**Folate, Red Blood Cells**

ADVIA Centaur System

**Principle of the Test**

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 biotin-labeled folate binding protein. Biotin-labeled folate binding protein binds to avidin that is covalently coupled to paramagnetic particles in the Solid Phase. In the ADVIA Centaur Folate assay the sample is pretreated to release the folate from endogenous binding proteins in the sample.

The system automatically performs the following steps:

- Dispenses 150 μL of sample into a cuvette
- Dispenses 50 μL of DTT/Releasing Agent
- Dispenses 100 μL of Folate Binding Protein and 200 μL of Solid Phase and incubates for 5.0 minutes at 37°C
- Dispenses 100 μL of Lite Reagent and incubates for 2.5 minutes at 37°C
- Separates, aspirates, and washes the cuvettes with reagent water
- Dispenses 300 μL each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction
- Reports results according to the selected option, as described in the system operating instructions or in the online help system

An inverse relationship exists between the amount of folate present in the patient sample and the amount of relative light units (RLUs) detected by the system.

**Clinical Application and Usefulness**

For in vitro diagnostic use in the quantitative determination of folate in red blood cells using the ADVIA Centaur System.

**Specimen Collection and Handling**

**Specimen Collection**

**BIOHAZARD**

All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

- EDTA whole blood is the only acceptable sample type for this assay.
• This assay requires 150 µL of sample for a single determination. Additional volume is required for onboard dilutions.
• Collect whole blood samples in EDTA tubes.
• Samples are free of fibrin or other particulate matter.
• Samples are free of bubbles.

Specimen Storage and Stability

CAUTION: Folates are light sensitive. Minimize exposure to light during sample handling and storage.

Whole Blood Samples

• Do not use samples that have been stored unfrozen for longer than 3 hours.
• Determine the hematocrit and freeze the whole blood specimen at or below -20°C if the sample is not assayed within 3 hours.
• Frozen whole blood specimens can be stored at -20°C in a non-frost free freezer for up to 2 months.
• Freeze specimens only once and mix thoroughly after thawing.

Hemolysates

• Freeze sample hemolysates at or below -20°C immediately if testing cannot be completed within 4 hours after hemolysate preparation.
• Sample hemolysates prepared with the reconstituted Folate Ascorbic Acid can be stored at -20°C in a non-frost free freezer for up to 3 months.
• If the hemolysate is frozen, thaw the hemolysate and mix it by inverting the tube several times. Let the hemolysate stand for 30 minutes at room temperature before testing. Test the hemolysate within 3 hours after thawing.

Prepare Red Blood Cell Hemolysate

CAUTION: Folates are light sensitive. Minimize exposure to light during sample handling and storage.

1. Allow the frozen EDTA sample to thaw at room temperature.
2. Invert the sample several times to mix.
3. Dispense 1.0 mL of reconstituted Folate Ascorbic Acid into a test tube or sample cup.
4. Add 50 µL of the sample into the Folate Ascorbic Acid.
5. Cap and invert the tube several times or vortex gently to mix. Avoid foaming.
6. Let the hemolysate stand protected from light, at room temperature, for a minimum of 90 minutes, but less than 3 hours. Freeze hemolysate if you cannot assay within 3 hours.
7. Do not mix the hemolysate again before placing the sample on the system.
**Reagents**

**Storage and Stability**

*Ready Packs® and Diluent*

- Store the reagents upright at 2–8 °C.
- Primary reagents stable until the expiration date on the pack label, or for 28 days onboard the system.
- DTT/Releasing Agent stable for 108 hours (4.5 days) after preparation; do not use beyond this date.
- Diluent stable until the expiration date on the pack label, or for 28 consecutive days after accessing the ancillary reagent pack.

**CAUTION:**

- Discard the primary reagent packs at the end of the onboard stability interval.
- Do not use reagents beyond the expiration date.

*Folate Calibrator Vials*

- Store the reagents upright at 2–8 °C.
- Reconstituted calibrators may be stored at ≤–20°C.
- Lyophilized calibrators stable until the expiration date on the vial label.
- Reconstituted calibrators stable for 7 days at 2–8 °C, 28 days at ≤–20°C, or for 8 hours onboard the system.

**CAUTION:**

- Discard the lyophilized calibrators at the end of the reconstituted onboard stability interval.
- Do not use lyophilized calibrators beyond the expiration date.

**Ingredients**

Reagent ingredients for the ADVIA Centaur Folate assay are as follows:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lite Reagent</td>
<td>10.0 mL/ReadyPack</td>
<td>folate labeled with acridinium ester (~9.8 ng/mL) in buffer with bovine serum albumin, sodium azide (0.1%), and preservatives</td>
</tr>
<tr>
<td>Solid Phase</td>
<td>20.0 mL/ReadyPack</td>
<td>purified avidin (~20 μg/mL) covalently coupled to paramagnetic particles in buffer with human serum albumin and preservatives</td>
</tr>
<tr>
<td>Folate Binding Protein</td>
<td>10.0 mL/ReadyPack</td>
<td>purified folate binding protein (~1.0 μg/mL) covalently coupled to biotin in buffer with bovine serum albumin and preservatives</td>
</tr>
</tbody>
</table>
DTT 5.0 mL/vial  dithiothreitol (~95 mg/mL) in liquid form
Releasing Agent 2.5 mL/vial  sodium hydroxide (~1.1N)
Calibrators 3.0 mL/vial  after reconstitution, low or high levels of N-5-methyl-tetrahydrofolic acid in buffer with human serum albumin with sodium azide (< 0.1%) and preservatives
Folate Ascorbic Acid/Folate Ascorbic Acid Diluent 30.0 mL/vial  lyophilized ascorbic acid (~0.30 g/vial)/bovine serum albumin in buffer with sodium azide (< 0.1%) and preservatives
Folate Diluent 10.0 mL/ReadyPack  human plasma with sodium azide (< 0.1%) and preservatives

**WARNING:** 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, if disposal into a drain is in compliance with federal, state, and local requirements.

**BIOHAZARD**
All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

**Reagents Special Preparation**

**Prepare the Folate Ascorbic Acid, as follows:**

**CAUTION:** Folates are light sensitive. Minimize exposure to light during sample handling and storage.

1. Reconstitute the Folate Ascorbic Acid by adding the contents of one vial of 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.
3. Use the prepared Folate Ascorbic Acid to prepare RBC hemolsyates for Folate analysis.

**Prepare the DTT/Releasing Agent, as follows:**

**CAUTION:** Irritant of the eyes and skin. In case of contact with the eyes or skin, wash immediately and copiously with water and then contact a physician.

1. Carefully transfer the contents of one vial of Releasing Agent into one vial of DTT. For convenience, the Releasing Agent can be poured or transferred by pipette into the DTT vial. This 12 mL volume is sufficient to perform 200 tests.

**Note:** This volume of DTT/Releasing Agent is required for each disposable ancillary reagent pack. If you need to perform more than 200 tests, you must prepare additional DTT/Releasing agent as
described in step 1 and add the contents to a second disposable ancillary reagent pack. The system will not aspirate more than 200 tests per ancillary reagent pack.

2. Firmly screw the cap on the DTT vial and invert the vial several times to mix.

3. Pour or pipette the entire contents of the DTT vial into the disposable ancillary reagent pack provided.

4. Place a pack seal on the disposable ancillary reagent pack. Ensure that the seal is centered over the opening of the pack, and press firmly on the adhesive portion of the seal.

**CAUTION:** 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. The prepared DTT/Releasing Agent must be used within 108 hours after placing in a disposable ancillary reagent pack.

**DTT/Releasing Agent Barcode Labels**

DTT/Releasing Agent barcode labels are lot number specific. Do not use barcode labels from one lot of DTT/Releasing Agent with any other lot of DTT/Releasing Agent.

**Prepare calibrators, as follows:**

1. Add 3.0 mL of reagent water into each calibrator vial using a volumetric pipet.

2. Let the calibrators stand for 15 to 20 minutes at room temperature to allow the lyophilized material to dissolve.

3. Gently swirl and invert the vials until homogeneous.

4. Aliquot 1.5 mL of the prepared calibrators into false bottom tubes. Label the tubes with the corresponding barcodes found in the package with the lyophilized calibrators. The Folate calibrators are stored at -20ºC and are stable for 28 days.

For detailed procedural information about scheduling a calibration, refer to the ADVIA Centaur Reference Manual or to the online help system.

**Two-point Calibration Interval**

Use the Folate Calibrator to perform two-point calibrations.

Perform a two-point calibration every 7 days. Additionally, calibrate when the following conditions occur:

- the lot numbers of the primary reagent packs have changed
- system components are repaired or replaced
- quality control results do not fall within established ranges

**Defining Calibrator Values for Two-point Calibration**

1. At the workspace, select Calibration.
2. Select **Calibrator Definition**.

3. Select **Scan Data**.

4. Scan the barcodes on the Calibrator Assigned Value Card.

5. Define the LIS field.

6. Review the values on the Calibrator Assigned Value card to ensure that they are correct. Scan again, if required.

7. Select **Save**.

**Master Curve**

Use the barcode reader to enter the Master Curve values from the Master Curve card onto the system. Ensure that the lot number on the Master Curve matches the lot number of the ReadyPack.

**Defining the Master Curve Using the Barcode Scanner**

1. At the workspace, select **Calibration**.

2. Select **Master Curve Definition**.

3. At the Calibration-Master Curve Definition window, select **Scan Data**.

4. Scan the barcodes on the Folate Master Curve Card.

5. Select **Save**.

**Quality Control (QC)**

**BioRad Lyphochek Whole Blood levels 1 & 3**

Whole Blood controls 1 & 3 are reconstituted with 2.0 mL of Reagent water. Let stand for 20 minutes, then gently mix to ensure complete reconstitution. Aliquot 250 µL into plastic cups and freeze at –20°C. Reconstituted Whole Blood controls are stable for 30 days at –20°C.

See posted QC chart for acceptability limits.

**BioRad Immunoassay Plus control level Level 2**

Each control is reconstituted with 5.0 mL of Reagent water. The reconstituted controls are stable for 7 days when stored at 2-8°C.

See posted QC chart for acceptability limits.

**QC Frequency**

Analyze all three levels of quality control material on each day that samples are analyzed.
Analyze all three levels of quality control material each time a two-point calibration is performed.

**Troubleshooting Out-of-Range QC Values**

A QC run is acceptable when all values fall within the expected ranges.

If the Folate QC results do not fall within the defined ranges then the run is rejected, and you must take the following corrective action:

- review these instructions to ensure that the assay was performed according to the procedures recommended by Bayer Diagnostics
- verify that the materials are not expired
- verify that required maintenance was performed
- if necessary, recalibrate the system and then rerun the quality control samples or contact Bayer Diagnostics for more assistance

**Instrument Operation and System Description**

The ADVIA Centaur system is an automated, random access, direct chemiluminescent immunoassay analyzer that offers no-pause reloading of reagents, samples, and supplies. Comprehensive assay groups are available.

When the sample start button is pressed, the barcode labels on the sample cups are read, sample is aspirated, reagent is dispensed, and the assay process begins. Paramagnetic 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 Acid and Base. The chemiluminescent reaction occurs in the luminometer. The PMT measures the chemical light reaction that takes place.

Refer to Section 4 in the ADVIA Centaur Reference Manual for detailed procedures that describe how to schedule samples and manage the worklist.

**A. System Start Button**

There is one (1) main system operation button on the ADVIA Centaur, the Start button. Pressing this button performs the following actions:

- Homes the subsystems.
- Starts specimen sampling.
- If the Start button is pressed while the system is processing samples, it stops sampling additional specimens, however it continues to process the specimens in the incubation ring.

**B. Start-up**

1. Put the samples racks on the sample entry queue.
2. Press the Start button.
C. Verify Supplies:
While the system is running, you can manage the following supplies without interrupting the run.

- Reagent water supply
- Liquid waste container
- Acid and Base reagents
- Cuvette waste bin
- Sample tip waste bin and tip tray waste area
- Cuvette supply
- Sample tip supply
- Ancillary reagent packs
- Primary reagent packs

D. Scheduling a Run/Entering a Worklist
You can enter a worklist by different methods:

**Automated Worklist**
You can send the worklist to the ADVIA Centaur from a Lab Information System (LIS). This is done in a unidirectional manner (Dynamic Download) or in a bi-directional manner (Host Query).

**Manual (Operator-initiated) Worklist**

**Scheduling Calibrators**
1. At the workspace, select Worklist and then select Schedule.
2. Select the Calibrator box.
3. Select the test you want to schedule for calibration.
4. The reagent lot that is on the system and any defined calibrators are displayed. Select the appropriate reagent and calibrator lot combination.
5. Select Save.
6. Ensure that the lot numbers of reagent and calibrator are available for system use.

**Scheduling Controls**
1. At the workspace, select Worklist and then select Schedule.
2. Select the Control box.
3. Select each test you would like to schedule.
4. Select each control level and lot number you need to run.
5. Select Save.

**Scheduling Patient Samples**

1. At the workspace, select Worklist and then select Schedule.
2. Select the Patient box.
3. Enter a patient sample identification number (SID).
4. Press the Enter key.
5. Select each test, profile, dilution, and/or replicates needed.
6. Select Save.

For detailed operating procedural information, refer to the ADVIA Centaur Reference Manual or to the online help system.

**E. Loading Sample Racks**

**BIOHAZARD**

All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

**CAUTION:** Do not load more than one size of sample container in each rack. The rack indicator must be positioned at the correct setting for the size of sample container.

**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. Affix the Folate Calibrator barcode labels to the sample cups.
2. Gently mix the calibrators and quality controls before dispensing into the sample cups.
3. Load the sample cups containing the calibrators, controls, or patient specimen into any Centaur rack.
4. Load racks onto the sample entry queue.
5. Press the Start button.

**F. Loading Ancillary Reagents**

Place the ancillary reagent pack on the ancillary entry queue until the green light comes on. The ADVIA Centaur moves the pack into the ancillary queue.
G. Loading Primary Reagents

**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 resuspended. For detailed information about preparing reagents for use, refer to Appendix C, *Handling Reagents*, in your ADVIA Centaur Assay Manual.

1. Gently mix the Folate ReadyPack before placing it on the reagent shelf for the first time.
2. Load the Folate ReadyPack by matching the color on the pack to the color-coded reagent shelf.
3. Close the reagent compartment door.

H. Loading STAT Samples in the STAT Entry Queue

1. Press the Start button, if the green Start light is not lit.
2. Place the STAT sample tube or sample cup in the rack.
3. Load the rack in the STAT entry queue until the green STAT light comes on, indicating the system has accepted the rack.

**Performance Characteristics**

**Precision**

Four serum samples were assayed 9 times in 30 runs, on 6 systems (n = 270 for each sample), over a period of 5 days. The following results were obtained:

<table>
<thead>
<tr>
<th>Mean (ng/mL)</th>
<th>Mean (nmol/L)</th>
<th>Within-run % CV</th>
<th>Run-to-run % CV</th>
<th>Total % CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.70</td>
<td>3.85</td>
<td>7.93</td>
<td>6.11</td>
<td>10.00</td>
</tr>
<tr>
<td>5.30</td>
<td>12.00</td>
<td>5.51</td>
<td>5.26</td>
<td>7.61</td>
</tr>
<tr>
<td>10.01</td>
<td>22.67</td>
<td>4.54</td>
<td>7.19</td>
<td>8.50</td>
</tr>
<tr>
<td>14.95</td>
<td>33.86</td>
<td>6.26</td>
<td>6.36</td>
<td>8.93</td>
</tr>
</tbody>
</table>

**Standardization**

The ADVIA Centaur Folate assay is traceable to an internal standard manufactured using highly purified material (N-5-methyl tetrahydrofolate). Assigned values of calibrators and ranges of Ligand Plus controls are traceable to this standardization.

**Analytical Sensitivity**

The ADVIA Centaur Folate assay has an analytical sensitivity of 0.4 µg/L (0.79 nmol/L).

Analytical sensitivity is defined as the concentration of folate that corresponds
to the RLUs that are two standard deviations less than the mean RLUs of 20 replicate determinations of the Folate zero standard.

**Analytical Measuring Range (AMR)**

The ADVIA Centaur Folate assay has an analytical measuring range (AMR) of 0.4 to 24.0 µg/L.

**Reportable Range**

The reportable range of the ADVIA Centaur Folate assay is 0.4 µg/L (0.79 nmol/L) to 24.0 µg/L (54.36 nmol/L). Values below 0.4 µg/L are reported as <0.4 µg/L. Values above 24.0 µg/L are reported as >24.0 µg/L. Results <0.4 µg/L will be calculated and reported by Sunquest as less than the calculated RBC Folate result. Example: Measured Folate (IFOL) <0.4 µg/L

- Hematocrit 45%
- RBC Folate (RFOL) will be reported as <20 µg/L

Results >24.0 µg/L will be calculated and reported by Sunquest as greater than the calculated RBC Folate result. Example: Measured Folate (IFOL) >24.0 µg/L

- Hematocrit 45%
- RBC Folate (RFOL) will be reported as >1120 µg/L

All patient samples with a Folate result (IFOL) less than 2.0 µg/L should be repeated before reporting results.

**Reference Interval**

- RBC folate: 280 to 791 µg/L (634 to 1792 nmol/L)

**Units for Reporting Results**

The system reports serum folate results in µg/L (mass units) or nmol/L (SI units). The conversion formula is 1 µg/L = 2.265 nmol/L. Units are user-defined in the system software.

**Procedure Notes**

**Calculations**

For detailed information about how the system calculates results, refer to the ADVIA Centaur Reference Manual or to the online help system.

**Red Blood Cell Folate**

Use this procedure to manually calculate RBC folate values.
1. Multiply the folate result for the hemolysate by 21 (a 1:21 dilution was made when preparing the RBC hemolysate). This value represents the folate concentration of whole blood in µg/L.

2. Divide this result by the hematocrit, and multiply by 100 to adjust for the hematocrit, which is a percentage.

\[
\text{RBC folate (µg/L)} = \frac{\text{(Folate result for hemolysate [IFOL], µg/L)}}{\text{hematocrit}} \times 21 \times 100
\]

Example:
Hemolysate folate value (IFOL) = 5.7 µg/L
Hematocrit = 43

\[
\text{RBC folate (RFOL) (µg/L)) = \frac{5.7 \times 21 \times 100}{43} = 278 \text{ µg/L RBC Hemolysate}}
\]

**Dilutions**

Dilutions are not performed

**Disposal**

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner, and in compliance with all federal, state, and local requirements.

**Method Limitations**

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

Do not pour the calibrators back into the vials after calibration because evaporation could occur, which may affect performance.

Dispose of any calibrator remaining in the sample cups after 8 hours.

Do not refill calibrator sample cups when the contents are depleted. If required, dispense fresh calibrators.

<table>
<thead>
<tr>
<th>Serum specimens that are . . .</th>
<th>Have an insignificant effect on the assay up to . . .</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipemic</td>
<td>2000 mg/dL of triglycerides</td>
</tr>
<tr>
<td>Icteric</td>
<td>20 mg/dL of bilirubin</td>
</tr>
</tbody>
</table>

Interference testing was determined according to NCCLS Document EP7-P.

For additional information on performance characteristics including dilution recovery, see the product information in the ADVIA Centaur Assay Manual.

**Equipment and Supplies**

- ADVIA Centaur Folate ReadyPack
• ADVIA Centaur Folate DTT/Releasing Agent ReadyPack
• ADVIA Centaur Folate Ascorbic Acid/Folate Ascorbic Acid Diluent
• ADVIA Centaur Folate Calibrator
• ADVIA Centaur Folate Diluent
• ADVIA Centaur Sample Cups and Caps
• ADVIA Centaur Cuvettes
• ADVIA Centaur Tips
• ADVIA Centaur Cleaning Solution Concentrate
• ADVIA Centaur Acid Reagent (0.5% H₂O₂, 0.1N HNO₃)
• ADVIA Centaur Base Reagent (0.25N NaOH and surfactant)
• Reagent Water

References

1. Bayer Diagnostics ADVIA Centaur Folate product insert, Revision B.
2. Bayer Diagnostics ADVIA Centaur Reference Manual, Revision D.

Technical Assistance
Bayer Diagnostics Technical Care Center: 1-877-229-3711
Customer Service: 1-800-255-3232
Serial Number: IRL 80600727
Customer Account Number: 619504