than (>) whatever result is obtained, up to 2000 ng/mL. If calculated result is greater than 2000, report as '> 2000 ng/ml'.

X. Procedural Notes/Problem-Solving Tips

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

B. Methotrexate and leucovorin interfere with folate measurement because these drugs cross-react with folate binding proteins.

C. Moderately lipemic (<1000 mg/dL of triglycerides), and icteric (<20 mg/dL of bilirubin) samples have no clinically significant effect on the Folate method.

XI. References

1. Bayer ADVIA Centaur Assay Manual for FOLATE; Rev B; 2001-10.
2. Bayer ADVIA Centaur Reference Manual; Rev C; 11/99; Bayer Corporation
3. Siemens Centaur, Centaur XPT Instructions for use: Document 10629859_EN. Rev. R, 2011-07.

POLICY CREATION :		
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REVISION HISTORY (began tracking 2011)			
Rev	**Description of Change**	**Author**	**Effective Date**
1	Added corrected rbc folate calculation, clarified result reporting methods, and changed QC performance frequency for Immunoassay Plus control.	K. Neikirk	5/10/12
2	Updated to CentaurXP, Biorad 1 & 2 controls being used instead of all three levels, serum folates are no longer being corrected so removed comment, removed comment about two centaurs	D Roth/ S Burton	06/12/14
3	Updated LIS code due to Sunquest LIS installation.	M. Greer	6/1/2016
4	Updated from XP to XPT	A. Gibbs	02/13/17

Reviewed by

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