Estimation of glucose in blood serum

Principle:

Enzymatic estimation of glucose uses a reagent containing two enzymes and a chromogen. Glucose oxidase catalyses the oxidation of glucose to gluconolactone with the formation of hydrogen peroxide as a side product. Hydrogen peroxide produced is determined by oxidative copulation of a substituted phenol with 4-aminophenazone, catalyzed by peroxidase, yielding a coloured compound, the amount of which can be measured photometrically.

Procedure:

1. Pipette into three test tubes:

<table>
<thead>
<tr>
<th></th>
<th>sample</th>
<th>standard</th>
<th>blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>sample (ml)</td>
<td>0.02</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>standard (ml)</td>
<td>-</td>
<td>0.02</td>
<td>-</td>
</tr>
<tr>
<td>distilled water (ml)</td>
<td>-</td>
<td>-</td>
<td>0.02</td>
</tr>
<tr>
<td>reagent (ml)</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

2. Mix properly and incubate for 15 min at 37°C.
3. Measure the absorbances of the sample and the standard at 498 nm against the blank.

4. Calculate the concentration:

\[
\text{Glucose (mmol/l)} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times c_{\text{standard}} (10 \text{ mmol/l})
\]
Estimation of total cholesterol in blood serum

Principle:

Enzymatic estimation of cholesterol uses a reagent containing three enzymes and a chromogen. Cholesterol esters are hydrolyzed to free cholesterol by cholesterol esterase. The free cholesterol produced is oxidized by cholesterol oxidase to cholestenone with the simultaneous production of hydrogen peroxide, which, in the presence of peroxidase allows oxidative copulation of 4-aminoantipyrine with phenol to yield a coloured compound suitable for the photometric determination.

Procedure:

1. Pipette into three test tubes:

<table>
<thead>
<tr>
<th></th>
<th>sample (ml)</th>
<th>standard (ml)</th>
<th>blank (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sample</td>
<td>0.015</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>standard</td>
<td>-</td>
<td>0.015</td>
<td>-</td>
</tr>
<tr>
<td>distilled water</td>
<td>-</td>
<td>-</td>
<td>0.015</td>
</tr>
<tr>
<td>reagent</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

2. Mix properly and incubate for 20 min at 37°C.

3. Measure the absorbances of the sample and the standard at 498 nm against the blank.

4. Calculate the concentration:

\[
\text{Total cholesterol (mmol/l)} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times C_{\text{standard}} (5.17 \text{ mmol/l})
\]
Estimation of HDL cholesterol in blood serum

Principle:

Traditional approach used a two-step method, first step was a chemical precipitation of lipoproteins containing apoprotein B (all the other types than HDL) followed by centrifugation, in the second step cholesterol remaining in the supernatant (=HDL cholesterol) was quantified as described for total cholesterol. Such precipitation-based methods are time-consuming.

Recently, an immunoinhibition method is used to bind lipoproteins other than HDL with anti-human β-lipoprotein antibodies to form antigen-antibody complexes so that cholesterol esterase and cholesterol oxidase react only with HDL cholesterol.

Procedure:

1. Pipette into three Eppendorf tubes:

<table>
<thead>
<tr>
<th></th>
<th>sample</th>
<th>standard</th>
<th>blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>sample (ml)</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>standard (ml)</td>
<td>-</td>
<td>0.01</td>
<td>-</td>
</tr>
<tr>
<td>distilled water (ml)</td>
<td>-</td>
<td>-</td>
<td>0.01</td>
</tr>
<tr>
<td>reagent 1 (ml)</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
</tr>
</tbody>
</table>

2. Mix properly and incubate in the thermoblock for 5 min at 37°C.

3. Measure the absorbances of the sample, standard and blank at 593 nm against distilled water.

The total volume of the reaction mixtures is rather small, transfer by pipetting all the content from the Eppendorf tube into a special cuvette for low volume samples and make the measurements with cooperation of lab assistant using spectrophotometer suitable for this purpose. Do not throw away the Eppendorf tubes, you will return there the solutions from cuvettes after the measurement.

4. Transfer by pipetting all the content from the cuvettes back into original Eppendorf tubes.

5. Add 0.3 ml of reagent 2 into each Eppendorf tube, close the cap and gently mix by turning upside down.
6. Incubate in the thermoblock for 5 min at 37°C.

7. Measure the absorbances of the sample, standard and blank at 593 nm against distilled water.

   The same procedure as you did at step 3 of this instruction sheet.

   \[
   \begin{array}{|c|c|c|}
   \hline
   \text{A}_2 \text{ sample} & \text{A}_2 \text{ simple} & \text{A}_2 \text{ standard} \\
   \hline
   \text{A}_2 \text{ standard} & \text{A}_2 \text{ standard} & \text{A}_2 \text{ standard} \\
   \hline
   \text{A}_2 \text{ blank} & \text{A}_2 \text{ blank} & \text{A}_2 \text{ blank} \\
   \hline
   \end{array}
   \]

8. Calculate the differences in absorbances:

   \[
   \begin{array}{|c|c|}
   \hline
   \Delta \text{A} \text{ sample} = & \text{A}_2 \text{ sample} - \text{A}_1 \text{ sample} \\
   \hline
   \Delta \text{A} \text{ standard} = & \text{A}_2 \text{ standard} - \text{A}_1 \text{ standard} \\
   \hline
   \Delta \text{A} \text{ blank} = & \text{A}_2 \text{ blank} - \text{A}_1 \text{ blank} \\
   \hline
   \end{array}
   \]

9. Calculate the concentration:

   \[
   \text{HDL cholesterol (mmol/l)} = \frac{\Delta \text{A} \text{ sample} - \Delta \text{A} \text{ blank}}{\Delta \text{A} \text{ standard} - \Delta \text{A} \text{ blank}} \times c_{\text{standard}} \times (1.42 \text{ mmol/l})
   \]
Estimation of triglycerides in blood serum

Principle:

Enzymatic estimation of triglycerides (triacylglycerols) uses a reagent containing four enzymes and a chromogen. Triglycerides are hydrolyzed to fatty acids and glycerol by a lipase. The glycerol produced is phosphorylated by glycerol kinase to glycerol-3-phosphate, which is then oxidized by glycerol-3-phosphate oxidase to dihydroxyacetone phosphate with the simultaneous production of hydrogen peroxide, which in the presence of peroxidase allows oxidative copulation of some chromogens to yield a coloured compound suitable for the photometric determination.

Procedure:

1. Pipette into three Eppendorf tubes:

<table>
<thead>
<tr>
<th></th>
<th>sample</th>
<th>standard</th>
<th>blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>sample (ml)</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>standard (ml)</td>
<td>-</td>
<td>0.01</td>
<td>-</td>
</tr>
<tr>
<td>distilled water (ml)</td>
<td>-</td>
<td>-</td>
<td>0.01</td>
</tr>
<tr>
<td>reagent (ml)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

2. Mix properly and incubate in the thermoblock for 10 min at 37°C.

3. Measure the absorbances of the sample and the standard at 546 nm against the blank.

   The total volume of the reaction mixtures is rather small, transfer by pipetting all the content from the Eppendorf tube into a special cuvette for low volume samples and make the measurements with cooperation of lab assistant using spectrophotometer suitable for this purpose.

4. Calculate the concentration:

   \[
   \text{Triglycerides concentration (mmol/l)} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times c_{\text{standard}} \ (2.26 \ \text{mmol/l})
   \]
Calculation of LDL cholesterol

Calculation of LDL cholesterol concentration can be done using the Friedewald formula, based on the known values of total cholesterol, HDL cholesterol and triglycerides:

$$\text{LDL cholesterol} = \text{total cholesterol} - \text{HDL cholesterol} - \frac{\text{triglycerides}}{2.2}$$

Atherosclerosis risk assessment

Cardiovascular diseases are a serious concern, accounting for approximately one-third to one-half of all deaths. Parameters of lipid metabolism can be used to calculate some indexes predicting the risk:

Atherogenic index (AI) = \frac{\text{total cholesterol}}{\text{HDL cholesterol}}

Atherogenic index of plasma (AIP) = \log \frac{\text{triglycerides}}{\text{HDL cholesterol}}

There are many other risk factors of atherosclerosis (age, gender, systolic blood pressure, smoking status). In clinical practice, overall risk can be simply predicted using the charts originating in the SCORE project (Systematic COronary Risk Evaluation). The charts show 10-year risk of fatal cardiovascular disease event.

SCORE risk chart (European Society of Cardiology)