Folate

Customer Service: Contact your local representative or find country specific contact information on www.abbottdiagnostics.com

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Key to symbols used

- **REF** List Number
- **IVD** In Vitro Diagnostic Medical Device
- **LOT** Lot Number
- **Expiration Date**
- **Store at 2-8°C**
- **Store at -10°C or colder**
- **Store at 15-30°C**
- **Caution**
- **Manufacturer**

- **STANDARD CAL A** Standard Calibrator (A-F)
- **CONTROL L** Control Low, Medium, High (L, M, H)
- **REAGENT PACK** Reagent Pack
- **REACTION VESSELS** Reaction Vessels
- **SAMPLE CUPS** Sample Cups
- **MATRIX CELLS** Matrix Cells
- **CONTAINS: AZIDE** Contains Sodium Azide. Contact with acids liberates very toxic gas.

See REAGENTS section for a full explanation of symbols used in reagent component naming.
The AxSYM Folate Reagents and sample are pipetted in the following sequence:

**SAMPLING CENTER**
- All AxSYM Folate Reagents required for one test are pipetted by the Sampling Probe into various wells of a Reaction Vessel (RV).
- MEIA Buffer, Denaturant #1, and sample are pipetted into the Incubation Well of the RV forming the Reaction Mixture.
- The RV is immediately transferred into the Processing Center. Further pipetting is done in the Processing Center by the Processing Probe.

**PROCESSING CENTER**
- Denaturant #2 is added to the Reaction Mixture in the Incubation Well of the RV.
- Capture Reagent and MEIA Buffer are added to the Reaction Mixture. Folate binds to the FBP component of the Capture Reagent forming a polyanion FBP analyte complex.
- The matrix cell is coated with Solution 2 (Ion Capture Solution) and incubated.
- An aliquot of the reaction mixture, containing polyanion FBP analyte complexes, is transferred to the matrix cell. The positively charged glass fibers electrostatically capture the negatively charged analyte complexes.
- The matrix cell is washed to remove unbound materials.
- The Folate: Alkaline Phosphatase Conjugate is dispensed onto the matrix cell and binds to the unoccupied FBP sites.
- The matrix cell is washed to remove unbound materials.
- The substrate, 4-Methylumbelliferyl Phosphate, is added to the matrix cell and the fluorescent product is measured by the MEIA optical assembly.

For further information, refer to the AxSYM System Operations Manual, Ion Capture Technology Addendum.

**REAGENTS**

**REAGENT PACK, 100 Tests**
- AxSYM Folate Reagent Pack (Dual Pack), (7K46-20)*

**Folate Denaturant #2**
- **DENATURANT [2]**
  - 1 Bottle (13.0 mL) Folate Denaturant #2 (7K46T).
  - 0.8 N Potassium Hydroxide supplied in a separate bottle from the Dual Pack.

**NOTE:** Prior to performing the AxSYM Folate assay for the first time, transfer the entire contents of the Denaturant #2 bottle into the reagent pack position of Reagent Pack B with the colored flipper bar.

An AxSYM System error code “3065 - Liquid Not Found” will occur if the Denaturant is not transferred into the colored flipper bar position of Reagent Pack B.

**Reagent Pack A:**
- 1 Bottle (13.4 mL) Folate: Alkaline Phosphatase (bovine) Conjugate in TRIS buffer with human albumin stabilizers. Minimum Concentration: 0.1 µg/mL. Preservative: sodium azide. (Reagent Bottle 1)
- 1 Bottle (25.3 mL) Capture Reagent. Monoclonal (mouse) Anti-Folate Binding Protein Coupled to Polyanion/Folate Binding Protein in borate buffer with human albumin and chemical stabilizers. Preservative: sodium azide. (Reagent Bottle 3)

**Reagent Pack B:**
- 1 Bottle (10.9 mL) Denaturant #1. Dithiothreitol in acetate buffer. (Reagent Bottle 1)
- 1 Bottle (13.0 mL) Denaturant #2. Potassium Hydroxide. Maximum concentration: 0.8 N. (Reagent Bottle 2)
- 1 Bottle (50.2 mL) Diluent. 0.3 M Sodium Chloride in TRIS buffer. Preservatives: sodium azide and antimicrobial agents. (Reagent Bottle 4)

**NOTE:** Prior to performing the AxSYM Folate assay for the first time, transfer the entire contents of the Denaturant #2 bottle into the reagent pack position of Reagent Pack B with the colored flipper bar.

- 7K46-20 includes an AxSYM Folate Reagent Pack (Dual Pack)(100 tests) plus Reaction Vessels (100 each) and Matrix Cells (100 each).

**NAME**
- AxSYM Folate

**INTENDED USE**
- AxSYM Folate is an Ion Capture assay for the quantitative determination of folic acid in human serum, plasma or red blood cells on the AxSYM System.

**SUMMARY AND EXPLANATION OF THE TEST**
- Folic acid are a class of vitamin compounds related to pteroylglutamic acid (PGA) which serve as cofactors in the enzymatic transfer of single carbon units in a variety of metabolic pathways. Folate mediated one-carbon metabolism represents one of the most important biochemical reactions that occur in cells. Folic acid are necessary for nucleic acid and mitochondrial protein synthesis, amino acid metabolism, and other cellular processes that involve single carbon transfers. Folic acid can serve as carbon donors or acceptors. Since different metabolic pathways require carbon groups with different levels of oxidation, cells contain numerous enzymes that change the oxidation state of carbon groups carried by folates resulting in different metabolically active forms of folic acid.
- The predominant form of circulating folic acid is 5-methyltetrahydrofolic acid (5mTHF). Folic acid are linked with vitamin B12 as much as vitamin B12 is necessary to convert 5mTHF into tetrahydrofolate (THF), another metabolically active form of folic acid. This occurs during the biosynthesis of methotrexate, which is catalyzed by the enzyme methotrexate synthetase and the cofactor methylcobalamin (a form of vitamin B12). Methionine synthetase uses 5mTHF as the source of a methyl group during the production of methotrexate. This methyl transfer converts 5mTHF to THF, a form of folic acid necessary for other key cellular processes. Thus, a deficiency of vitamin B12 prevents cells from producing usable THF, which in turn prevents cells from synthesizing purines or thymines for DNA replication, and metabolizing histidine and serine. These metabolic deficiencies can lead to megaloblastic anemia, wherein blood cells cannot divide because they are unable to make DNA.
- Folic acid are derived entirely from the diet. Folate deficiency can be caused by low dietary intake, malabsorption due to gastrointestinal diseases, inadequate utilization due to enzyme deficiencies or folate antagonist therapy, drugs such as alcohol and oral contraceptives, and excessive folic acid demand, such as during pregnancy and cellular proliferation disorders. Because deficiencies of both vitamin B12 and folate can lead to megaloblastic (macrocytic) anemia, appropriate treatment requires differential diagnosis of the deficiency, thus, both vitamin B12 and folic acid values are needed. Low serum folic acid levels reflect the first stage of negative folic acid balance, and precede tissue depletion. Low red blood cell folate values reflect the second stage of negative folic acid balance, and more closely correlate with tissue levels and megaloblastic anemia.

**BIOLOGICAL PRINCIPLES OF THE PROCEDURE**
- AxSYM Folate is based on ion capture technology. In this technology, a high molecular weight quaternary ammonium compound, Ion Capture solution (Bulk solution 2), is dispensed on the glass fiber matrix of the matrix cell. This imparts a positive charge to the matrix which enables capture of negatively charged analyte complexes. During the assay, negatively charged polyanion-analyte complexes are formed. These complexes are captured through electrostatic interaction with the positively charged glass fiber matrix. The AxSYM Folate assay utilizes a soluble affinity reagent composed of folate binding protein (FBP) affinity coupled to monoclonal antibodies, which are in turn covalently coupled to carboxymethylamylose (a polyanion). Negatively charged analyte complexes are formed during the AxSYM Folate assay through the binding reaction between folate and the soluble affinity reagent. The negatively charged analyte complexes are then captured through electrostatic interaction with the positively charged glass fiber matrix. Folate is quantified by measuring the population of unoccupied FBP sites bound to the matrix using a conjugate of pteric acid (a folate analog) and alkaline phosphatase as the signal-generating molecule and a substrate, 4-methylumbelliferyl phosphate.
AxSYM Folate Specimen Diluent (7K46-50)

**SPECIMEN DILUENT**

Conversion factor used by the AxSYM System is 2.265. The AxSYM Folate default result unit is ng/mL. An alternate unit (nmol/L) may be selected for reporting results (Assay Parameter 45). The Folate Lysis Reagent contains Guanidine Hydrochloride and is classified per applicable European Community (EC) Directives as: Harmful (Xn). The following are the appropriate Risk (R) and Safety (S) phrases.

- S22 Do not breathe dust.
- S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- S35 This material and its container must be disposed of in a safe way.
- S36 Wear suitable protective clothing.
- S46 If swallowed, seek medical advice immediately and show this container or label.

The Denaturant #2 contains Potassium Hydroxide and is classified per applicable European Community (EC) Directives as: Corrosive (C). The following are the appropriate Risk (R) and Safety (S) phrases.

- R22 Harmful if swallowed.
- R36/38 Irritating to eyes and skin.
- S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- S35 This material and its container must be disposed of in a safe way.
- S36 Wear suitable protective clothing.
- S46 If swallowed, seek medical advice immediately and show this container or label.

The human albumin used in the AxSYM Folate Conjugate, Capture Reagent, Calibrators A-F, Low, Medium and High Controls, Specimen Diluent and RBC Protein Diluent has had its donor units tested and found to be nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2 and anti-HCV.

The Folate Lysis Reagent contains Guanidine Hydrochloride and is classified per applicable European Community (EC) Directives as: Harmful (Xn). The following are the appropriate Risk (R) and Safety (S) phrases.

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

AxSYM Probe Cleaning Solution (9A35-05)

AxSYM Probe Cleaning Solution containing 2% Tetraethylammonium Hydroxide (TEAH).

**WARNINGS AND PRECAUTIONS**

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

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

**SAFETY PRECAUTIONS**

- Risks: R36/38 Irritating to eyes and skin. S35 This material and its container must be disposed of in a safe way.
- S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- S36 Wear suitable protective clothing.
- S46 If swallowed, seek medical advice immediately and show this container or label.
- S22 Do not breathe dust.
All reagents are stable until expiration date when stored and handled as printed on the bottle. Do not exceed the expiration date printed on the bottle. Write the expiration date of the reconstituted Folate Lysis Reagent before reconstitution at 2-8°C for up to 7 days. After reconstitution, store the Folate Lysis Reagent at 2-8°C for up to 7 days. Write the expiration date of the AxSYM Folate Medium Control before thawing at 2-8°C for up to 7 days. After thawing, store the AxSYM Folate Medium Control at 2-8°C for up to 7 days. Mix by gentle inversion (3 to 5 times) prior to use. The AxSYM Folate Low and High Controls must be stored at 2-8°C. The Reagent Pack A and RBC Protein Diluent, and Specimen Diluent must be stored at 2-8°C (do not freeze). The AxSYM Folate Low and High Controls must be stored at 2-8°C. The Reagent Pack, RBC Protein Diluent, Specimen Diluent, and Controls may be used immediately after removal from the refrigerator.

The AxSYM Folate Standard Calibrators must be stored at -10°C or colder. Remove from box and allow calibrators to thaw at room temperature (15-30°C) until completely thawed (approximately 45 minutes). Mix by gentle inversion (3 to 5 times) prior to use. Return calibrators to -10°C or colder immediately after use. The AxSYM Folate Medium Control must be stored at -10°C or colder until use. Remove one vial at a time from the box. Allow the Medium Control to thaw at room temperature (15-30°C) until completely thawed (approximately 45 minutes). Mix by gentle inversion (3 to 5 times) prior to use. After thawing, store the AxSYM Folate Medium Control at 2-8°C for up to 7 days. Write the expiration date of the AxSYM Folate Medium Control on the space provided on the bottle. Do not exceed the expiration date printed on the bottle.

Unreconstituted Folate Lysis Reagent must be stored at 15-30°C. After reconstitution, store the Folate Lysis Reagent at 2-8°C for up to 7 days. Write the expiration date of the reconstituted Folate Lysis Reagent on the space provided on the bottle. Do not exceed the expiration date printed on the bottle.

All reagents are stable until expiration date when stored and handled as directed.

### STORAGE CONDITIONS OF AxSYM FOLATE REAGENTS

<table>
<thead>
<tr>
<th>Storage</th>
<th>Expiration Date</th>
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<tbody>
<tr>
<td>at -10°C or colder</td>
<td>As printed on bottle</td>
</tr>
<tr>
<td>Calibrators</td>
<td>As printed on bottle</td>
</tr>
<tr>
<td>Medium Control (before thawing)</td>
<td>As printed on bottle</td>
</tr>
<tr>
<td>Storage at 2-8°C</td>
<td>Expiration Date</td>
</tr>
<tr>
<td>Low and High Controls</td>
<td>Seven (7) days after thawing</td>
</tr>
<tr>
<td>Medium Control (after thawing)</td>
<td>(Write expiration date on bottle)</td>
</tr>
<tr>
<td>RBC Protein Diluent</td>
<td>As printed on bottle</td>
</tr>
<tr>
<td>Lysis Reagent (after reconstitution)</td>
<td>Seven (7) days after reconstitution</td>
</tr>
<tr>
<td>(Write expiration date on bottle)</td>
<td></td>
</tr>
<tr>
<td>Reagent Pack (Tightly Close Caps)</td>
<td>As printed on pack</td>
</tr>
<tr>
<td>Specimen Diluent</td>
<td>As printed on bottle</td>
</tr>
<tr>
<td>Storage at 15-30°C</td>
<td>Expiration date</td>
</tr>
<tr>
<td>Lysis Reagent (before reconstitution)</td>
<td>As printed on bottle</td>
</tr>
</tbody>
</table>

The AxSYM Folate Reagent Pack may be on-board the AxSYM System for a maximum of 224 cumulative hours; for example 28 eight hour shifts. Recalibration may be required to obtain maximum on-board reagent stability. More frequent use of controls may be required to monitor reagent performance within the same lot. Refer to the AxSYM System Operations Manual, Sections 2, 5, and Appendix C for further information on tracking on-board time.

Solution 1 (MUP) must be stored at 2-8°C (do not freeze). It may be used immediately after removing it from the refrigerator. MUP may be on-board the AxSYM System for a maximum of 14 days. After 14 days, it must be discarded. The AxSYM Solution 2 (Ion Capture Solution), Solution 3 (Matrix Cell Wash), Solution 4 (Line Diluent), and Probe Cleaning Solution must be stored at 15-30°C.

### INSTRUMENT PROCEDURE

#### ASSAY FILE OPTIONS

Two assay files are available for use with the AxSYM Folate reagents. The AxSYM Folate assay file may be used with serum, plasma, and whole blood specimens. Results obtained using this assay file will not specify specimen type. The FolateRBC assay file may be used for whole blood specimens. The primary use of the FolateRBC assay file is to differentiate between orders for serum folate and red blood cell folate. Results obtained using this assay file are identified as FolateRBC. For each assay file, the red blood cell folate concentration must be calculated. Refer to the RESULTS section in this package insert.

#### ASSAY FILE INSTALLATION

The AxSYM Folate Assay File must be installed on the AxSYM System from one of the following assay software disks, prior to performing AxSYM Folate or FolateRBC assay:

- 3C62-01
- 3D52-02
- 7G35-01 or higher.

Refer to the AxSYM System Operations Manual, Section 2, for proper installation procedures.

#### AxSYM FOLATE ASSAY PARAMETERS

The default values for the visible assay parameters used for the AxSYM Folate and FolateRBC assays are listed in the following table. These parameters can be displayed and edited according to the procedure in the AxSYM System Operations Manual, Section 2. Press PRINT to print the assay parameters. Assay parameters that can be edited contain a (>) symbol. In order to obtain values for the parameters with an asterisk (*), review the specific Assay Parameter screen.
### Folate Assay Parameters

<p>| | |</p>
<table>
<thead>
<tr>
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<td>1</td>
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<td>6</td>
<td>Abbrev Assay Name (English): Folate</td>
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<td>11</td>
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<td>Assay File Revision: *</td>
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<td>18</td>
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<td>Cal B Concentration: 1.5</td>
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<tr>
<td>23</td>
<td>Cal C Concentration: 3.0</td>
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<td>Blank I-Max background intensity: 0.0000</td>
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<td>Max Intercept-Max MUP intercept: *</td>
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<td>Min Intercept-Min MUP intercept: *</td>
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<td>Upper limit for NRMSE for low rates: *</td>
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<td>67</td>
<td>Upper limit for NRMSE for high rates: *</td>
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<td>68</td>
<td>Max Rate-Max rate used to check Min MUP Intercept: *</td>
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<tr>
<td>69</td>
<td>Min Rate-Rate cutoff for NRMSE and Corr Coef: *</td>
</tr>
<tr>
<td>70</td>
<td>Min correlation coefficient for low rates: *</td>
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<td>71</td>
<td>Min correlation coefficient for high rates: *</td>
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<td>72</td>
<td>MUP T Delay-Time delay following MUP: *</td>
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<td>Low Limit-Normal/Therapeutic Range lower limit &gt;*</td>
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<tr>
<td>74</td>
<td>High Limit-Normal/Therapeutic Range upper limit &gt;*</td>
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<td>Low Extreme Value &gt;*</td>
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<td>76</td>
<td>High Extreme Value &gt;*</td>
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<td>92</td>
<td>High Range Undiluted: 20.0</td>
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</tbody>
</table>

**NOTE:** Parameter 45 can be edited to the alternate result unit, nmol/L.

### FolateRBC Assay Parameters

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<td>Calibration Version: *</td>
</tr>
<tr>
<td>14</td>
<td>Assay File Revision: *</td>
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</table>

**NOTE:** All remaining FolateRBC parameters are identical to Folate assay parameters.

An AxSYM System error code “3065 - Liquid Not Found” will occur if the Denaturant #2 is not transferred into the colored flipper bar position of Reagent Pack B. Refer to the AxSYM System Operations Manual for a detailed description of the Instrument Procedures. For details on Automatic Sample Retest Configuration, refer to the AxSYM System Operations Manual, Section 2.

### SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

#### SPECIMEN COLLECTION

- Human serum (including serum from serum separator tubes [SST]), plasma (collected in tripotassium EDTA, potassium oxalate, or sodium citrate tubes), or whole blood (collected in tripotassium EDTA) may be used in the AxSYM Folate assay. Sodium heparin has been found to interfere with the AxSYM Folate assay; other anticoagulants have not been tested. Follow the manufacturer’s processing instructions for serum or plasma collection tubes.
- Human serum, plasma, or whole blood specimens should be tested for folate should be protected from light. 0°-11°
- Serum and plasma specimens should be collected from fasting individuals. Recent food intake may appreciably increase the serum folate concentration. 11°
- When shipped, specimens must be packaged and labeled in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.
- **Serum Specimens**
  - Ensure that complete clot formation has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.
  - Remove serum from the clot within 24 hours of draw.
  - If testing will be delayed more than 24 hours, serum specimens may be stored at 2-8°C for up to 1 week or frozen (-20°C or colder) for up to 1 month prior to being tested.
- **Plasma Specimens**
  - Remove plasma collected in sodium citrate or potassium oxalate tubes from red blood cells within 24 hours of draw. If testing will be delayed more than 24 hours, plasma specimens collected in sodium citrate or potassium oxalate tubes may be stored at 2-8°C or frozen (-20°C or colder) for up to 1 week prior to being tested.
  - Remove plasma collected in tripotassium EDTA tubes from red blood cells within 8 hours of draw. If testing will be delayed more than 8 hours, plasma specimens collected in tripotassium EDTA tubes may be stored at 2-8°C or frozen (-20°C or colder) for up to 24 hours prior to testing.
- **Whole Blood Specimens**
  - Whole blood specimens collected in tripotassium EDTA tubes may be stored at 2-8°C for up to 5 days or frozen (-10°C or colder) for up to 30 days.
  - Determine hematocrit before storage.

#### PREPARATION FOR ANALYSIS

- Inspect all samples for bubbles. Remove bubbles prior to analysis.
- Specimens with obvious microbial contamination should not be used.
- Hemolyzed specimens should not be used. Hemolyzed serum or plasma specimens may give falsely elevated folate levels.
- Multiple freeze-thaw cycles should be avoided. Specimens must be mixed thoroughly after thawing, by LOW speed vortexing or by gently inverting, and centrifuged prior to use to remove particulate matter and to ensure consistency in the results.
- Patient specimens should be mixed and centrifuged after any freeze-thaw cycle or to remove fibrin, red blood cells, or particulate matter. Serum or plasma specimens containing red blood cells may give falsely elevated folate levels.
- The AxSYM System does not provide the capability to verify sample type. It is the responsibility of the operator to verify the correct sample type(s) is (are) used in the AxSYM Folate assay.
- All samples (patient specimens, Controls, and Calibrators) should be tested within 3 hours of being placed on-board the AxSYM System. Refer to the AxSYM System Operations Manual, Section 5, for a more detailed discussion of on-board storage constraints.
- For red blood cell folate determinations, determine the hematocrit and PREPARE A RED BLOOD CELL HEMOLYSATE for each specimen to be assayed as described in PREPARATION OF RED BLOOD CELL HEMOLYSATE in this section.
AxSYM FOLATE PROCEDURE

MATERIALS REQUIRED

- 7K46-20 AxSYM Folate Reagent Kit, containing:
  - AxSYM Folate REAGENT PACK
  - 100 REACTION VESSELS
  - 100 MATRIX CELLS

MATERIALS REQUIRED BUT NOT PROVIDED

- AxSYM System
- Ion Capture Technology Addendum
- 7K46-10 AxSYM Folate Low and High Controls
- 7K46-11 AxSYM Folate Medium Control
- 7K46-01 AxSYM Folate Standard Calibrators
- 7K46-50 AxSYM Folate Specimen Diluent
- 7K46-60 Folate Lysis Reagent
- 7K46-65 AxSYM Folate RBC Protein Diluent
- 8A47-04 SOLUTION 1 (MUP)
- 6C98-04 SOLUTION 2 (ION CAPTURE)
- 8A81-04 SOLUTION 3 MATRIX CELL WASH
- 8A46 SOLUTION 4 LINE DILUENT
- 9A35-05 AxSYM PROBE CLEANING SOLUTION
- 8A76-01 SAMPLE CUPS
- 8A73-02 MATRIX CUPS
- 8A75-02 REACTION VESSELS
- Pipettes or pipette tips (optional) to deliver the volumes specified on the Orderlist screen
- Clean glass tubes for PREPARATION OF RED BLOOD CELL HEMOLYSATE

CAUTION:

- When manually dispensing sample into sample cups, verify that dispensing equipment does not introduce cross contamination and delivers the specified sample volume. Use a separate pipette tip for each sample. Use accurately calibrated equipment.
- For optimal performance, it is important to follow the routine maintenance procedures defined in the AxSYM System Operations Manual, Section 9, and the Ion Capture Technology Addendum (Solution 2 line maintenance). If your laboratory requires more frequent maintenance, follow those procedures.

ASSAY PROCEDURE

Sections 5 and 6 of the AxSYM System Operations Manual contain detailed steps for performing assay calibration and sample testing procedures. Prior to ordering tests, confirm that the System inventory of matrix cells, bulk solutions, and waste levels are acceptable. Confirm Solution 2 (Ion Capture Solution) is placed on platform 2 of the AxSYM System. Refer to the Ion Capture Technology Addendum for instructions on loading Solution 2.

The Orderlist Report contains sample placement information and sample cup volume requirements for all ordered tests. It is recommended that this report be referenced when loading samples into sample segments. When using Host Order Query, the Orderlist Report is not available. Refer to the AxSYM System Operations Manual, Section 5, for a description of the Host Order Query option.

CAUTION: When operating the AxSYM System, always observe the following:

- Open Reagent Bottle 4 of Reagent Pack B containing Diluent before placing Dual Pack on-board the AxSYM System.
- The system status must be WARMING, PAUSED, READY, or STOPPED before adding or removing sample segments, reagent packs, or reaction vessels (RVs).
- Do not open the Interior Waste Door or the AxSYM Processing Center Cover while any test is in progress. If opened, all processing will stop. Tests in process will be terminated and must be repeated.
- When testing is completed, it is recommended that samples and the AxSYM Folate Reagent Pack are removed from the Sampling Center to maximize the on-board reagent pack use. Store at 2-8°C.
**SPECIMEN DILUTION PROCEDURES**

Patient specimens with a folate assay value exceeding 20 ng/mL (HIGH RANGE, assay parameter 92), are flagged with the code ">20 ng/mL". To quantitate the concentration of these specimens, perform the Manual Dilution Protocol. Do not dilute below the sensitivity (0.9 ng/mL) of the assay.

**Automated Dilution Protocol**

Specimens cannot be diluted for folate concentration determinations using the Automated Dilution Protocol.

**Manual Dilution Protocol**

Patient specimens with analyte concentrations reported as greater than 20 ng/mL may be diluted using a suggested manual dilution of 1:2 (e.g. 100 µL of patient specimen to 100 µL of AxSYM Folate Specimen Diluent). The dilution should be performed so that the diluted test results read greater than the sensitivity (0.9 ng/mL) of the assay. Dilutions greater than 1:4 are not recommended. The concentration reported by the AxSYM System must be multiplied by the manual dilution factor to obtain the final sample concentration.

\[
\text{Final Specimen Concentration} = \frac{\text{Printed Concentration} \times \text{Manual Dilution Factor}}{(\text{Volume of Specimen} + \text{Volume of Specimen Diluent}) / \text{Volume of Specimen}}
\]

**QUALITY CONTROL PROCEDURES**

**CALIBRATION**

The AxSYM Folate assay must be calibrated using a Standard Calibration (6 point) procedure.

**Standard Calibration**

To perform an AxSYM Folate Standard Calibration, test Standard Calibrators A, B, C, D, E, and F in duplicate. A single sample of all levels of Folate Controls must be tested as a means of evaluating the assay calibration.

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

- A Dual Reagent Pack with a new lot number is used.
- Controls are out of range.
- Refer to the AxSYM System Operations Manual, Section 6, for:
  - Setting up an assay calibration
  - When recalibration may be necessary
  - Calibration verification

The AxSYM System verifies that the results of an assay calibration meet the specifications assigned to selected validity parameters. An error message occurs when the calibration fails to meet a specification. Refer to the AxSYM System Operations Manual, Section 10, and the Ion Capture Technology Addendum for an explanation of the corrective actions for the error code. Refer to the AxSYM System Operations Manual, Appendix E, for an explanation of the calibration validity parameters that may be used by the AxSYM System.

**QUALITY CONTROL**

The recommended control requirement for an AxSYM Folate assay is a single sample of all Folate Control levels, tested once every 24 hours each day of use.

**NOTE:** Subtle changes in assay performance can be best detected by the AxSYM Folate Medium Control. If the AxSYM Folate Medium Control value is outside of its specified range, the assay is invalid and must be repeated.

If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow those procedures.

To achieve maximum on-board reagent stability, more frequent use of controls may be required to monitor reagent performance within the same lot.

Ensure that the AxSYM Folate assay control values are within the concentration ranges specified in the REAGENTS, CONTROLS section of this package insert.

**INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS**

When a Folate Control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid and require retesting. Assay recalibration may be necessary. Refer to the AxSYM System Operations Manual, Section 10, and the Ion Capture Technology Addendum for further troubleshooting information.

The AxSYM System has the capability to generate a Levey-Jennings plot of each assay’s quality control performance. Refer to the AxSYM System Operations Manual, Section 5. At the discretion of the laboratory, selected quality control rules may be applied to the quality control data.

**FLUORESCENCE BACKGROUND ACCEPTANCE CRITERIA**

Quality control with regard to the MUP substrate blank is automatically determined by the instrument and checked under Assay Parameter 64 Max Intercept-Max MUP intercept, each time a test result is calculated. If the MUP intercept value is greater than the maximum allowable value, the test result is invalid. The test request will be moved to the Exceptions List where it will appear with the message “1064 Invalid test result, intercept too high” and the calculated intercept value. Refer to the AxSYM System Operations Manual, Section 10, and the Ion Capture Technology Addendum when this error message is obtained.

Refer to AxSYM System Operations Manual, Section 2, for further information on this parameter.

**RESULTS**

**CALCULATION**

The AxSYM Folate assay utilizes a 4-parameter logistic data reduction method (4PLC) to generate a standard calibration curve. Refer to the AxSYM System Operations Manual, Appendix F, for further information.

**ALTERNATE RESULT UNIT**

The default result unit for AxSYM Folate is ng/mL. When selecting the alternate results unit, nmol/L, the conversion factor used by the AxSYM System is 2.265.

\[
\text{AxSYM Folate Printed Result (ng/mL)} \times 22 \times 100 = \text{RBC Folate Concentration (ng/mL)}
\]

\[
\% \text{Hematocrit} = \frac{5.0 \times 22 \times 100}{47} = 234.0 \text{ (ng/mL)}
\]

**CALCULATION OF THE RBC FOLATE CONCENTRATION**

To calculate the RBC folate concentration from the value printed on the AxSYM test results printout, use the following formula:

\[
\text{AxSYM Folate Printed Result (ng/mL)} \times 22 \times 100 = \text{RBC Folate Concentration (ng/mL)}
\]

\[
\% \text{Hematocrit} = \frac{5.0 \times 22 \times 100}{47} = 234.0 \text{ (ng/mL)}
\]

**CALCULATION OF THE CORRECTED RBC FOLATE CONCENTRATION**

Folate values from serum or plasma are very small as compared to the RBC folate value, in most cases. It is possible for the serum folate value to be above the deficient range while the RBC folate value is deficient. The following calculation will correct for serum folate concentrations:

\[
\text{RBC Folate Concentration (ng/mL)} = \frac{\text{Serum Folate Concentration (ng/mL)} - \left(100 - \text{Hematocrit} \times \text{Hematocrit}ight)}{47}
\]

**Example:**

- RBC Folate Concentration = 234.0 ng/mL
- Serum Folate Concentration = 9.5 ng/mL
- % hematocrit = 47

\[
\frac{234.0 - 9.5}{47} = 223.3 \text{ (ng/mL)}
\]

**Formulas and examples indicate ng/mL as the result unit. If the chosen AxSYM Folate result unit is nmol/L, the final result would be in nmol/L.**

**Flags**

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the AxSYM System Operations Manual, Sections 1 and 2.
Folate deficiency is typically associated with serum levels less than 3 ng/mL or RBC values less than 150 ng/mL. Patients with RBC folate values ranging from 150 to 250 ng/mL have been associated with megaloblastic erythropoiesis, but folate values in patients with normal erythropoiesis can also fall within this range. Often, the diagnosis of folate deficiency cannot be based solely on serum or RBC folate levels, and further testing may be required. Serum and plasma samples containing red blood cells can give falsely elevated serum or plasma folate levels. These samples must be centrifuged prior to use in the AxSYM Folate assay. Serum or plasma samples that are hemolyzed will give falsely elevated serum or plasma folate levels.

Some serum and plasma samples in the upper region of the dynamic range may read lower in the AxSYM Folate assay when compared to some other commercial assays. This can result in a decreased correlation coefficient value.

Methotrexate, aminopterin, and folic acid (Leucovorin) are chemotherapeutic agents whose molecular structures are similar to folate. These agents cross react with folate binding protein in folate assays. Some serum and plasma specimens with various concentrations of triglycerides, protein, or bilirubin. Testing was performed by supplementing multiple human serum specimens with various concentrations of triglycerides, protein, or bilirubin. The AxSYM Folate assay demonstrated the interference as stated in the following table:

### SPECIFIC PERFORMANCE CHARACTERISTICS

**PRECISION**
The AxSYM Folate assay is designed to have precision of ≤ 19 % total CV for concentrations within the low control range and ≤ 10 % total CV for concentrations in the range of the medium and high controls. Precision was determined as described in the Clinical and Laboratory Standards Institute (CLSI) document EP5-A2. Each of the Controls (Low, Medium, and High) was assayed using three lots of reagents, in replicates of two at two separate times per day for 20 days on two instruments. Data from this study are summarized below.

### LIMITATIONS OF THE PROCEDURE

- For diagnostic purposes, the AxSYM Folate result should be used in conjunction with other data; e.g. other clinical testing, symptoms, clinical impressions, etc.
- If the folate level is inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- Specimens from subjects who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). HAMA, present in serum or plasma specimens, may interfere with immunoassays which utilize mouse monoclonal antibodies.12,13 These specimens should not be assayed with the AxSYM Folate assay.

### SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

**EXPECTED VALUES**
It is recommended that each laboratory establish its own reference range, which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.

**FOLATE NORMALS**
A study was performed based on guidance from Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) document C28-A2. The nutritional status of the specimen donors was unknown. However, all specimens tested were from apparently healthy US adults, a population typically supplemented with dietary folate.

Serum samples from 124 fasting individuals were assayed for serum folate using the AxSYM Folate assay. The median concentration for the population tested was 13.0 ng/mL with a range of 5.6 to 19.8 ng/mL. Using a non-parametric analysis, the central 95% of the population was 7.2 to 15.4 ng/mL.

Whole blood samples from 145 individuals were assayed for red blood cell (RBC) folate using the AxSYM Folate assay. The median concentration for the population tested was 473.4 ng/mL with a range of 218.4 to 1019.8 ng/mL. Using a non-parametric analysis, the central 95% of the population was 252.6 to 813.7 ng/mL.

<table>
<thead>
<tr>
<th>Folate Level</th>
<th>Concentration (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Normal Range</td>
<td>7.2 to 15.4</td>
</tr>
<tr>
<td>RBC Normal Range</td>
<td>252.6 to 813.7</td>
</tr>
</tbody>
</table>

### Cross-Reactivity

**AxSYM Folate Cross-Reactivity**

<table>
<thead>
<tr>
<th>Cross Reactant</th>
<th>Concentration (ng/mL)</th>
<th>Cross-Reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminopterin</td>
<td>500</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Folic Acid</td>
<td>100</td>
<td>&lt; 3</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>100</td>
<td>&lt; 2</td>
</tr>
</tbody>
</table>

**INTERFERENCE**
Testing was performed by supplementing multiple human serum specimens with various concentrations of triglycerides, protein, or bilirubin. The AxSYM Folate assay demonstrated the interference as stated in the following table:

---

The following is based on literature sources:

- **FOLATE DEFICIENTS / INDETERMINATES**

  Folate deficiency is typically associated with serum levels less than 3 ng/mL or RBC values less than 150 ng/mL. Patients with RBC folate values ranging from 150 to 250 ng/mL have been associated with megaloblastic erythropoiesis, but folate values in patients with normal erythropoiesis can also fall within this range. Often, the diagnosis of folate deficiency cannot be based solely on serum or RBC folate levels, and further testing may be required.
**AxSYM Folate Interference**

<table>
<thead>
<tr>
<th>Interfering Substance</th>
<th>Maximum Concentration of Interfering Substance</th>
<th>Interference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>20 mg/dL</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Protein</td>
<td>12 g/dL</td>
<td>&lt; 11</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>2000 mg/dL</td>
<td>&lt; 10</td>
</tr>
</tbody>
</table>

**RECOVERY**

Accuracy was evaluated by measuring the recovery of folate from three human serum samples which were supplemented with approximately 4 and 8 ng/mL concentrations of pteroylglutamic acid (PGA) or 5-methyltetrahydrofolic acid (5mTHF). The calculated recoveries ranged from 86.7 to 106.2% (mean = 96.5%) for PGA and 86.9 to 109.3% (mean = 98.6%) for 5mTHF.

**AxSYM Folate Recovery**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Analyte</th>
<th>Endogenous Folate* (ng/mL)</th>
<th>Supplemented Folate* (ng/mL)</th>
<th>Value Obtained (ng/mL)</th>
<th>Recovery** (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PGA</td>
<td>7.8</td>
<td>3.5</td>
<td>12.0</td>
<td>106.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.8</td>
<td>7.08</td>
<td>15.5</td>
<td>104.2</td>
</tr>
<tr>
<td></td>
<td>10.3</td>
<td>10.3</td>
<td>7.08</td>
<td>15.6</td>
<td>89.8</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>10.0</td>
<td>7.08</td>
<td>14.8</td>
<td>86.7</td>
</tr>
<tr>
<td>2</td>
<td>PGA</td>
<td>7.8</td>
<td>4.0</td>
<td>12.9</td>
<td>95.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.8</td>
<td>8.0</td>
<td>15.6</td>
<td>98.7</td>
</tr>
<tr>
<td></td>
<td>10.3</td>
<td>10.3</td>
<td>4.0</td>
<td>14.0</td>
<td>97.9</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>10.0</td>
<td>8.0</td>
<td>15.9</td>
<td>86.9</td>
</tr>
<tr>
<td>3</td>
<td>PGA</td>
<td>7.8</td>
<td>4.0</td>
<td>14.0</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.8</td>
<td>8.0</td>
<td>15.8</td>
<td>87.8</td>
</tr>
</tbody>
</table>

* Concentrations were determined using the folate concentrates as samples in the AxSYM Folate assay.

**% Recovery = 
(\frac{\text{Value Obtained (ng/mL)}}{\text{Endogenous + Supplemented Folate Level}} \times 100)**

**DILUTION LINEARITY**

Dilution linearity of the AxSYM Folate assay has been verified to Dilution linearity of the AxSYM Folate assay has been verified to

**Folate Dilution Linearity**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution Factor</th>
<th>AxSYM Expected (mean ng/mL)</th>
<th>AxSYM Observed (mean ng/mL)</th>
<th>AxSYM Recovery* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cal. F**</td>
<td>Undiluted</td>
<td>20.0</td>
<td>19.30</td>
<td>96.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10.0</td>
<td>10.15</td>
<td>101.5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5.0</td>
<td>4.50</td>
<td>90.0</td>
</tr>
<tr>
<td>1</td>
<td>Undiluted</td>
<td>-</td>
<td>12.3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.15</td>
<td>7.75</td>
<td>126.0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3.08</td>
<td>4.45</td>
<td>144.5</td>
</tr>
<tr>
<td>2</td>
<td>Undiluted</td>
<td>-</td>
<td>12.7</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.35</td>
<td>8.5</td>
<td>133.9</td>
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<tr>
<td></td>
<td>4</td>
<td>3.18</td>
<td>4.9</td>
<td>154.1</td>
</tr>
<tr>
<td>3</td>
<td>Undiluted</td>
<td>-</td>
<td>9.7</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.85</td>
<td>5.45</td>
<td>112.4</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2.43</td>
<td>2.9</td>
<td>119.3</td>
</tr>
</tbody>
</table>

* % Recovery = \left(\frac{\text{AxSYM Observed (ng/mL)}}{\text{AxSYM Expected (mean ng/mL)}} \right) \times 100

**** Average Recovery of Folate Calibrator F = 96.0%.

**ACCURACY BY CORRELATION**

The AxSYM Folate assay was compared to the IMX Folate assay. The results of serum and red blood cell folate testing follow.

**AxSYM Folate Correlation**

<table>
<thead>
<tr>
<th>Method</th>
<th>Number of Observations</th>
<th>Slope</th>
<th>Correlation Coefficient</th>
<th>Y-Intercept (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMx serum samples</td>
<td>878</td>
<td>0.97</td>
<td>0.95</td>
<td>0.6</td>
</tr>
<tr>
<td>red blood cell samples</td>
<td>513</td>
<td>0.99</td>
<td>0.97</td>
<td>13.8</td>
</tr>
</tbody>
</table>

In this study, serum specimens ranged from 0.9 to 18.8 ng/mL for the AxSYM Folate assay and from 1.4 to 18.9 ng/mL for the IMx Folate assay. Whole blood specimens ranged from 132.6 to 2158.8 ng/mL for the AxSYM Folate assay and from 116.1 to 2170.0 ng/mL for the IMx Folate assay.

**BIBLIOGRAPHY**


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July 2010
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