OBJECTIVES

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

1. Determine serum glucose values with 90% accuracy using a colorimetric method.
2. Identify and explain specific differences between glucose oxidase and hexokinase methods.

PRINCIPLE

The Trinder method for glucose analysis utilizes the coupled enzyme reactions shown below:

$$\text{Glucose + O}_2 \xrightarrow{\text{glucose oxidase}} \text{glucuronic acid + H}_2\text{O}_2$$

$$\text{H}_2\text{O}_2 + \text{reduced dye} \xrightarrow{\text{horseradish peroxidase}} \text{oxidized dye + H}_2\text{O}$$

The reaction is linear to approximately 10,000 mg/L (1000 mg/dL) and reaches its endpoint in 15-20 minutes at 37°C.

GLOSSARY

**End-point** - refers to the final state to be attained. In the case of chemical assays, this term refers to the point when the reaction is essentially completed and there is no net production of reaction product.

**Water-blank** - when the absorbance of a solution is measured vs pure water; water is used to “zero” the instrument (100% T).

MATERIALS

- Glucose standard
- Glucose Oxidase Reagent
- Controls
- Test Tubes (13 x 100 mm)
- Pipets Incubator
- Spectrophotometer

PROCEDURE

1. Label sufficient 13 x 100 mm test tubes for each standard, control, and patient sample to be tested.
2. Pipet 2.0 mL of glucose reagent into each tube.
3. Add 10 \(\mu\)L of standard, control, or serum to each appropriate tube.
4. Mix well.
5. Incubate each tube at 37°C for 20 minutes.
6. Using appropriate cuvettes, measure the absorbance of each reaction mixture at 525 nm against a water blank.
7. Calculate each glucose concentration using the absorbance and concentration of the standard by the proportion method (see Exercise #1).
8. Record your results on the data sheet.
9. Record the appearance of the serum samples on the data sheet.

**OPTIONAL EXERCISE**

1. Prepare a hemolysate as described in Exercise #15 Bilirubin
2. Add approximately 10 \(\mu\)L of the hemolysate to 1 mL of a sample whose glucose was previously measured. Remeasure the glucose concentration.
3. Record the value on the data sheet.

**DATA SHEET, EXERCISE #10**

| NAME: ___________ | DATE: ___________
|------------------|--------------------|

**RESULTS**

<table>
<thead>
<tr>
<th>Glucose, mg/L</th>
<th>A525</th>
<th>Measured Value</th>
<th>Target Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Control</td>
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<td></td>
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<tr>
<td>Abnormal Control</td>
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<td></td>
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<tr>
<td>Sample #</td>
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<td>Sample #</td>
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</tbody>
</table>

**OPTIONAL EXERCISE**

Sample #____:
Previously measured glucose ____ x 0.99 = ____________ mg/L

Hemolyzed sample’s glucose concentration = __________ mg/L

DISCUSSION QUESTIONS

1. What is (would be) the effect of hemolysis on the measurement of glucose by this method? How could you correct for the presence of hemolysis in a sample when using this method?

2. Is this reaction more or less specific than a glucose oxidase/oxygen electrode method? Why?

3. Bilirubin will cause interference in this method. Why can this interference not be corrected by the procedure discussed in question 1?

4. What are the major advantages and disadvantages of the glucose oxidase/peroxidase method of determining glucose?