RELATED READING: Chapter 27. See Methods in CD-ROM for Albumin.

OBJECTIVES

Upon completion of this exercise, discussion, and appropriate reading, the student will be able to:

1. Perform serum albumin determinations on 4 samples with 90% accuracy.
2. Explain the principle behind the Bromcresol Green method for albumin.
3. List the factors that will cause interference with this method.

PRINCIPLE

Albumin is known for its ability to bind many types of organic compounds, including organic dyes. When albumin binds with Bromcresol Green (BCG) it causes a change in the absorbance maximum of BCG. This change can be measured spectrophotometrically and used to determine albumin concentration.

GLOSSARY

Reagent blank -a solution used to zero a spectrophotometer (set 100% T); usually contains all diluent and reagent in the reaction solution, but no sample. Some reagent blanks do contain the sample as well, but they lack one crucial reagent component needed to produce a color-yielding reaction.

MATERIALS

- Calibrator
- Serum samples
- Controls
- Brom cresol green reagent
- Spectrophotometer
- Pipets
- 13 x 100 mm test tubes

PROCEDURE

1. Label sufficient 13 x 100 mm test tubes for all samples, controls, calibrators, and a reagent blank.
2. Using a 5 mL serological pipet, pipet 2.5 mL of BCG reagent into each tube.
3. Pipet 10 μL of each sample, control or calibrator into the appropriate tubes. Add 10 μL of distilled water to the reagent blank tube.
4. Mix all tubes by inversion.
5. Read the absorbance of each tube at 628 nm against the reagent blank and record your results on the data sheet.
6. Calculate the albumin concentration of each sample in g/L using the absorbance/concentration proportion method (see Exercise #1 Basic Spectrophotometry).

**OPTIONAL EXERCISE: FAST KINETIC MEASUREMENT**

1. Follow steps 1 and 2 in the procedure.
2. Add 10 μL of sample or calibrator and mix rapidly.
3. At 10 seconds after mixing, measure the absorbance at 628 nm.
4. Read absorbance again at 1 minute and record on data sheet.
5. Calculate the \( \Delta A \) (60 second reading minus the 10 second reading) for each specimen.
6. Calculate the albumin concentrations of the unknowns by methods of proportions (see Exercise #1).
   \[
   \text{Concentration albumin} = \frac{\Delta A_{\text{stand}} \times [\text{Alb}]_{\text{stand}}}{\Delta A_{\text{unknown}}}
   \]

**DATA SHEET, EXERCISE #13**

- **NAME:** ___________
- **DATE:** ___________

**RESULTS**

<table>
<thead>
<tr>
<th>Absorbance</th>
<th>Albumin g/L (Target Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator</td>
<td></td>
</tr>
<tr>
<td>Normal Control</td>
<td></td>
</tr>
<tr>
<td>Abnormal Control</td>
<td></td>
</tr>
<tr>
<td>Sample #</td>
<td></td>
</tr>
<tr>
<td>Sample #</td>
<td></td>
</tr>
</tbody>
</table>

**OPTIONAL EXERCISE**

<table>
<thead>
<tr>
<th>Absorbance at 628 nm</th>
<th>Albumin, g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 sec.</td>
<td>60 sec.</td>
</tr>
</tbody>
</table>

- Calibrator
- Normal Control
Absorbance at 628 nm     Albumin, g/L

Abnormal Control

Sample #
Sample #

DISCUSSION QUESTIONS

1. A hemolyzed sample is brought to the laboratory for albumin analysis. Can the sample be used? Discuss.
2. Why is it not desirable to incubate the reaction before measuring the absorbance?
3. Some analyzers can measure the absorbance of the BCG reaction within 30 seconds after adding sample. Does this tend to increase the specificity of the reaction?