Vitamin B12 (Vit B12) Test System
Product Code: 7675-300

1.0 INTRODUCTION

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

2.0 SUMMARY AND EXPLANATION OF THE TEST

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 caused by a 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.

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 sufficiently supplement their diet are at risk. The elderly are also at high risk because of their diet, as well as the less efficient functioning of their digestive system.

Intake of Vitamin B12 starts by ingestion and then digestion by the body. Once Vitamin B12 has been absorbed into the blood, it binds to proteins called intrinsic factors, which are present in the food. These proteins then bind the vitamin B12 to the intestines where it can be absorbed into your body through its association with IF. 1,2,3

Two very useful tests to distinguish between Vitamin B12 deficiency and folate deficiency are methylmalonyl CoA (MMA) and homocysteine. While both are represented by similar symptoms; however, even though both show increased 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 cause increased oxidative stress to cells therefore causing increased apoptosis, which can lead to various disease manifestations in the form of atherosclerosis, coronary heart disease and/or neurodegeneration (ex. Parkinson’s disease). 1,2,3

3.0 PRINCIPLE

Delayed Competitive Enzyme Immunoassay (TYPE E): The essential reagents required for an enzyme immunoassay include antibody containing 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:

\[
Ag + Ab = AgAb
\]

Ag = Antigen (Variable Quantity)  Ab = Antigen (Constant Quantity)

After a short incubation, the enzyme conjugate is added (this delayed addition permits an incubation in sample vials at 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 binding sites (not consumed in the first incubation).

\[
Ag + AgAb = AgAgAb + AgAb
\]

Ag = Enzyme-antigen Conjugate (Constant Quantity)  Ab = Biotinylated antibody (Variable Quantity)  AgAb = Immuno-complex

The enzyme in the antibody binding fraction after decarboxylation or aspiration.

\[
AgAb + Strepavidin = immobilized complex
\]

Strepavidinimmobilized on well

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 close dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. Vitamin B12 Calibrators – 1ml/vial – Incon

B. Vitamin B12 Tracer Reagent – 7ml/vial – Icon

C. Vitamin B12 Biotin Reagent – 7ml/vial – Icon

D. Light Reaction Wells – 96 wells – Icon

E. Wash Solution Concentrate – 20ml/vial - Icon

F. Signal Reagent A – 7.0 ml/vial – Icon CA

G. Signal Reagent B – 0.7 ml/vial – Icon CA

H. Neutralizing Buffer – 7.0 ml/vial – Icon

I. Stabilizing Agent – 0.7 ml/vial – Icon

J. Neutralizing Buffer – 7.0 ml/vial – Icon

K. Timer – 1.0 ml/vial – Icon

L. Quality control materials

5.0 PRECAUTIONS

For In Vivo Diagnostic Use

Not for 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) 88-8385.

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 in type, and taken with the usual precautions. The collection of venous blood samples. For accurate comparison to establish normal values, a fasting morning serum sample should be obtained. The blood shall be collected in a red top with or without gel additives) venipuncture tube(s) without anticoagulants. Allow the blood to clot undisturbed for 1-2 hours. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the serum should be stored at -70°C. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.10ml (100µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. The manufacturer recommends that laboratories use one unknown and five levels of determined in every test procedure performed. Quality control charts should be maintained and the goals of the manufacturer for consistent performance. Pertinent statistical details of the five (5) controls should be employed to ascertain trends. The individual laboratory should set its own assay performance acceptance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variation.

8.0 REAGENT PREPARATION

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, calibrators and controls to room temperature (20 - 27°C).

**“You should be proficient by an skilled individual or trained professional”**

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 patient (see note 1) into individual test wells.

3. Add 0.050 ml (50 µl) of the Biotin Reagent to all wells.

4. Swirl the microplate gently for 20-30 seconds to mix.

5. Incubate at room temperature.

6. Add 0.050 ml (50 µl) of Vitamin B12 Tracer Reagent to all wells.

7. Add directly on top the reagents dispensed in the wells.

8. Swirl the microplate gently for 20-30 seconds to mix.

9. Cover the microplate with cover for 30 minutes at room temperature.

10. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper and replace cover.

11. Add 0.350 ml (350 µl) of wash solution (see Reagent Preparation Section, decad), (see note 5) to each test tube, shake (see note 3) after each addition. Let the reagents 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).

12. Add 0.100 ml (100 µl) of working signal reagent solution to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time differences.

DO NOT SHAKE THE PLATE AFTER SIGNAL ADDITION

Incubate at room temperature for five (5) minutes.

Read the signal within 10 seconds (2-10 µl) 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: Dilute the samples suspected of concentrations higher than 2000 pg/ml by 1:10 and 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 Vitamin B12 in unknown specimens.
1. Record the RLUs obtained from the printout of the microplate reader as shown in Example 1.
2. Plot the RLUs for each duplicate calibrator versus the following criteria should be met:
   - The above data and table below is for example only. Do not use it for calculating your results.

11.0 QC PARAMETERS
In order for the assay results to be considered valid, the following criteria should be met:

1. The absorbance (OD) of calibrator 0 pg/ml should be ≥ 1.3.
2. Plot the RLUs of each duplicate calibrator should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The signal of a reagent solution initiates a kinetic reaction; therefore, the solution should be added in the same sequence to all test tubes as indicated.
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 IV Directive 98/79/EC for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

EXAMPLE 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Well</th>
<th>RLU (A)</th>
<th>RLU (B)</th>
<th>Mean</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cal A</td>
<td>A1</td>
<td>102903</td>
<td>100000</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B1</td>
<td>97097</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>84051</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D1</td>
<td>83328</td>
<td></td>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Cal C</td>
<td>E1</td>
<td>75125</td>
<td>74866</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>74607</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal D</td>
<td>G1</td>
<td>52757</td>
<td>64706</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1</td>
<td>56566</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal E</td>
<td>A2</td>
<td>17699</td>
<td>17304</td>
<td>1000</td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td>16910</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal F</td>
<td>C2</td>
<td>4602</td>
<td>4091</td>
<td>2000</td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td>3580</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P8×1</td>
<td>G2</td>
<td>41954</td>
<td>42160</td>
<td>601.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H2</td>
<td>42365</td>
<td></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.

Vitamin B12

12.0 RISK ANALYSIS

12.1 Assay Performance

1.1 It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipette graph paper (do not average the duplicates of the calibrator and re-assay.
3. Recorded the RLUs obtained from the printout of the Vitamin B12 in unknown specimens.
4. If test kits are altered, such as by mixing parts of different concentrations.
5. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
6. Use components from the same lot. No intermixing of reagents from different batches.
7. 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.
8. 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.
9. 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.
10. Risk Analysis - as required by CE Mark IV 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 diagnostic information.
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 MC. Heterophilic antibodies: a problem for all immunoassays’ Clin.Chem.1986:327-33).
4. For valid test results, accurate controls and other parameters must be within the listed ranges and assay requirements.
5. 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 calibrator 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 Vitamin B12 AccuLite® CLIA Test System are detailed in Table 1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>Least Square Regression Analysis</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>20</td>
<td>y = 1263x + 72.462</td>
<td>0.9516</td>
</tr>
<tr>
<td>Normal</td>
<td>517.2</td>
<td>y = 1263x + 72.462</td>
<td>0.9516</td>
</tr>
<tr>
<td>High</td>
<td>927.5</td>
<td>y = 1263x + 72.462</td>
<td>0.9516</td>
</tr>
</tbody>
</table>

* Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean value of “normal” persons, as 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 in an in-house range can be determined by the analyst using his own laboratory with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between 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 2 and Table 3.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean Value (pg/ml)</th>
<th>Within Assay Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>20</td>
<td>105.2</td>
</tr>
<tr>
<td>Normal</td>
<td>517.2</td>
<td>105.2</td>
</tr>
<tr>
<td>High</td>
<td>927.5</td>
<td>105.2</td>
</tr>
</tbody>
</table>

* As measured in ten experiments in duplicate over a ten day period.

14.2 Sensitivity

The Vitamin B12 AccuLite® CLIA Test System has a sensitivity of 37.92 pg/ml. The sensitivity was ascertained by determining the variability of the 0 pg/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

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 273.9 pg/ml – 1865.0 pg/ml). The total number of such specimens was 57. The least square regression equation and the correlation coefficient were computed for this test system comparison with the reference method. The data obtained is displayed in Table 4.

15.0 REFERENCES

7. Liu, Y.K. Blood 1072, 3(9), 426-432.

Revision: 4 Date: 2014-NOV-07 Product Code: 7675-300

For Orders and Inquiries, please contact
Monobind Inc.
190 North Pointe Drive
Lake Forest, IL 92690 USA
Tel: +1-949.861.2665 Email: info@monobind.com Fax: +1-949.861.0389 Web: www.monobind.com
Please visit our website to learn more about our other interesting products and services.