Upon mixing the biotinylated antibody with a serum containing antibody binging sites (not consumed in the first incubation). 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).

The essential reagents required for an enzyme immunoassay are:

- Enzyme-antigen Conjugate -Antibody Complex
- Biotinylated antibody not reacted in first incubation
- Rate Constant of Dissociation

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.

\[ E_{\text{Ag-AbBtn}} + \text{EnzAgAbBtn} \rightarrow \text{Immune Complex} \]

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

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 form of atherosclerosis, coronary heart disease and/or thrombosis.

Two of the most common causes of Vitamin B12 deficiency are diet and age. Because most sources of dietary Vitamin B12 come from foods that do not sufficiently 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.

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 the intrinsic factor. Once Vitamin B12 is stable into your body through of its association with IF.

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

4.0 REAGENTS

Materials Provided:

- Vitamin B12 Calibrators - Tmviial - Ions A-F
- (50 µL) of the neutralizing buffer, vortex
- 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.

Materials Provided:

- Vitamin B12 Calibrators - Tmviial - Ions A-F
- (50 µL) of the neutralizing buffer, vortex
- 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.

Materials Provided:

- Vitamin B12 Calibrators - Tmviial - Ions A-F
- (50 µL) of the neutralizing buffer, vortex
- 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.

Materials Provided:

- Vitamin B12 Calibrators - Tmviial - Ions A-F
- (50 µL) of the neutralizing buffer, vortex
- 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.

Materials Provided:

- Vitamin B12 Calibrators - Tmviial - Ions A-F
- (50 µL) of the neutralizing buffer, vortex
- 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.

Materials Provided:

- Vitamin B12 Calibrators - Tmviial - Ions A-F
- (50 µL) of the neutralizing buffer, vortex
- 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.

Materials Provided:

- Vitamin B12 Calibrators - Tmviial - Ions A-F
- (50 µL) of the neutralizing buffer, vortex
- 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.

Materials Provided:

- Vitamin B12 Calibrators - Tmviial - Ions A-F
- (50 µL) of the neutralizing buffer, vortex
- 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.
A dose response curve is used to ascertain the concentration of Vitamin B12 in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate calibrator versus the corresponding Vitamin B12 concentration in pg/ml on linear graph paper (do not average the duplicates of the calibrators before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of Vitamin B12 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 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 absorbance (1.53) must be plotted on the graph paper (do not average the duplicates of the calibrators corresponding Vitamin B12 concentration in pg/ml on linear software is utilized, the validation of the software should be ascertained.

### 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 other devices made by Monobind, can be requested via email from Monobind@monobind.com.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.

### 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 determinations.
3. Risk Analysis, as required by CE Marking Directive 98/79/EC, for this and other devices made by Monobind, can be requested via email from Monobind@monobind.com.
4. It is important to keep in mind that establishment of a range of 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 analyst using the method with a population with a unknown may be averaged as indicated. In the following example, the average absorbance (1.53) must be plotted on the graph paper (do not average the duplicates of the calibrators corresponding Vitamin B12 concentration in pg/ml on linear software is utilized, the validation of the software should be ascertained.

### 12.3 Expected Ranges of Values
In agreement with established reference intervals for a “normal” population the expected ranges for the Vitamin B12 AccuBind® ELISA Test System are detailed in Table 1.

#### Table 1
**Expected Values - Vit B12 AccuBind® ELISA Test System**

<table>
<thead>
<tr>
<th>Population</th>
<th>pg/ml</th>
<th>pmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn</td>
<td>160 - 590</td>
<td>118-591</td>
</tr>
<tr>
<td>Adult</td>
<td>590 - 148</td>
<td>140</td>
</tr>
</tbody>
</table>

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 analyst using the method with a population with a unknown may be averaged as indicated. In the following example, the average absorbance (1.53) must be plotted on the graph paper (do not average the duplicates of the calibrators corresponding Vitamin B12 concentration in pg/ml on linear software is utilized, the validation of the software should be ascertained.

### 12.4 Specificity
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.

### 15.0 REFERENCES