University Of Fallujah
College Of Medicine
Medical Biochemistry

Lecture 6: Estimation of Serum Amylase and Lipase

Stage: 2nd Year

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Learning Objectives

Understand the physiological role of amylase and lipase

Recognize clinical conditions that raise or lower these enzymes

Interpret enzyme levels in acute vs chronic pancreatitis

Identify interfering substances and sample requirements

Serum Amylase

- **Amylase** in serum arises mainly from the pancreas (P-isoamylase) and the salivary glands (S-isoamylase).
- Serum P-isoamylase activity is a more sensitive and more specific test than total amylase for the detection of acute pancreatitis.
- Amylase is filtered by the glomeruli and excreted in urine.
- It catalyzes the hydrolysis of starch and glycogen.
- This test measures the amount of amylase in the blood or urine.

Elevation of serum amylase occurs in:

- Pancreatic diseases: acute and chronic pancreatitis
- Parotitis

- Increase in serum levels is also seen in mumps, obstruction of the pancreatic duct, and Gallstones.
- Amylase tests are sometimes used to monitor treatment of cancers involving the pancreas and after the removal of gallstones that cause gallbladder attacks.

- In acute pancreatitis, serum amylase begins to rise within 3-6 hours, peaks at 24 hours, and returns to normal levels by 2-3 days.

- Urinary amylase is also high in acute pancreatitis and remains elevated for 7-10 days.

- Normal serum level is: 50-120 U/L.

Sample collection:

- Serum, heparinized plasma, urine

Interferences Substance :

- Some drugs that may cause elevation of amylase include aspirin, diuretics, oral contraceptives and morphine.

Procedure

PROCEDURE

1. Bring the Reagent and the instrument to reaction temperature.
2. Pipette into a cuvette: (Notes 1,2)

	Serum or plasma		Urine	
	37°C	25°C,30°C	37°C	25°C,30°C
Reagent (A)	1.0 mL	1.0 mL	1.0 mL	1.0 mL
Sample	20 µL	50 µL	10 µL	20 µL

3. Mix and insert the cuvette into the photometer. Start the stopwatch.
4. Record initial absorbance and at 1 minute intervals thereafter for 3 minutes.
5. Calculate the difference between consecutive absorbances, and the average absorbance difference per minute (ΔA/min).

CALCULATIONS

The α-Amylase concentration in the sample is calculated using the following general formula:

$$\Delta A/min \times \frac{Vt \times 10^{6}}{\varepsilon \times l \times Vs} = U/L$$

The molar absorbance (ε) of CNP at 405 nm is 15490 and the lightpath (l) is 1 cm. For serum and plasma samples, the total reaction volume (Vt) is 1.02 at 37°C and 1.05 at 25-30°C and the sample volume (Vs) is 0.02 at 37°C and 0.05 at 25-30°C. For urine samples, the total reaction volume (Vt) is 1.01 at 37°C and 1.02 at 25-30°C and the sample volume (Vs) is 0.01 at 37°C and 0.02 at 25-30°C. 1 U/L are 0.0166 µkat/L. The following formulas are deduced for the calculation of the catalytic concentration:

		37°C	25-30°C
ΔA/min	Serum, plasma	x 3292 = U/L x 54.9 = µkat/L	x 1355 = U/L x 22.6 = µkat/L
	Urine	x 6520 = U/L x 108.7 = µkat/L	x 3292 = U/L x 54.9 = µkat/L

lipase

- Lipase is an enzyme produced by the pancreas to help digest dietary fats, especially TG.
- Lipase is more specific than Amylase for pancreatic pathology because lipase remains elevated longer than amylase.
- Serum lipase starts to increase within 3-6 hours, reaches maximum at 24 hours, and remains elevated for 8-14 days.
- Lipase has a longer half-life than amylase and, therefore, may be more specific in the diagnosis of late-presenting acute pancreatitis.

- The blood test for lipase is ordered, often along with an amylase test, to help diagnose and monitor acute pancreatitis, chronic pancreatitis, and other disorders that involve the pancreas.

Serum lipase is elevated in:

- pancreatic diseases
- intestinal diseases
- acute cholecystitis
- Carcinoma of the pancreas.

- **Normal lipase range : 50-175 IU/L.**

- A lipase test may be ordered when a person has symptoms of a pancreatic disorder, such as severe abdominal or back pain, fever, loss of appetite, or nausea.

Sample collection:

- Serum, heparinized plasma, urine

Interferences Substance :

- Some drugs that may cause elevation of lipase include aspirin, diuretics, and morphine.

Procedure

SAMPLES

Serum or sodium, lithium or ammonium heparin plasma collected by standard procedures.

Lipase in the sample is stable for 7 days at 2-8°C.

PROCEDURE

1. Bring the Reagents and the instrument to 37°C.
2. Pipette into a cuvette: (Note 1)

Reagent A	1000 µL
Serum / Standard (S)	10 µL

3. Mix and insert the cuvette into the instrument. Start the stopwatch. After 1-3 minute, add:

Reagent B	200 µL

4. Mix.
5. After 1 minute, record initial absorbance and at 1 minute intervals thereafter for 3 minutes.
6. Calculate the difference between consecutive absorbances, and the average absorbance difference per minute (ΔA/min).

CALCULATIONS

The lipase concentration in the sample is calculated using the following general formula:

$$\frac{\Delta A/\text{min}_{\text{Sample}}}{\Delta A/\text{min}_{\text{Standard}}} \times C_{\text{Standard}} = U/L$$