INTRODUCTION

The clinical usefulness of the measurement of serum digoxin (D) is due to its low therapeutic ratio; a very small difference exists between therapeutic and toxic tissue levels. In addition, individuals may vary in their response to digoxin with an apparent one-to-one ratio of the myocardial concentrations of digoxin to serum levels remaining constant during normal renal function. This distribution is, however, altered in patients receiving digitalis or digoxin. Thus, monitoring digoxin therapy by measurement of serum levels is feasible from the pharmacological standpoint, since serum levels are related to tissue levels.8 Post absorption equilibration.1 A practical and sensitive method of determining digoxin concentration in human serum or plasma by a Microplate Enzyme Immunoassay, Colorimetric

SUMMARY AND EXPLANATION OF THE TEST

The myocardial concentrations of digoxin to serum levels remain relatively constant during normal renal function. This distribution is altered in patients receiving digitalis or digoxin. Thus, monitoring digoxin therapy by measurement of serum levels is feasible from the pharmacological standpoint, since serum levels are related to tissue levels.8 Post absorption equilibration.1 A practical and sensitive method of determining digoxin concentration in human serum or plasma by a Microplate Enzyme Immunoassay, Colorimetric

REAGENTS

Materials Provided:
A. Dig Calibrators – 1ml/vial - Icons A-F
B. Dig Enzyme Reagent– 6ml/vial - Icon
C. Streptavidin Coated Plate – 96 wells – Icon
D. Streptavidin Coat Plate – 96 wells – Icon
E. Wash Solution Concentrate – 20ml/vial - Icon
F. Substrate A – 7ml/vial - Icon SA
G. Substrate B – 7ml/vial - Icon SB
H. Stop Solution – 8ml/vial - Icon SH

PRODUCT INSTRUCTIONS

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Do not use reagents that have been heated or have had light exposure. Opened reagents are stable for up to 60 days when stored at 2-8°C. Kit and component stability are identified on the label.

Calibration

Sample ID. Well Number Abs (A) Mean Abs (B) Value (ng/ml)
Cal A 1.214 2.175 1.758
Cal B 1.374 2.175 1.840
Cal C 1.142 2.175 1.241
Cal D 1.344 2.175 1.280
Cal E 1.142 2.175 1.300
Cal F 0.254 2.175 0.300
Patient 1.374 2.175 1.250

Sample Preparation

1. Format the microplates’ wells for each serum reference and unknown specimen. Stabilize the unknown specimen’s activity can be correlated with digoxin concentration.

Quality control materials

PRECAUTIONS

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibody. FDA recommends all kits be used for reagents that have been heated or have had light exposure. Opened reagents are stable for up to 60 days when stored at 2-8°C. Kit and component stability are identified on the label.

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Sample Preparation

1. Format the microplates’ wells for each serum reference and unknown specimen. Stabilize the unknown specimen’s activity can be correlated with digoxin concentration.
The data presented in Example 1 and Figure 1 is for illustration only and should not be used in lieu of a standard curve prepared with each assay.

Figure 1

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" ng/ml should be > 1.3.
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 specimens 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 substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. 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 would 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.

12.2 Interpretation
1. Measurements and interpretation of results must be performed by a skilled individual (not required 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. The reagents for AccuBind® ELISA procedures 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. 1983:39:52-7). 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 mixing parts from different kits, which could produce false test results, or if results are grossly 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.
7. Certain disease states are known to increase a patient's susceptibility to digoxin toxicity. The following are examples of such disease states: hypokalaemia, hypothyroidism, renal failure, and advanced Heart Disease.
8. A number of researchers have reported relatively high serum digoxin levels in infants. However, digoxin treated-children older than two years of age demonstrate serum digoxin levels more closely resembling adult values.
9. Patients receiving simultaneous quinidine and digoxin therapy should be monitored closely. Serum digoxin levels may rise to greater than twice the stabilized level within 24 hours after initiation of quinidine therapy and may remain higher for several days.
10. Patients receiving the diuretic furosemide may not display digoxin values that correspond to the clinical picture, when furosemide and digitalis preparations are used concurrently, monitoring patients is desirable.
11. Individuals on large doses of biotin supplements should discontinue use one day before blood draw in order to eliminate possible interferences.

13.0 EXPECTED RANGES OF VALUES

The usual therapeutic range of digoxin in adults is 0.5-2.0 ng/ml. However, there is an overlap of serum digoxin concentrations in groups of patients with and without clinical toxicity. A significant number of toxic patients have serum concentrations greater than 2.0 ng/ml and a correspondingly significant number of toxic patients have serum values in the range of 1.4-2.0 ng/ml.

Also, the addition of anticoagulants or antifibrinolytics may require higher doses to control their cardiac rate: these patients' digoxin levels are dependent upon a multiplicity of factors: the specificity of the method, the population tested and the dose and patient characteristics. A significant number of non-toxic patients have serum concentrations greater than 2.0 ng/ml and a correspondingly significant number of toxic patients have serum values in the range of 1.0-1.5 ng/ml.

For these reasons, the physician should make a definite clinical diagnosis before laboratory findings are evaluated.

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 and between assay precision of the DIG AccuBind® ELISA test system were determined by analyses on three different levels of pool control sera. The number (N), mean values (X), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>12</td>
<td>0.048</td>
<td>0.04</td>
<td>9.0</td>
</tr>
<tr>
<td>Normal</td>
<td>12</td>
<td>1.62</td>
<td>0.13</td>
<td>8.0</td>
</tr>
<tr>
<td>High</td>
<td>12</td>
<td>3.14</td>
<td>0.16</td>
<td>5.0</td>
</tr>
</tbody>
</table>

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

14.2 Sensitivity
The DIG AccuBind® ELISA test system has an analytical sensitivity of 0.072 ng/ml. The sensitivity was ascertained by determining the variability of the "0" calibrator and using the 95 (95%) certainty statistic to calculate the minimum concentration.

14.3 Accuracy
The DIG AccuBind® ELISA test system was compared against a predicate Digoxin method. Biological specimens from a general population were used. The values ranged from 0.5 - 2.917 ng/ml. The correlation is presented in Table 4.

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean</th>
<th>Least Square Regression Analysis</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monobind</td>
<td>1.249</td>
<td>y = 0.9702x + 0.1384</td>
<td>0.9288</td>
</tr>
</tbody>
</table>

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient also indicates excellent method agreement.

14.4 Specificity
The cross-reactivity of the digoxin 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 doses of interfering substance to dose of digoxin needed to displace the same amount of tracer.

<table>
<thead>
<tr>
<th>SUBSTANCE</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digitoxin</td>
<td>1.00</td>
</tr>
<tr>
<td>Acetyldigoxin</td>
<td>1.00</td>
</tr>
<tr>
<td>β-Méthyldigoxin</td>
<td>1.00</td>
</tr>
<tr>
<td>a-Acetyldigoxin</td>
<td>1.00</td>
</tr>
<tr>
<td>Digoxin</td>
<td>0.819</td>
</tr>
<tr>
<td>Digitoxigen</td>
<td>0.017</td>
</tr>
<tr>
<td>Lanatoside A</td>
<td>0.016</td>
</tr>
<tr>
<td>Quabain</td>
<td>0.001</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>0.001</td>
</tr>
<tr>
<td>Prednisone</td>
<td>0.001</td>
</tr>
<tr>
<td>Predargrenol</td>
<td>0.001</td>
</tr>
<tr>
<td>Digitoxol</td>
<td>0.001</td>
</tr>
</tbody>
</table>

15.0 REFERENCES