

BCH 447 Practical Metabolism Lipase Assay (Using Turbidimetric Method)

Mark Distribution

Task	Marks		
Attendance and Performance	5 Marks		
Report	15 Marks		
Homework	3 Marks		
Presentation	5 Marks (due 15 Apr 2025)		
Quiz	12 Marks		
Midtorm (20 Marks)	Practical	15 Marks	
Midterm (20 Marks)	Theoretical	5 Marks	
Final (10 Marks) 22	Practical	25 Marks	
Final (40 Marks) ??	Theoretical	15 Marks	
Total	100 Marks		



Introduction

Lipase

gl

• Is an enzyme that breaks down dietary fats into smaller molecules, fatty acids and

lycerol.	CH ₂ OCOR ₁		CH ₂ OH		R ₁ COOH
	CHOCOR ₂ + 3 H ₂ O	Lipase	снон	+	R ₂ COOH
	CH ₂ OCOR ₃		і сн ₂ он		+ R₃COOH
	Triglyceride		Glycerol		Free Fatty Acids

Figure 1. Schematic representation of lipase action. Source: sciencedirect.com

 It is produced by the pancreas in large quantity and secrete them into the small intestine.

Serum lipase concentration

- The measurement of lipase activity in serum and other fluids evaluate the conditions associated with **pancreas**.
- Lipase concentrations is <u>increased</u> in **acute pancreatitis.**
- Acute pancreatitis is a <u>sudden inflammation</u> of the pancreas. Its most common causes are:
 - Gallstones
 - Pancreatic cancer, and other pancreatic disease
 - Gallbladder inflammation.



Acute pancreatitis

- The common bile duct and the pancreatic
 duct join together to transport digestive
 enzymes and bile to the small intestine.
- A gallstone in the common bile duct can cause back pressure in the pancreatic duct leading to pancreatitis (*elevated blood lipase levels*).

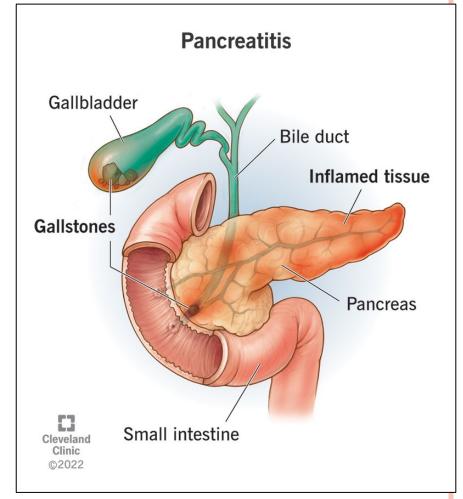


Figure 2. Pathology of acute pancreatitis. Source: my.clevelandclinic.org



• A low level of lipase in the blood may indicate <u>permanent damage</u> to the lipase-producing cells

in the pancreas and this can occur in <u>chronic diseases</u> that affect the pancreas such as **cystic fibrosis.**

Principle of Turbidimetric Method:

 Hydrolysis of triglycerides present in the olive oil by serum lipase causes a decrease in the turbidity of the reaction mixture.

Triglycerides (olive oil) + H_2O _____ mono + di-glycerides + fatty acids

 The decrease in turbidity at 400 nm (after incubation) is proportional to lipase activity in the specimen and reflects the activity of lipase in the sample



Objective:

• To determine lipase activity in a serum sample using turbidimetric method.

Method:

1. <u>Set two test tubes as:</u>

	Test	Blank
Reagent (substrate+ buffer)	3 ml	3 ml
Pre-incubate for 5 minutes at 37° C		
Sample (contains lipase)	0.1 ml	_

- 1. Read the absorbance (A°) immediately at <u>400 nm</u> against <u>distilled water</u>.
- Then transfer to water bath at 37° C and incubate for 5 min then read the absorbance
 (A₁) at <u>400 nm</u> against <u>distilled water</u>.

Calculations:

Note:

- Reagent blank: if $(A_{\circ} A_{1})$ is a negative value, it should be <u>considered as zero</u>.
- However, it should normally be **<u>between 0.000 and 0.005</u>**.

Normal range:

- In adults: 10-150 U/L
- In old individuals (more than 60 years): **18-180** U/L