Monobind Inc.
Lake Forest, CA 92630, USA

VAST™
Versatile Analyte Selection Technology
2 Analytes 1 Kit
CLIA Microwells

# Folate / Vitamin B12 *(Folate/Vit B12)*
## Anemia Panel VAST® Test System
### *Product Code: 7875-300*

## 1.0 INTRODUCTION

**Intended Use: The Quantitative Determination of Vitamin B12 and Folate Concentration in Human Serum and Plasma by a Microplate Enzyme Immunoassay, Chemiluminescence.**

## 2.0 SUMMARY AND EXPLANATION OF THE TEST

Folate plays an important role in brain development and is therefore vital during growth. The most common defects resulting from folate deficiencies are neural tube defects. With a vital role in nucleic acid synthesis, folate supplementation has been found to be beneficial during pregnancy and other times of rapid tissue growth. Folate also plays a vital role in maintaining proper balance of homocysteine, a contributing factor in occurrences of occlusive vascular diseases and stroke. Individuals with susceptibility to heart disease and several forms of cancer may also benefit from supplementation.[1,2,3]

Major sources of folate include green leafy vegetables, legumes, beans and fortified cereals. Foods fortified with folate are actually fortified with folic acid because of the higher bioavailability for absorption by the body. In circulation, folate is present in several different forms, some of which are more stable than others. Folic acid and N-methyltetrahydofolate are two common forms, the latter being more stable and found in higher concentrations in serum. Due to the stability of the molecule, methytetrahydrofolate is very often used as the form focused on during methods of analysis.[3,4]

Folate binding proteins are responsible for folate metabolism. Two types exist in circulation: one type aids in binding to the cell surface 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 N-methytetrahydrofolate. The interaction between folic acid and folate binding protein is greater than methyltetrahydrofolate. Current assays on the market require an extraction step to release the folate derivatives from the folate binding protein.[4,5]

Vitamin B12 is one of the nine water soluble vitamins important for healthy body functioning. The most important roles Vitamin B12 plays in the human body are in the formation of red blood cells and the formation of the myelin sheath around the nerves. Since the effects are seen in body systems with a large range of function, the symptoms of Vitamin B12 deficiency can sometimes be very ambiguous. A deficiency may also take from months to years to manifest depending on the cause and severity.[6,7,8]

Two of the most common causes of Vitamin B12 deficiency are diet and age. Because most sources of dietary Vitamin B12 come from animals, vegans who do not efficiently supplement their diet are at risk. The elderly community is also at high risk because of their diet, as well as the less efficient functioning of their digestive system.[6,8,9]

Two very useful tests to distinguish between Vitamin B12 deficiency and folate deficiency are methylmalonyl CoA (MMA) and homocysteine (hcy). Both deficiencies are represented by similar symptoms; however, even though both show increased levels of homocysteine, only Vitamin B12 deficiency causes an increase in methylmalonyl CoA. The increase in levels of methylmalonyl CoA and homocysteine is thought to be the root cause of any symptoms that accompany a Vitamin B12 deficiency. High levels of these two analytes in the blood stream causes increased oxidative stress to cells therefore causing increased apoptosis. In turn, vascular disease results in the form of atherosclerosis, coronary heart disease and/or neurodegeneration (ex. Parkinson's Disease).[6,10,11]

## 3.0 PRINCIPLE

**Folate - Competitive Binding Protein Assay (TYPE 8):**
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 enzyme-antigen conjugate for a limited number of binding sites. The interaction is illustrated by the followed equation:

$$^{Enz}Ag + Ag + BP_{Btn} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgBP_{Btn} + {}^{Enz}AgBP_{Btn}$$

$BP_{Btn}$ = Biotinylated Binding Protein (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
$^{Enz}Ag$ = Enzyme-antigen Conjugate (Constant Quantity)
$BP_{Btn}$ = Antigen-Binding Protein Complex
$^{Enz}AgBP_{Btn}$= Enzyme-Antigen-Binding Protein Complex
$k_a$ = Rate Constant of Association
$k_{-a}$ = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

A simultaneous reaction between the biotin attached to the binding protein and the streptavidin immobilized on the microwell occurs. This effects the separation of the binding protein enzyme bound fraction after decantation or aspiration.
$AgAb_{Btn} + {}^{Enz}AgBP_{Btn} + \underline{Streptavidin}_{CW} \Rightarrow \underline{immobilized\ complex}$
$\underline{Streptavidin}_{CW}$ = Streptavidin immobilized on well
$\underline{Immobilized\ complex}$ = sandwich complex bound to the solid surface

The enzyme activity in the protein binding protein bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

**Vitamin B12 - Delayed Competitive Enzyme Immunoassay (TYPE 9):**
The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing the biotinylated antibody with a serum containing the antigen, a reaction results between the antigen and the antibody. The interaction is illustrated by the following equation:

$$Ag + Ab_{Btn} \rightleftharpoons AgAb_{Btn}$$

$Ab_{Btn}$ = Biotinylated antibody
Ag = Antigen (Variable Quantity) $AgAb_{Btn}$ = Immune Complex

After a short incubation, the enzyme conjugate is added (this delayed addition permits an increase in sensitivity for low concentration samples). Upon the addition of the enzyme conjugate, competition reaction results between the enzyme analog and the antigen in the sample for a limited number of antibody binging sites (not consumed in the first incubation).

$$^{Enz}Ag + Ag + rAb_{Btn} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{Btn} + {}^{Enz}AgAb_{Btn}$$

$^{Enz}Ag$ = Enzyme-antigen Conjugate (Constant Quantity)
$^{Enz}Ag\ Ab_{Btn}$ = Enzyme-antigen Conjugate -Antibody Complex
$rAb_{Btn}$ = Biotinylated antibody not reacted in first incubation
$k_a$ = Rate Constant of Association
$k_{-a}$ = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.
$AgAb_{Btn} + {}^{Enz}AgAb_{Btn} + \underline{Streptavidin}_{CW} \Rightarrow \underline{immobilized\ complex}$
$\underline{Streptavidin}_{CW}$ = Streptavidin immobilized on well
$\underline{Immobilized\ complex}$ = sandwich complex bound to the solid surface

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

## 4.0 MATERIALS

**Reagents for 96 well Microplate, provided**

**A. Combi-Cal® Folate/Vit B12 Calibrators – 1 ml/vial – Icons A-F**
Six (6) vials containing references for markers at levels indicated below. A preservative has been added. The calibrators, human serum based, were calibrated using a reference preparations indicated in the chart.

| Analyte | Folate (ng/ml) | Vitamin B12 (pg/ml) |
|---|---|---|
| A | 0.0 | 0 |
| B | 1.0 | 125 |
| C | 2.5 | 250 |
| D | 5.0 | 500 |
| E | 10.0 | 1000 |
| F | 25.0 | 2500 |

**B. Folate Tracer Reagent – 7.0 ml/vial – Icon Ⓔ**
One (1) vial containing Folate (Analog)-horseradish peroxides (HRP) conjugate in a protein-stabilizing matrix with dye. Store at 2-8°C.

**C. Folate Biotin Reagent – 7.0 ml/vial – Icon ∇**
One (1) vial containing biotinylated purified folate binding protein conjugate in buffer, dye and preservative. Store at 2-8°C.

**D. Vitamin B12 Tracer Reagent – 7.0 ml/vial – Icon Ⓔ**
One (1) vial containing Vitamin B12 (Analog)-horseradish peroxides (HRP) conjugate in a protein-stabilizing matrix. Store at 2-8°C.

**E. Vitamin B12 Biotin Reagent – 7.0 ml/vial – Icon ∇**
One (1) vial containing anti-Vitamin B12 biotinylated purified rabbit IgG conjugate in buffer, dye and preservative. Store at 2-8°C.

**F. Wash Solution Concentrate – 20.0 ml/vial – Icon 💧**
One (1) vial containing surfactant in buffered saline. A preservative has been added. Store at 2-8°C for up to 60 days.

**G. Light Reaction Wells – 96 wells – Icon ⇓**
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

**H. Signal Reagent A – 7.0 ml/vial – Icon $C^A$**
One (1) vial containing luminol in buffer. (See 'Reagent Preparation' section). Store at 2-8°C.

**I. Signal Reagent B – 7.0 ml/vial – Icon $C^B$**
One (1) vial containing hydrogen peroxide ($H_2O_2$) in buffer. (See 'Reagent Preparation' section). Store at 2-8°C.

**J. Releasing Agent – 14.0 ml/vial – Icon Ξ**
One (1) vial containing a strong base (sodium hydroxide) and potassium cyanide. Store 2-8°C.

**K. Stabilizing Agent – 0.7 ml/ vial – Icon Π**
One (1) vial containing tris (2-carboxyethyl) phosphine (TCEP) solution. Store at 2-8°C.

**L. Neutralizing Buffer – 7.0 ml/ vial – Icon NZ**
One (1) vial containing buffer with dye that reduces the pH of sample extraction. Store at 2-8°C.

**M. Product Insert**

**Note 1:** Do not use reagents beyond the kit 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 label.**
**Note 3:** Above reagents are for a single 96-well microplate

**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 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Adjustable volume (200-1000µl) dispenser(s) for conjugate.
4. Glass test tubes for serum reference, control, and patient sample preparation.
5. Microplate washer or a squeeze bottle (optional).
6. Microplate Luminometer
7. Absorbent Paper for blotting the microplate wells.
8. Plastic wrap or microplate covers for incubation steps.
9. Vacuum aspirator (optional) for wash steps.
10. Timer.

## 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, HHS Publication No. (CDC) 88-8395.

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

## 6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum or plasma in type, and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

**In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.**

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) can not be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml (100µl) of the specimen is required for each anemia marker assayed.

## 7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicated unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for variations.

## 8.0 REAGENT PREPARATION

**1. Wash Buffer**
Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at room temperature (2-30°C) for up to 60 days.

2. **EXTRACTION AGENT**
   Add an aliquot of the stabilizing agent in order to prepare a 1/40 (stabilizing agent / releasing agent) dilute solution. For example, to make 4ml (4000µl), add 0.100ml (100µl) stabilizing agent to 3.9ml (3900µl) releasing agent.
3. **SAMPLE EXTRACTION (*See Note 3)***
   Obtain enough test tubes for preparation of all patient samples, controls, and calibrators. Dispense 0.10ml (100µl) of all samples into individual test tubes. Pipette 0.050ml (50µl) of the prepared extraction agent to each test tube, shaking *(see note 3)* after each addition. Let the reaction proceed for 15 min. At end of the 15 min, dispense 0.050 ml (50µl) of the neutralizing buffer, vortex *(see note 3)*.
   ***For Folate:*** after the neutralization buffer is added and mixed, let the reaction go to completion by waiting an additional 5 min before dispensing into the microwells. Wait time is not needed for Vitamin B12.
4. **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) eight well 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.

***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 by adding near the bottom of the glass tubes at an angle while touching the side of the tubes.***
***Note 4: Samples with high protein concentration should be diluted 1:1 with a saline solution before performing the extraction.***
***Note 5: See www.monobind.com/education-center for Step-by-Step Guide on Sample Extraction for Vit B12 (& Folate) in Lab Tips***

## 9.0 TEST PROCEDURE

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

**For Folate:**
1. Prepare all samples according to the "Sample Extraction" procedure in section "8.0 Reagent Preparation"; **it is important to wait 5 min before proceeding to allow the neutralization reaction to go to completion (see above).**
2. Format the microplates' wells for each calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
3. Pipette 0.050 ml (50 µL) of the appropriate extracted Folate calibrator, control or specimen into the assigned well.
4. Add 0.050 ml (50 µl) of Folate Tracer Reagent to all wells
5. Mix the microplate gently for 20-30 seconds.
6. Add 0.050 ml (50 µl) of the Folate Biotin Reagent to all wells.
7. Mix the microplate gently for 20-30 seconds.
8. Cover and incubate for 45 minutes at room temperature.
9. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
10. Add 0.350 ml (350 µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat four (4) additional times.**
11. Add 0.100 ml (100 µl) of signal reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**
    **DO NOT SHAKE THE PLATE AFTER SIGNAL ADDITION**
12. Incubate at room temperature for five (5) minutes.
13. Read the relative light units (RLUs) in each well for 0.2 – 1.0 seconds. **The results should be read within thirty (30) minutes of adding the signal reagent solution.**

**Note 1**: Dilute the samples suspected of concentrations higher than 25ng/ml 1:5 with Folate '0' ng/ml calibrator and re-assay.
**Note 2:** It is very important to dispense all reagents in the center of the coated well. Always add reagents in the same order to minimize reaction time differences between wells.

**For Vitamin B12:**
1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.050 ml (50 µL) of the appropriate extracted Vitamin B12 calibrator, control or specimen into the assigned well.
3. Add 0.050 ml (50 µl) of the Vitamin B12 Biotin Reagent to all wells.
4. Mix the microplate gently for 20-30 seconds to mix.
5. Cover and incubate for 45 minutes at room temperature.
6. Add 0.050 ml (50 µl) of Vitamin B12 Tracer Reagent to all wells. **Add directly on top the reagents dispensed in the wells**.
7. Mix the microplate gently for 20-30 seconds to mix.
8. Cover and incubate for 30 minutes at room temperature.
9. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
10. Add 0.350 ml (350 µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat four (4) additional times.**
11. Add 0.100 ml (100 µl) of signal reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**
    **DO NOT SHAKE THE PLATE AFTER SIGNAL ADDITION**
12. Incubate at room temperature for five (5) minutes.
13. Read the relative light units (RLUs) in each well for 0.2 – 1.0 seconds. **The results should be read within thirty (30) minutes of adding the signal reagent solution.**

**Note 3**: Dilute the samples suspected of concentrations higher than 2000pg/ml 1:5 and 1:10 with Vitamin B12 '0' pg/ml calibrator and re-assay.

## 10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of each corresponding marker in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Examples 1 and 2.
2. Plot the absorbance for each duplicate serum reference versus the corresponding marker concentration in appropriate units on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of corresponding cancer marker for an unknown, locate the average absorbance 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 ng/ml) from the horizontal axis of the graph.

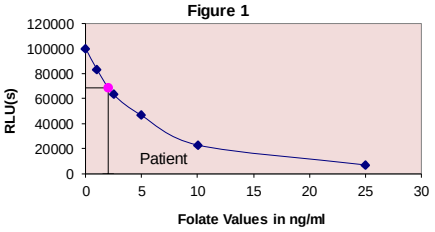
**Note:** Computer data reduction software designed for CLIA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

* The data, figures and tables below are for example only. Do not use them for calculating your results.

**EXAMPLE 1 - FOLATE**

| Sample I.D. | Well Number | RLU (A) | Mean RLU (B) | Value (ng/ml) |
|---|---|---|---|---|
| **Cal A** | A1 | 101286 | 100000 | 0.0 |
| | B1 | 98714 | | |
| **Cal B** | C1 | 82657 | 83377 | 1.0 |
| | D1 | 84097 | | |
| **Cal C** | E1 | 63819 | 63255 | 2.5 |
| | F1 | 62691 | | |
| **Cal D** | G1 | 46841 | 46476 | 5.0 |
| | H1 | 46111 | | |
| **Cal E** | A2 | 22775 | 22903 | 10.0 |
| | B2 | 23031 | | |
| **Cal F** | C2 | 7243 | 6991 | 25.0 |
| | D2 | 6739 | | |
| **Sample** | E2 | 68250 | 68907 | 2.012 |
| | F2 | 69565 | | |

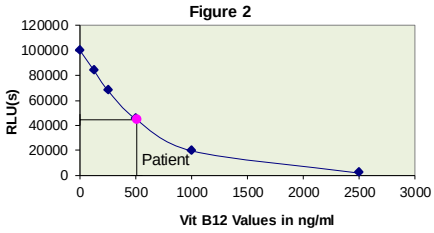
**Figure 1**




**EXAMPLE 2 – VITAMIN B12**

| Sample I.D. | Well Number | RLU (A) | Mean RLU (B) | Value (pg/ml) |
|---|---|---|---|---|
| **Cal A** | A1 | 100687 | 100000 | 0 |
| | B1 | 99313 | | |
| **Cal B** | C1 | 83533 | 84032 | 125 |
| | D1 | 84531 | | |
| **Cal C** | E1 | 67325 | 68008 | 250 |
| | F1 | 68692 | | |
| **Cal D** | G1 | 45061 | 45073 | 500 |
| | H1 | 45086 | | |
| **Cal E** | A2 | 20558 | 19645 | 1000 |
| | B2 | 18732 | | |
| **Cal F** | C2 | 1809 | 1851 | 2500 |
| | D2 | 1894 | | |
| **Sample** | E2 | 43135 | 44390 | 509.8 |
| | F2 | 45646 | | |

**Figure 2**




## 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 (DRC) should be within established parameters.
2. Four out of six quality control pools should be within the established ranges.

## 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) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of signal solution initiates a kinetic reaction; therefore, the substrate and stop solution 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 time and temperature requirements prescribed are 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 interactions 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 MC. 'Heterophilic antibodies: a problem for all immunoassays.' *Clin. Chem.* 1988: 3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient 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 VALUES

In agreement with established reference intervals for a "normal" population the expected ranges for the Folate/Vit B12 VAST AccuLite® CLIA Test System are detailed in Table 1 and 2.

**TABLE 1**
**Expected Values - Folate[6]**

| |
|---|
| Normal Adult Population > 3.0 ng/ml |

**TABLE 2**
**Expected Values - Vitamin B12[12]**

| Population | pg/ml | pmol/L |
|---|---|---|
| Newborn | 160 - 1300 | 118-959 |
| Adult | 200 – 835 | 148 – 616 |
| Adult > 60 y | 110 – 800 | 81 – 590 |

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

## 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 3.

**TABLE 3**
Folate Within Assay Precision (Values in ng/ml)

| Sample | N | X | σ | C.V.% |
|---|---|---|---|---|
| Level 1 | 24 | 3.947 | 0.304 | 7.7 |
| Level 2 | 24 | 10.213 | 0.504 | 4.9 |
| Level 3 | 24 | 14.932 | 0.613 | 4.1 |

The within assay precision of the Vitamin B12 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 4.

**TABLE 4**
Vitamin B12 Within Assay Precision (Values in pg/ml)

| Sample | N | X | σ | C.V.% |
|---|---|---|---|---|
| Low | 24 | 424.9 | 36.4 | 8.6 |
| Normal | 24 | 592.4 | 30.2 | 5.1 |
| High | 24 | 724.4 | 35.2 | 4.9 |

### 14.2 Sensitivity

The Folate/Vit B12 VAST® AccuLite® CLIA Test System has sensitivity for different analytes as listed in the following Table 5. The sensitivity was ascertained by determining the variability of the 0ng/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

**TABLE 5**

| Analyte | Sensitivity |
|---|---|
| Folate | 0.23 ng/ml |
| Vitamin B12 | 42.12 pg/ml |

### 14.3 Accuracy

The Folate AccuLite® CLIA Test System was compared with a reference method. Biological specimens were used with values that ranged from 3.2ng/ml – 13.7ng/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 6.

**TABLE 6 - FOLATE**

| Method | Mean (x) | Least Square Regression Analysis | Correlation Coefficient |
|---|---|---|---|
| This Method (Y) | 7.76 | y= 0.162 + 1.07x | 0.984 |
| Reference (X) | 8.46 | | |

The Vitamin B12 AccuLite® CLIA Test System was compared with a reference method. Biological specimens from low, normal and relatively high Vitamin B12 level populations were used (values ranged from 156 pg/ml – 1830 pg/ml). The total number of such specimens was 56. The least square regression equation and the correlation coefficient were computed for this Vitamin B12 test in comparison with the reference method. The data obtained is displayed in Table 7.

**TABLE 7 - VITAMIN B12**

| Method | Mean (x) | Least Square Regression Analysis | Correlation Coefficient |
|---|---|---|---|
| This Method (Y) | 654.3 | y= 1.0186x - 48.82 | 0.951 |
| Reference (X) | 690.2 | | |

Only slight amounts of bias between the Folate/Vit B12 VAST® AccuLite® CLIA Test System and the reference methods are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

### 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 8 – FOLATE**

| Substance | Interference |
|---|---|
| Bilirubin | ND* |
| Biotin | ND* |
| Lipemia | ND* |

**ND=Not Detectable*

The % cross reactivity of the Vitamin B12 antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of Vitamin B12 needed to displace the same amount of labeled analog.

**TABLE 9 – VITAMIN B12**

| Substance | Cross Reactivity |
|---|---|
| Bilirubin | 0.0003 |
| Rheumatoid Factor | 0.0008 |
| Cobinamide | <0.0001 |
| Lipemia | <0.0001 |
| Hemoglobin | <0.0001 |

## 15.0 REFERENCES


1. Scholl, TO. *The American Journal of Clinical Nutrition*. 1996, 63, 520-525.
2. Clarke, R. *Age and Ageing*. 2004, 33, 34-41.
3. Arcot, J. *Trends in Food Science and Technology*. 2005, 16, 253-266.
4. Mantovani, LT. *European Journal of Cancer*. 1994, 30, 363-369.
5. Nygren-Babol, L. *International Dairy Journal*. 2009, 19, 437-442.
6. Snow, C.F., M.D. *Archives of Internal Medicine*. 1999, 159, 1289-1298.
7. Gruber, K. *Chemical Society Reviews*. DOI: 10.1039 / cs15118e.
8. Nijst, T.Q. *Journal of Neurology, Neurosurgery, and Psychiatry*. 1990, 53, 951-954.
9. Steele, T.J. *Clinician Reviews*. 2010, 20(8), 16-19.
10. de Lau, L.M.L., M.D., Ph.D. *Neurology*. 2006, 67, 315-318.
11. Ubbink, J.B. *Clinical Chemistry*. 1995, 41(7), 1033-1037.
12. Tietz. Reference Information for the Clinical Laboratory. In *Textbook of Clinical Chemistry*, 3rd Ed. Burtis, C.A., Ashwood, R.A. W.B. Saunders: Philadelphia, 1999; 1831.



**Revision: 1 Date: 2019-Jul-16 DCO: 1353**
**MP7875 Product Code: *7875-300***


| Size | | 96(A) | 192(B) |
|---|---|---|---|
| Reagent (fill) | A) | 1ml set | 1ml set |
| | B) | 1 (7ml) | 1 (7ml) |
| | C) | 1 (7ml) | 1 (7ml) |
| | D) | 1 (7ml) | 2 (7ml) |
| | E) | 1 (7ml) | 2 (7ml) |
| | F) | 1 (20ml) | 1 (20ml) |
| | G) | 1 plate | 2 plates |
| | H) | 1 (7ml) | 2 (7ml) |
| | I) | 1 (7ml) | 2 (7ml) |
| | J) | 1 (14ml) | 2 (14ml) |
| | K) | 1 (0.7ml) | 2 (0.7ml) |
| | L) | 1 (7ml) | 2 (7ml) |


**For Orders and Inquires, please contact**

**Monobind Inc.**
100 North Pointe Drive
Lake Forest, CA 92630 USA

Tel: +1 949.951.2665 Mail: info@monobind.com
Fax: +1 949.951.3539 Fax: www.monobind.com

IVD 2°-8° CE
EC REP **CEpartner4U,** Esdoornlaan 13
3951 DBMaarn, The Neatherlands
**www.cepartner4u.eu**


**Please visit our website to learn more about our products and services.**

### Glossary of Symbols
(EN 980/ISO 15223)