Upon mixing the biotinylated antibody with a serum containing the Delayed Competitive Enzyme Immunoassay (TYPE 9):

Intended Use: The Quantitative Determination of Vitamin B12 levels of homocysteine, only Vitamin B12 deficiency causes an apoptosis. In turn, vascular disease results in the form of cause of any symptoms that accompany a Vitamin B12 deficiency.

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 are varied and can sometimes be very ambiguous. A deficiency may also take from months to years to manifest depending on the cause and severity.1,2,3

The major causes of Vitamin B12 deficiency are diet and age related. Most sources of dietary Vitamin B12 comes 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 systems.1,3,4

Intake of Vitamin B12 starts by ingestion and then digestion by saliva. Once reaching the gut, Vitamin B12 bound to proteins in food are released by the acids present. The B12 can then bind to intrinsic factor. Once bound to IF, Vitamin B12 is stable enough to travel into the intestines where it can become absorbed into your body through its association with other intrinsic factors.1,3

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 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.2

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 arteriosclerosis, atherosclerotic heart disease and/or neurodegeneration (ex. Parkinson’s Disease).1,3,5

Vitamin 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 are varied and can sometimes be very ambiguous. A deficiency may also take from months to years to manifest depending on the cause and severity.1,2,3

Two of the most common causes of Vitamin B12 deficiency are diet and age related. Most sources of dietary Vitamin B12 comes 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 systems.1,3,4

The major causes of Vitamin B12 deficiency are diet and age related. Most sources of dietary Vitamin B12 comes 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 systems.1,3,4

3.0 PRINCIPLE

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:

\[ \text{AgAb} + \text{Ag} + \text{AgAb} \rightarrow \text{AgAbAg} \]

4.0 REAGENTS

Materials Provided:

A. Vitamin B12 Calibrators – 4ml vial – Icon A
B. Vitamin B12 Sample 1 – 3ml vial – Icon B
C. Vitamin B12 Sample 2 – 3ml vial – Icon C
D. Vitamin B12 Sample 3 – 3ml vial – Icon D
E. Vitamin B12 Sample 4 – 3ml vial – Icon E
F. Signal Reagent A – 7.0 ml/vial – Icon CA
G. Signal Reagent B – 7.0 ml/vial – Icon CB
H. Signal Reagent C – 7.0 ml/vial – Icon CC
I. Neutralizing Buffer – 2.0 ml/vial – Icon NZ
J. Buffer Solution – 1.0 ml/vial – Icon BU
K. Wash Solution – 1.0 ml/vial – Icon WS
L. Control Solution – 1.0 ml/vial – Icon CO
M. standards are reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV
N. All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum, in type, and taken with the usual precautions in the collection of venipuncture samples. For appropriate cardiovascular disease, such as heart disease, a fasting morning serum sample should be obtained. The blood should be collected in a well (with or without gel additives) venipuncture tube(s) without preservatives. The patient sample serum is drawn from the serum of the patients.

In patients receiving therapy with high doses of vitamin B12, no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure faster sampling.

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

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high 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 acceptable ranges for individual laboratory acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from the mean for one or more controls necessitates 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

Dilute contents of wash solution to 1000ml with distilled or deionized water. Allow the blood to clot for serum samples. Dilute to 1:1 with a saline solution before performing the extraction.

Note 1: Do not use reagents that are contaminated or have been allowed to grow.

Note 2: Use of multiple (3) touch vitamin is recommended.

Note 3: It is extremely important to accurately dispense the correct volume of a calibration buffer strip into the sample well by adding near the bottom of the glass tube at an angle while touching the side of the tube.

Note 4: protein concentration should be diluted 1:1 with a saline solution before performing the extraction.

Note 5: See www.mbnolinc.com/educationcenter for Step-By-Step Guide on Sample Extraction for Vitamin B12 (A) Folates in Lab Top

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, controls and controls to room temperature (20-25°C).

TEST PROCEDURE SHOULD BE PERFORMED BY A SKILLED INDIVIDUAL OR TRAINED PROFESSIONAL

1. Format the microplates' wells for each serum reference calibrator, control and patient sample to be assayed in duplicate. Place the wells in a microwell strip for use in the microplate reader.

2. Pipette 0.050 ml (50 µl) of the appropriate extracted Vitamin B12 sample, or control and capacity the assigned well.

3. Add 0.050 ml (50 µl) of the B12 calibrator to all wells.

4. Add an aliquot of the stabilizing agent in order to prepare a 1:10 dilution of the extracted sample.

5. Cover and incubate for 15 minutes. At the end of the 15 minute, dispense 0.50 ml (50 µl) of the neutralizing buffer, vortex (see note 3).

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

Add an aliquot of the stabilizing agent in order to prepare a 1:10 dilution of the extracted sample.

7. Add directly to the test reagents dispensed in the wells.

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

9. Incubate at room temperature.

10. Incubate at room temperature.

11. Add 0.350 ml (350 µl) of wash buffer (see Reagent Preparation Section) made up with 3900 µl volume to a 350 µl volumes with a precision of better than 1.5%.

12. Add 0.100 ml (100 µl) of working signal reaction solution to all wells. Mix for 20-30 seconds to mix.

13. Incubate at room temperature for five (5) minutes.

14. Read the relative light units (RLUs) in each well for 0.2 - 1.0 seconds with a precision of better than 1.5%.

15. Add 0.060 ml (60 µl) of the signal reagent solution.
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 Vitamin B12 in unknown specimens.

2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.

3. The absorbance (OD) of calibrator 0 pg/ml should be > 1.3.

4. To determine the concentration of Vitamin B12 for an unknown, locate the average RLU of the duplicates for each control. The number of RLUs, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

5. The addition of signal reagent solution initiates a kinetic reaction; therefore, the solution should be added in the same 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. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor performance.

8. 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 RLU (42160) intersect the dose response curve at 601.3 pg/ml Vitamin B12 concentration (Figure 1).

11.0 Q.C. PARAMETERS

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

2. The absorbance (OD) of calibrator 0 pg/ml should be > 1.3.

3. 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 piping graph paper (pH 7.1) plates, it is recommended to repeat the dose response curve.

5. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, to perform routine preventative maintenance.

6. Risk Analysis as required by CE Mark IVD Directive 98/79/EC - as measured in ten experiments in duplicate over a ten day period.

12.2 Interpretation

1. Measurements and interpretation of results must be performed by a skilled and trained professional.

2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, patient care and should not be the sole basis for therapy, patient care and should not be the sole basis for therapy, patient care and should not be the sole basis for therapy.

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 potential problems for all kinds of immunoassays (Bioscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem. 1991; 37(7): 721-726). The total number of such specimens was 57. The least square regression equation and the correlation coefficient were computed for this test system in comparison with the reference method. The data obtained is displayed in Table 4.

12.3 Precision

1. The Vitamin B12 AccuLite® CLIA Test System was compared with a reference method. Biological specimens from low, normal and relatively high Vitamin B12 levels were used (values ranged from 27.9 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 in comparison with the reference method. The data obtained is displayed in Table 4.

12.4 Sensitivity

1. The Vitamin B12 AccuLite® CLIA Test System has a sensitivity of 0.0 pg/ml and the 2x (95% certainty) to calculate the minimum dose.

12.5 Accuracy

1. The Vitamin B12 AccuLite® CLIA Test System was compared with a reference method. Biological specimens from low, normal and relatively high Vitamin B12 levels were used (values ranged from 27.9 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 in comparison with the reference method. The data obtained is displayed in Table 4.

12.6 Specificity

1. 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 the dose of interfering substance to dose of Vitamin B12 needed to displace the same amount of labeled analog.

15.0 REFERENCES
