2.0 SUMMARY AND EXPLANATION OF THE TEST

Folate supplementation has escalated over recent years with the knowledge of its many benefits. As one of the B vitamins, folate, or Vitamin B9, is involved in many bodily functions and can cause disease in not only the elderly but infants too. Folate deficiency is associated with megaloblastic anemia, neural tube defects, and cardiovascular diseases.1,2,3

3.0 PRINCIPLE

Folate binding proteins are responsible for folate metabolism. Two types exist in circulation; one targets in binding to the cell surfaces and the other soluble form exists in circulation. These folate binding proteins also have the capability of binding several different folate derivatives including folic acid and methylenetetrahydrofolate. The interaction between folic acid and folate binding protein is greater than methylenetetrahydrofolate. Current assays on the market require an extraction step to release the folate derivative binding protein.1,2

In the past, folate has been quantified in samples using such methods as microbiological assays, bio-specific procedures and HPLC-MS techniques. Overall, this rapid rise in knowledge of folate, its importance, and subsequently folate supplementation has caused a higher demand for improved testing methods.3

3.0 PRINCIPLE

Competitive Binding Protein Assay (TYPE B):

The essential reagents required for a competitive binding assay include specific binding protein, enzyme-antigen conjugate and native antigen. Upon mixing enzyme-antigen conjugate, biotinylated binding protein and a serum containing the native antigen, a competition reaction results between the native antigen and the biotinylated binding protein for the limited number of binding sites. The interaction is illustrated by the following equation:

\[
\frac{k_a \cdot [AgBPBtn \cdot + \text{Ag} \cdot + \text{BPBtn} \cdot]}{k_b \cdot [AgBPBtn \cdot + \text{Ag}\cdot + \text{BPBtn} \cdot]}
\]

Note 1: Do not use reagents beyond the expiration date.

Note 2: Avoid extended exposure to heat and light. Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the product insert.

4.0 REAGENTS

Materials Provided:

- Folate Calibrators – 1ml/ov - High A
- 1 ml (100 µl) of 0.050 M standard folate solution in 0.9% saline reference for Folate at concentrations of 0, (A), 1.0, 2.5, 5.0, 10.0, 20.0, and 50.0 (µg/ml) reference standard for the folate assay.

Note: The calibrators, human serum based, were calibrated using a highly purified N-methyltetrahydrofolate preparation.

- Folate Tracer Reagent – 7.0 ml/vial - immobilized complex

One vial containing a highly purified folate binding protein conjugate in buffer, dye and preservative. Store at 2-8°C.

- Folate Biotin Reagent – 7.0 ml/vial - immobilized complex

One vial containing biotinylated purified folate binding protein conjugate in buffer, dye and preservative. Store at 2-8°C.

- Light Reaction Wells 96 wells - immobilized complex

One 96-well light reaction plate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

- Wash Solution Concentrate – 20.0 ml/vial - immobilized complex

One vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

- Signal Reagent A – 7.0 ml/vial - immobilized complex

One vial containing luminol in buffer. Store at 2-8°C.

- Signal Reagent B – 7.0 ml/vial - immobilized complex

One vial containing hydrogen peroxide (H2O2) in buffer. Store at 2-8°C.

- Releasing Agent – 14.0 ml/vial - immobilized complex

One vial containing 0.1 M potassium hydroxide (sodium hydroxide) and potassium cyanide. Store at 2-8°C.

- Neutralizing Buffer – 7.0 ml/vial - immobilized complex

One vial containing buffer that reduces the pH of the sample extract. Store at 2-8°C.

- Product Insert

Note 1: Do not use reagents beyond the expiration date.

4.1 Required But Not Provided:

1. Pipette capable of delivering 0.050 ml (50 µl) and 0.100 ml (100 µl) with a precision of better than 1.5%.

2. Dispenser(s) for repetitive deliveries of 0.100 ml (100 µl) and 0.350 ml (350 µl) volumes with a precision of better than 1.5%.

3. Adjustable volume (200-1000µl) dispenser(s) for conjugate.


5. Microplate washer or a squeeze bottle (optional).


7. Absorbent Paper for blunting the microplate wells.

8. Plastic wrap or micropore tape for incubation steps.

9. Vacuum aspirator (optional) for wash steps.

10. Microplate Luminometer.

11. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use

Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, “Biosafety in Microbiological and Biomedical Laboratories,” 2nd Edition, 1988, pHIS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirements.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum or heparinized plasma in a sterile vacutainer tube or for whole blood use either heparin or EDTA anticoagulant. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Samples may be refrigerated at 4°C for a maximum period of three (3) days. When assaying sample solution should be obtained. The blood should be collected in a red top (with or without gel additives) venipuncture tube(s) or for plasma use vacuum tubes containing heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance should indicate unnoticeable change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. Wash Buffer

Dilute contents of wash solution to 1000 ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at room temperature (20-20°C) for up to 60 days.

2. Working Signal Reagent Solution - Store at 2 - 8°C. Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 ml of A and 1ml of B per two (2) well eight strips (A slight excess of solution is made). Discard the unused portion if not used within 36 hours after mixing. If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

3. EXTRACTION AGENT

Add 0.050 ml (50 µl) of Signal Reagent A to the mixed reagents (see note 3) after each addition. Let the reaction proceed for 15 min. At the end of the 15 min, dispense 0.050 ml (50 µl) of the neutralizing buffer, vortex (see note 3). After the neutralization has occurred, add 0.050 ml (50 µl) of the washed buffer solution is added. Mix with the mixed reagents, vortex again, and let the mixture sit for another 5 min before proceeding to the microplate.

12.0 SPECIMEN EXTRACTIION

Obtain enough test tubes for preparation of all patient samples, controls, and calibrators. Dispense 0.100 ml (100 µl) of all samples into individual test tubes. Pipette 0.050 ml (50 µl) of the prepared extraction reagent to each test tube sample. Mix the contents of the tube(s) or plasma use either heparin or EDTA anticoagulant. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Note 1: Do not use reagents that are contaminated or have bacteria growth.

Note 2: Use of multiple (3) touch vortex is recommended. Note 3: It is extremely important to accurately dispense the correct volume with a calibrated pipette and touch near the bottom of the glass tubes at an angle while touching the side of the tubes.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, calibrators and controls to room temperature (20 - 27°C). "**Test Procedure should be performed by a skilled individual or trained professional**"
10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of Folate in unknown specimens.

1. Record the relative light units (RLU) obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the RLU's for each duplicate calibrator versus the corresponding Folate concentration in pg/ml on linear graph paper (do not average the duplicates of the calibrator before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of Folate for an unknown, locate the average RLU of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in pg/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average RLU (68907) intersects the dose response curve at 2.012 ng/ml Folate concentration (See Figure 1).

Note: Computer data reduction software designed for chemiluminescence assay may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number</th>
<th>Well</th>
<th>RLU (A)</th>
<th>Mean RLU</th>
<th>Conc. (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td></td>
<td></td>
<td>101286</td>
<td>100000</td>
<td>0.0</td>
</tr>
<tr>
<td>B1</td>
<td></td>
<td></td>
<td>98714</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td></td>
<td></td>
<td>82657</td>
<td>83777</td>
<td>1.0</td>
</tr>
<tr>
<td>D1</td>
<td></td>
<td></td>
<td>84097</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1</td>
<td></td>
<td></td>
<td>63819</td>
<td>63255</td>
<td>2.5</td>
</tr>
<tr>
<td>F1</td>
<td></td>
<td></td>
<td>62691</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td></td>
<td></td>
<td>48641</td>
<td>46476</td>
<td>5.0</td>
</tr>
<tr>
<td>H1</td>
<td></td>
<td></td>
<td>48111</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td></td>
<td></td>
<td>22775</td>
<td>22003</td>
<td>10.0</td>
</tr>
<tr>
<td>B2</td>
<td></td>
<td></td>
<td>23031</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td></td>
<td></td>
<td>7243</td>
<td>6991</td>
<td>25.0</td>
</tr>
<tr>
<td>D2</td>
<td></td>
<td></td>
<td>6739</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td></td>
<td></td>
<td>68250</td>
<td>68907</td>
<td>2.012</td>
</tr>
<tr>
<td>F2</td>
<td></td>
<td></td>
<td>65865</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The above data and table below is for example only. Do not use it for calculating your results.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The Dose Response Curve should be within established parameters.
2. Four out of six quality control pools should be within the established ranges.
3. The reagents for the test system procedure have been pipetted of samples should not extend beyond ten (10) microliters.
4. Highly lipemic, hemolized or grossly contaminated specimens should be determined as such.
5. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
6. The addition of signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.
7. Plate readers measure vertically. Do not touch the bottom of the wells.
8. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
9. Accurate and precise pipetting, as well as following the exact procedure, is essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC- for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) microliters.
3. Highly lipemic, hemolyzed or grossly contaminated specimens should be determined as such.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact procedure, is essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC- for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. Measurements and interpretation of results must be performed by a skilled individual or trained professional.
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM Stuart. Clin. Chem. 1988:34:27-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient's history and, all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a "normal" population the expected ranges for the Folate AccuLite® CLIA Test System are detailed in Table 1.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Interference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>ND*</td>
</tr>
<tr>
<td>Biotin</td>
<td>ND*</td>
</tr>
<tr>
<td>Lipemia</td>
<td>ND*</td>
</tr>
</tbody>
</table>

*ND=Not Detectable

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within assay precision of the Folate AccuLite® CLIA Test System, were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Within Assay Precision (Values in ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>24  3.947  0.504  7.7</td>
</tr>
<tr>
<td>Level 2</td>
<td>24  10.213  0.504  4.9</td>
</tr>
<tr>
<td>Level 3</td>
<td>24  14.932  0.613  4.1</td>
</tr>
</tbody>
</table>

14.2 Sensitivity

The Folate AccuLite® CLIA Test System has a sensitivity of 0.234 ng/ml. The sensitivity was ascertained by determining the variation of the 0 ng/ml serum calibrator and using the 2x (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The Folate AccuLite® CLIA Test System was compared with a reference chemiluminescence immunoassay method. Biological specimens were used with values that ranged from 3.2 ng/ml – 13.7 ng/ml. The total number of such specimens was 30. The least square regression equation and the correlation coefficient were computed for this Folate CLIA in comparison with the reference method. The data obtained is displayed in Table 3.

14.4 Specificity

The specificity of the Folate Binding Protein used to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Interference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>ND*</td>
</tr>
<tr>
<td>Biotin</td>
<td>ND*</td>
</tr>
<tr>
<td>Lipemia</td>
<td>ND*</td>
</tr>
</tbody>
</table>

*ND=Not Detectable

15.0 REFERENCES

7. Snow, CF, M.D. Archives of Internal Medicine. 1999, 159, 1289-1298

Rev. 1 Date: 2014-JUL-21 DCO: 1003 Product Code: 7575-300