Determination of plasma amylase
Amylase:

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

• It is produced in the salivary glands, pancreas, liver, and fallopian tubes 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 in Serum and urine:

• 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 specify of amylase measurement in the diagnosis of acute pancreatitis.
RANGE OF EXPECTED VALUES of amylase:

• Serum: 16-108 U/L

• Urine: 0 - 14 U/Hour

Increased plasma amylase:

• salivary gland inflammation.

• Pancreatitis.

• pancreatic cancer.

Decreased plasma amylase:

• Pancreatic insufficiency.
Practical Part
-Objective:

• To estimate the concentration of amylase in serum.

• Amylase kit:

• P-Nitrophenyl D-Maltoheptoside

• Glucosidase

• Glucoamylase

• Sodium Chloride 50 mM,

• Calcium Chloride

• Buffer, pH 6.9 + 0.01
**Principle:**

1. Amylase hydrolyzed p-nitrophenyl D-maltoheptoside (PNPG7) to P-nitrophenylmaltotriose (PNPG3) and maltotetrose:

   \[ \text{PNPG7} \xrightarrow{\text{AMYLASE (in the sample)}} \text{PNPG3} + \text{Maltotetrose} \]

2. Glucoamylase hydrolyzes PNG3 to P-nitrophenylglycosie (PNPG1) and glucose:

   \[ \text{PNPG3} \xrightarrow{\text{GLUCOAMYLASE}} \text{PNPG1} + \text{Glucose} \]

3. Then PNPG1 is hydrolyzed by glycosidase to glucose and P-nitrophenol which produce a yellow color which absorb at 405nm, the rate of increase in Ab is measured at 405nm and is proportional to the amylase activity in the sample:

   \[ \text{PNPG1} \xrightarrow{\text{GLUCOSIDASE}} \text{p-Nitrophenol} + \text{Glucose} \]
-Method:

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 ΔA/min.

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

Pre-warm at 37oC for 5 minutes and add:

| Sample1                  | 0.025 ml       |
-Results:

<table>
<thead>
<tr>
<th>Time (Seconds)</th>
<th>Absorbance at 405 nm</th>
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<tbody>
<tr>
<td>0</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
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<tr>
<td>60</td>
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<tr>
<td>90</td>
<td></td>
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<td>120</td>
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-Calculations:

Amylase Activity in TEST (U/L) = $\Delta A/min \times 4824$

$\Delta A/\text{Min} = (\Delta A_1 + \Delta A_2) \div 2$

$\Delta A_1 = (A_{60s} - A_{30s}) + (A_{30s} - A_{0s})$

$\Delta A_2 = (A_{120s} - A_{90s}) + (A_{90s} - A_{60s})$