Determination of plasma amylase

BCH 472
Amylase:

Amylase is an enzyme that catalyze the **breakdown of starch and glycogen** into smaller carbohydrate groups (maltose, oligosaccharides, glucose)

It is produced mainly in the **salivary glands** and **pancreas**, and is normally **excreted in small amounts in the urine**.

Among healthy individuals, the pancreas and the salivary glands account for almost all serum amylase, 40-45% from the pancreas and 55-60% from the salivary glands.
Amylase:

- When the pancreas is diseased or inflamed, amylase releases into the blood.

- A test can be done to measure the level of this enzyme in a blood.
Determination of amylase level:

- **This test of blood and urine** is most often used to **distinguish** acute pancreatitis and other causes of abdominal pain that require immediate surgery.

- It may also detect some digestive tract problems.

- **Serum and urine amylase** measurement in addition to other laboratory tests, amylase clearance, amylase isozyme, and measurement of serum lipase levels, increase the specificity of amylase measurement in the **diagnosis of acute pancreatitis**.
Amylase in Serum and urine:

- **Increased blood and urine amylase levels may occur due to:**
  - **Acute** pancreatitis (a sudden inflammation of the pancreas).
  - Obstruction of the pancreatic duct.
  - Infection of the salivary glands or a blockage.

- **Decreased level:**
  - Damage to the pancreas.
Practical Part
Objective:

To estimate the concentration of amylase in serum
Amylase Kit

- Amylase kit:
  - P-Nitrophenyl D-Maltoptoside
  - Glucosidase
  - Glucoamylase
  - Sodium Chloride 50 mM
  - Calcium Chloride
  - Buffer, pH 6.9 ± 0.01
Principle:

1- Amylase hydrolyzed p-nitrophenyl D-maltoheptoside (PNPG\textsubscript{7}) to P-nitrophenylmaltotriose (PNPG\textsubscript{3}) and maltotetrose.

- PNPG\textsubscript{7} $\xrightarrow{\text{AMYLASE}}$ PNPG\textsubscript{3} + Maltotetrose

2- Glucoamylase hydrolyzes PNG\textsubscript{3} to P-nitrophenylglycose (PNPG\textsubscript{1}) and glucose.

- PNPG\textsubscript{3} $\xrightarrow{\text{GLUCOAMYLASE}}$ PNPG\textsubscript{1} + Glucose

3- Then PNPG\textsubscript{1} is hydrolyzed by glucosidase to glucose and P-nitrophenol which produce a yellow color.

- PNPG\textsubscript{1} $\xrightarrow{\text{GLUCOSIDASE}}$ p-Nitrophenol + Glucose
Method:

<table>
<thead>
<tr>
<th>CHEMICALS</th>
<th>SAMPLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMYLASE SUBSTRATE (kit)</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

Pre-warm at 37°C for 5 minutes and add:

| Sample (serum) | 0.025 ml |

1. Mix and incubate at 37°C for **90 seconds** and read the absorbance at 405 nm against distilled water.

2. Continue readings every 30 seconds for 2 minutes and determine $\Delta A/\text{Min}$.

- The rate of increase in Ab is measured at 405 nm and is **proportional** to the amylase activity in the sample.
Results:

<table>
<thead>
<tr>
<th>Seconds</th>
<th>Absorbance at 405 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td></td>
</tr>
<tr>
<td>120</td>
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</tbody>
</table>
CALCULATIONS:

Amylase Activity in TEST (U/L) = \( \Delta A/\text{Min} \times 4824 \)

\[ \Delta A/\text{Min} = (\Delta A1 + \Delta A2) / 2 \]

\[ \Delta A1 = (A_{60\,s} - A_{30\,s}) + (A_{30\,s} - A_{0\,s}) \]

\[ \Delta A2 = (A_{120\,s} - A_{90\,s}) + (A_{90\,s} - A_{60\,s}) \]
RANGE OF EXPECTED VALUES of amylase:

- Serum: 16-108 U/L
- Urine: 0 - 14 U/Hour
Discussion:

Comment on the amylase concentration in the sample.