UNIT: Therapeutic Drug Monitoring

Overview
Because individuals vary greatly in their abilities to absorb and eliminate substances, a large number of drugs administered must be closely monitored by the laboratory. Often there is a narrow margin of safety between therapeutically effective and toxic levels. The following are brief discussions of two such drugs.

Theophylline is a bronchodilating drug widely prescribed for the treatment of asthma. A number of known and suspected factors that affect the absorption and metabolism rate of the drug, combined with a narrow therapeutic range, make it necessary to carefully monitor serum levels. In addition, the half-life of theophylline is longer in premature infants than in adults and side effects of therapy may be more serious. Therefore, it is important to carefully monitor infant serum levels. According to Bishop, et al., (page 524), the therapeutic range of theophylline is 10-20 \( \mu g/ml \), and toxic levels are >20 \( \mu g/ml \).

Digoxin is a cardiac glycoside drug commonly prescribed for congestive heart failure and rhythm abnormalities. Individual variation in dosage, age, bioavailability, renal and gastrointestinal function have been shown to affect desired pharmacological activity. Although research has shown good correlation between serum levels and the clinical state, overlap between therapeutic and toxic levels has been observed. The physician should base his/her patient evaluation on measured blood levels combined with other clinical data.

Therapeutic digoxin administration may result in inadequate or toxic serum levels. Serum digoxin levels accurately reflect corresponding cardiac muscle drug levels. There is a narrow margin of safety between therapeutic and toxic response. Symptoms of digoxin overdose include nausea, vomiting, suppressed appetite, diarrhea, confusion, seizures, hallucinations, light ‘halos’ around objects, fatigue, irregular heartbeat, slow or fast heartbeat. As with all medication, allergic reactions are possible.

Task
Use the ENDAB\textsuperscript{®} Digoxin Enzyme Immunoassay (EIA) test kit to determine the in-vitro concentration of digoxin in human serum. OR a procedure to be announced by instructor.

Objectives
After completion of this exercise, the student will be able to:

1. Review a TDM laboratory procedure to summarize the testing principle and determine the purpose of acting reagents.
2. Perform a TDM assay, as directed.
3. State the reason theophylline is administered.
4. State the reason digoxin is administered.
5. State the therapeutic ranges for digoxin and theophylline and state the clinical significance of abnormal levels / toxic levels.
6. Determine acceptability of run / procedure by analyzing and evaluating control results.
7. Provide answers to related study questions.

Supplies and Equipment
1. Endab\textsuperscript{®} Digoxin EIA kit #109
   a. Digoxin Standards (0, 0.5, 1.0, 2.0, 4.0, and 8.0 ng/mL)
   b. Digoxin Antiserum (rabbit anti-digoxin serum)
   c. Digoxin-alkaline Phosphatase Conjugate (digoxin – labeled alkaline phosphatase)
   d. Second Antibody Separating Reagent (goat anti-rabbit serum)
   e. Color Generating Substrate (p-nitrophenyl phosphate lyophilized powder)
UNIT: Therapeutic Drug Monitoring (continued)

f. Stopping Reagent* (sodium hydroxide)  *CAUTION: caustic liquid; avoid contact with face or eyes

g. DEA Buffer (diethanolamine)

h. Background Reagent (normal rabbit serum)

2. DI water
3. 12 x 75 mm glass test tubes and rack
4. 37°C waterbath
5. Pipets [25 µL, 50 µL, 100 µL, 5.0 mL (or 1 mL pipetter) and 10 mL]
6. Centrifuge capable of at least 1000X g force
7. Vortex mixer, moderate to high speed
8. Absorbent paper
9. Spectrophotometer capable of transmitting light at 400 nm

References

Endab® Digoxin Enzyme Immunoassay Kit product insert, Immunotech Corp., Boston, MA.

Reagent Preparation

1. Color Generating Substrate
   Reconstitute with 10 mL DEA buffer. Gently mix to completely dissolve and transfer entire contents to the remainder of the DEA buffer. Label as Working Substrate.

2. All other reagents are supplied ready for use.
3. Prior to assay, warm all reagents to room temperature. Gently mix all reagents prior to use.

Test Principle

The enzyme immunoassay method is based on the principle of competitive binding. Serum containing digoxin (i.e., standard, control, or patient sample) is mixed with a specified amount of enzymatically tagged digoxin [Digoxin-Enzyme Alkaline Phosphatase Conjugate]. The enzyme-tagged digoxin and untagged digoxin compete to bind with a specified amount of rabbit anti-digoxin serum added.

After a brief incubation period, antibody bound digoxin (both tagged and untagged) is separated from unbound digoxin (both tagged and untagged) by immunoprecipitation with Second Antibody Separating Reagent (goat anti-rabbit serum). Physical separation occurs as the result of centrifugation followed by the decantation of the supernatant.

The antibody bound digoxin precipitate in the bottom of the tube is re-suspended and the enzyme activity of the tagged digoxin determined. The enzyme activity is inversely proportional to the amount of serum digoxin (standard, control, or patient).

Specimen Collection and Handling

1. Serum is required for use in this assay. Blood samples should be drawn at least six (6) hours following administration of Digoxin.
2. Separate serum immediately from cells.
3. Specimens may be stored tightly capped at refrigerator temperature for seven days. Freezing is required to store specimens for a longer period of time.
4. Avoid multiple freeze-thaw cycles.
5. Prior to assay, frozen sera should be completely thawed and mixed well.
6. Moderately lipemic, hemolyzed or icteric specimens will not interfere with the assay. (Do not use grossly lipemic specimens.)
Procedural Notes
1. All reagents should be kept covered when not in use.
2. When pipetting reagents, maintain a consistent order of addition from tube to tube. (This will ensure equal incubation times for all times.)
3. Pipet all reagents directly to the bottom of the tube to prevent liquid from remaining on the side wall.
4. Inadequate removal of supernatant after centrifugation may lead to poor replication and incorrect patient values. (Start thinking of why.) For best results, remove tubes immediately after centrifuge has stopped. Keep tubes in an upright position.
   a. Take two tubes at a time and invert them to discard the liquid supernatant into a sink or container. Keeping the tubes inverted, touch the rims several times with absorbent paper. Turn the tubes upright and return them to the rack. Do not invert the tubes again as this may dislodge the pellet.
   b. Alternatively, a foam rack may be used to hold 1-50 tubes for batch decanting and blotting.

Assay Procedure
1. Label 13 x 100 mm round bottom tubes:
   a. (1) NSB (nonspecific binding; 0 ng/mL Standard)
   b. (5) standards (0.5, 1.0, 2.0, 4.0, and 8.0 ng/mL)
   c. (1) each control
   d. (1) each patient
2. Pipet 50 µL of each sample (NSB, standards, control(s), or patient serum) into the bottom of the appropriate tube.
3. Pipet 25 µL of Digoxin-Alkaline Phosphatase Conjugate into all tubes.
4. Pipet 100 µL of Digoxin Antiserum into all tubes except NSB. Pipet 100 µL of Background Reagent into NSB tube.
5. Mix reagents by shaking test tube rack and incubate all tubes at room temperature for 10 minutes.
6. Swirl or mix Second Antibody Separating Reagent and dispense or pipet 1.0 mL into all tubes.
7. Centrifuge all tubes for 10 minutes at maximum speed using a swing head centrifuge.
8. Decant supernatant from all tubes and blot on absorbent paper to remove excess liquid. (See procedural note #4.)
9. Dispense 1 mL Working Substrate Reagent to all tubes and also to a tube labeled BLANK.
10. Vortex all tubes vigorously and consistently at high speed for a minimum of two seconds each.
11. Incubate all tubes at 37°C for 30 minutes.
12. Remove tubes from the water bath and rapidly dispense 1.0 mL Stopping Reagent to all tubes, including the BLANK. Mix.
13. Measure the absorbance for all tubes on a spectrophotometer at 400 nm. Read against the blank for the initial calibration of the instrument to 0.

Results
1. Construct the standard curve by plotting the absorbance of the standards (vertical axis) versus the digoxin standard concentrations (horizontal axis) on the semi-logarithmic paper provided. Properly label the curve.
2. Draw the best curve through the points.
3. Use the standard curve to interpolate the control(s) and patient(s) serum values from each absorbance measured.
4. Record the value for each control and patient sample.

Name ___________________________
### Digoxin TDM Worksheet

#### Results 8 pts

<table>
<thead>
<tr>
<th>Standards</th>
<th>Absorbance</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ng/mL</td>
<td>0.000</td>
<td>0 ng/mL</td>
</tr>
<tr>
<td>0.5 ng/mL</td>
<td>0.055</td>
<td>0.5 ng/mL</td>
</tr>
<tr>
<td>1.0 ng/mL</td>
<td>0.105</td>
<td>1.0 ng/mL</td>
</tr>
<tr>
<td>2.0 ng/mL</td>
<td>0.211</td>
<td>2.0 ng/mL</td>
</tr>
<tr>
<td>4.0 ng/mL</td>
<td>0.398</td>
<td>4.0 ng/mL</td>
</tr>
<tr>
<td>8.0 ng/mL</td>
<td>0.810</td>
<td>8.0 ng/mL</td>
</tr>
<tr>
<td>Control 1 Stanbio Lot # 08661</td>
<td>0.135</td>
<td></td>
</tr>
<tr>
<td>Control 2 Stanbio Lot # 08662</td>
<td>0.319</td>
<td></td>
</tr>
<tr>
<td>Patient 1 Will Smith</td>
<td>0.023</td>
<td></td>
</tr>
<tr>
<td>Patient 2 Jill Shatner</td>
<td>0.423</td>
<td></td>
</tr>
</tbody>
</table>

#### Curve = 5 points

#### QC 5 pts.

<table>
<thead>
<tr>
<th>Quality Control</th>
<th>Your Results</th>
<th>Controls’ range of expected results.</th>
<th>In control? Yes / No</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>compare to Ortho-Clinical Vitros (spectrophotometric)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level 1 ID. Stanbio Lot # 08661</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level 2 ID. Stanbio Lot # 08662</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accepting Patient Results?</td>
<td>Reason</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Study Questions

Instructions: Legibly write your answers in the space provided. Unless otherwise indicated, each question is worth one point.

1. For what purpose is theophylline administered?

2. Why is theophylline assayed?

3. What is the therapeutic range for theophylline? (½ point) At what level would it be considered toxic? (½ point)

4. For what purpose is digoxin administered?

5. Why is digoxin assayed?

6. List the side effects of digoxin overdose, as presented in this lab. (2 points)

7. Explain why the digoxin procedure is classified as a competitive binding immunoassay. (2 points)
8. Complete the following information chart.

<table>
<thead>
<tr>
<th>Reagent Name</th>
<th>Constituent(s)</th>
<th>Functional Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digoxin Serum Standards</td>
<td>0.5, 1.0, 2.0, 4.0 and 8.0 ng/mL of digoxin respectively</td>
<td>Known concentrations of digoxin to be used in establishing a working curve</td>
</tr>
<tr>
<td>Digoxin antiserum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(2 points)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digoxin-Alkaline Phosphatase Conjugate</td>
<td>(2 points)</td>
<td></td>
</tr>
<tr>
<td>Second Antibody Separating Reagent</td>
<td>(2 points)</td>
<td></td>
</tr>
<tr>
<td>Color Generating Substrate</td>
<td>(2 points)</td>
<td></td>
</tr>
<tr>
<td>Stopping Reagent</td>
<td>(2 points)</td>
<td></td>
</tr>
<tr>
<td>Background Reagent</td>
<td>(1 point)</td>
<td>serves as reagent blank</td>
</tr>
</tbody>
</table>

9. What is the purpose of the vortex in this procedure? What effect could result if this process were not done (or done incorrectly)? (5 points)

To resuspend the precipitate and mix it with substrate. It would decrease the availability of the enzymes access to substrate – would result in falsely increased patient values.

10. What is the purpose of the absorbent paper in this procedure? What effect could result if this process were not done (or done incorrectly)? (5 points)

To blot away unbound (tagged and untagged) digoxin. Any enzyme tagged digoxin will react with substrate resulting in