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Biochemical Evaluation of Serum Lipids and Cardiac Enzymes: Techniques and Diagnostic Value

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Abstract
The biochemical evaluation of serum lipids and cardiac enzymes is central to understanding cardiovascular health and disease. Lipid profile estimation, including cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL), provides insight into atherogenic risk. At the same time, enzymatic assays for cardiac markers such as creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), troponins, and aspartate aminotransferase (AST) enable the diagnosis of myocardial injury. This article reviews the key methods used in lipid and enzyme estimation, presents their strengths and limitations, and highlights their diagnostic value in clinical practice.

Keywords: Serum Lipids, Cardiac Enzymes, Lipid Profile Estimation, Troponin Biomarkers, Cardiovascular Diagnostics

1. Introduction
Cardiovascular diseases (CVDs) remain the leading cause of morbidity and mortality globally (Gaziano, 2022) [8]. Biochemical markers play a critical role in the early detection, risk assessment, and monitoring of disease progression (Nazir *et al,* 2025) [18]. Two major categories of tests dominate clinical biochemistry in cardiovascular assessment: serum lipid profile, which evaluates long-term risk factors, and cardiac enzymes, which reflect acute cardiac injury. Understanding the principles, applications, and limitations of these tests is essential for accurate diagnosis and effective patient management.

2. Methods for Lipid Profile Estimation
Table 1 summarizes the common biochemical methods used in estimating the serum lipid profile and their clinical relevance. For total cholesterol, enzymatic oxidation with cholesterol oxidase is employed, producing hydrogen peroxide that reacts with a chromogen to yield a measurable color; elevated values are strongly associated with atherosclerosis risk. Triglyceride (TG) estimation involves lipase-mediated hydrolysis to glycerol, which undergoes subsequent enzymatic oxidation with hydrogen peroxide detection; high TG levels are linked to pancreatitis and metabolic syndrome. High-density lipoprotein (HDL) cholesterol is measured after precipitation of non-HDL lipoproteins or through direct homogeneous assays; HDL plays a protective role in cardiovascular disease due to its function in reverse cholesterol transport. Low-density lipoprotein (LDL) cholesterol is commonly calculated using the Friedewald formula or measured directly with enzymatic assays; elevated LDL remains a key predictor of atherogenesis and cardiovascular events.

Table 1: Common methods for lipid profile estimation

Lipid Component	Principle of Method	Clinical Significance
Total Cholesterol	Enzymatic oxidation with cholesterol oxidase → H_2O_2 formation measured with chromogen	High levels indicate risk of atherosclerosis
Triglycerides (TG)	Lipase hydrolysis → glycerol → enzymatic oxidation → H_2O_2 detection	Hypertriglyceridemia linked to pancreatitis and metabolic syndrome
HDL	Precipitation or direct homogeneous enzymatic assays	Protective against CVD (reverse cholesterol transport)
LDL	Calculated by Friedewald formula or direct enzymatic assays	Major risk factor for atherosclerosis

2.1 Total Cholesterol

- Method: Enzymatic colorimetric assays using cholesterol esterase and cholesterol oxidase are widely employed. Cholesterol is converted to cholestenone and hydrogen peroxide, the latter detected by a peroxidase reaction with a chromogen, yielding a measurable colored complex (Sabry *et al,* 2025) [22].
- Clinical Relevance: Serum cholesterol levels provide critical insight into lipid metabolism and cardiovascular health. Elevated total cholesterol is strongly associated with atherosclerosis, coronary artery disease (CAD), ischemic stroke, and peripheral vascular disease. Conversely, abnormally low cholesterol may be seen in conditions such as hyperthyroidism, malabsorption syndromes, and chronic illness. Total cholesterol, while clinically valuable, must often be interpreted alongside lipoprotein fractions (HDL, LDL, VLDL) for a comprehensive assessment of cardiovascular risk. Current guidelines emphasize total cholesterol as part of the lipid profile, guiding therapeutic decisions such as lifestyle interventions and statin therapy (Wazir *et al,* 2023) [27].

2.2 Triglycerides (TG)

- Method: Enzymatic hydrolysis by lipase produces glycerol, which is phosphorylated and oxidized to generate hydrogen peroxide (Martin *et al,* 2013) [15]. A peroxidase-catalyzed reaction then yields a chromogenic signal proportional to TG concentration (Sajja *et al,* 2021) [23].
- Clinical Relevance: Serum triglyceride measurement plays a crucial role in the assessment of metabolic and cardiovascular health. Hypertriglyceridemia is a major risk factor for acute pancreatitis, particularly when levels exceed 1000 mg/dL (11.3 mmol/L). Even moderate elevations are strongly associated with metabolic syndrome, type 2 diabetes mellitus, insulin resistance, and increased cardiovascular disease (CVD) risk (Kosmas *et al,* 2023) [12]. Elevated triglycerides often coexist with low HDL cholesterol and small, dense LDL particles, constituting an atherogenic lipid profile. Conversely, abnormally low triglyceride levels may be observed in conditions such as malnutrition, hyperthyroidism, and chronic wasting diseases. Clinical guidelines recommend routine fasting or non-fasting triglyceride testing as part of the standard lipid profile, aiding in both diagnosis and treatment monitoring (Boullart *et al,* 2012) [4].

2.3 High-Density Lipoprotein (HDL)

- Method: Precipitation methods remove non-HDL lipoproteins, followed by enzymatic measurement of cholesterol in the supernatant. Direct homogeneous assays, which selectively solubilize HDL, are increasingly common (Holzer *et al,* 2022) [9].
- Clinical Relevance: HDL is often referred to as the "good cholesterol" due to its role in reverse cholesterol transport, a process by which cholesterol is removed from peripheral tissues and delivered to the liver for excretion (Xiang *et al,* 2019) [28]. Higher HDL cholesterol levels are generally associated with a reduced risk of atherosclerotic cardiovascular disease (CVD), including coronary artery disease and ischemic stroke. Beyond cholesterol transport, HDL exhibits anti-inflammatory, antioxidant, and antithrombotic properties, contributing further to vascular protection. However, recent studies highlight that HDL functionality may be more important than absolute concentration, as dysfunctional HDL may fail to provide cardioprotective effects. Clinically, HDL measurement remains an essential component of the lipid profile, used in cardiovascular risk stratification and therapeutic decision-making (Wang *et al,* 2018) [26].

2.4 Low-Density Lipoprotein (LDL)

- Method: Calculated using the Friedewald equation (LDL = Total Cholesterol – HDL – [TG/5]) when TG < 400 mg/dL, or measured directly with homogeneous enzymatic assays (Islam *et al,* 2022) [10].
- Clinical Relevance: LDL cholesterol is the primary atherogenic lipoprotein and a central target in cardiovascular disease prevention. Elevated LDL-C levels promote endothelial dysfunction, oxidative modification of LDL particles, foam cell formation, and atherosclerotic plaque development, leading to coronary artery disease, stroke, and peripheral arterial disease. Clinical trials and meta-analyses consistently demonstrate that LDL-lowering with statins, PCSK9 inhibitors, or other lipid-lowering agents reduces cardiovascular morbidity and mortality. Current international guidelines classify LDL-C as the most important lipid parameter for risk stratification and therapeutic monitoring, with recommended target levels adjusted based on individual cardiovascular risk profiles (Mortensen & Nordestgaard, 2020; Nesti *et al*, 2020) [17, 19].

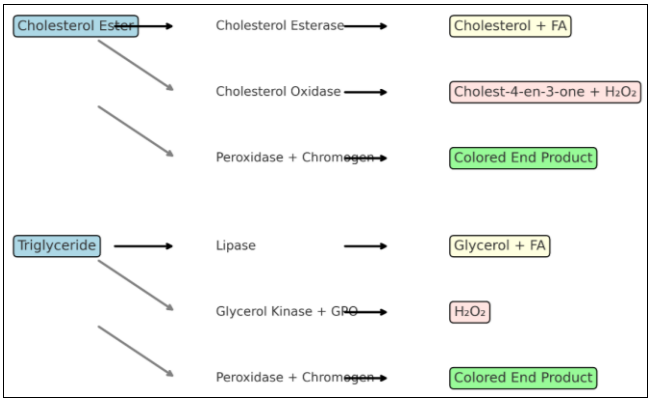

Fig 1: A schematic of enzymatic colorimetric assays for cholesterol and triglycerides (substrate → enzyme reaction → colored end product)

3. Enzymatic Assays for Cardiac Markers

Table 2 presents the major cardiac enzyme markers used in the biochemical diagnosis of myocardial infarction (MI), highlighting their analytical methods, temporal patterns of elevation after infarction, and diagnostic significance. Creatine kinase-MB (CK-MB) is measured by immune-inhibition or mass assays; it rises within 3–12 hours, peaks at 24 hours, and normalizes within 2–3 days, making it valuable for early detection but limited by false elevations due to skeletal muscle injury. Lactate dehydrogenase (LDH-1) is quantified by spectrophotometric measurement of NADH oxidation and rises later (after 24 hours), peaking at 2–3 days and remaining elevated for up to 2 weeks, which helps to diagnose late-presenting MI but lacks specificity.

Aspartate aminotransferase (AST), measured through coupled enzymatic assays, shows an intermediate pattern, rising within 6–12 hours and normalizing within 3–5 days; however, its use is restricted by nonspecific elevation in liver and muscle damage. Cardiac troponins (cTnI and cTnT), detected using high-sensitivity immunoassays, rise within 3–6 hours, peak within 12–24 hours, and remain elevated for up to 10 days, providing the highest sensitivity and specificity, and are therefore considered the current gold standard for MI diagnosis.

Table 2: Major cardiac enzyme markers and their diagnostic role

Marker	Method	Time Course Post-MI	Diagnostic Value
CK-MB	Immunoinhibition or mass assays	Rises 3–12 hrs, peaks 24 hrs, returns to normal in 48–72 hrs	Early but limited by skeletal muscle interference
LDH (LDH-1)	NADH oxidation measured spectrophotometrically	Rises 24 hrs, peaks 2–3 days, normal in 7–14 days	Useful for late MI detection but nonspecific
AST	Coupled enzymatic assays	Rises 6–12 hrs, peaks 24–36 hrs, normal in 3–5 days	Nonspecific, elevated in liver/muscle injury
Troponins (cTnI, cTnT)	High-sensitivity immunoassays	Rises 3–6 hrs, peaks 12–24 hrs, remains high 7–10 days	Gold standard for MI diagnosis

3.1 Creatine Kinase-MB (CK-MB)

- Method: Immuno-inhibition or mass assays selectively measure the MB isoenzyme (Yuan *et al,* 1982) [29].
- Diagnostic Value: CK-MB is a valuable marker for the early detection of myocardial infarction (MI). Serum levels typically begin to rise 3–12 hours after the onset of chest pain, peak at approximately 24 hours, and return to baseline within 48–72 hours. This time course makes CK-MB useful for diagnosing reinfarction, as recurrent elevation after normalization may indicate a new ischemic event. However, the diagnostic utility of CK-MB is limited by its presence in skeletal muscle, which can cause false-positive elevations in conditions such as trauma, surgery, muscular dystrophies, or intense physical exercise (PyAti *et al*, 2015; Robinson & Christenson,1999) [20, 21].

3.2 Lactate Dehydrogenase (LDH)

- Method: Spectrophotometric measurement of NADH oxidation during pyruvate-to-lactate conversion. Isoenzyme electrophoresis can distinguish cardiac-specific LDH-1 (Shetye *et al,* 2025) [25].
- Diagnostic Value: Serum LDH levels begin to rise approximately 24 hours after myocardial infarction (MI), peak at 2–3 days, and return to baseline within 7–14 days (Elsman *et al*, 2004) [6]. This prolonged elevation makes LDH useful in the retrospective diagnosis of MI, especially in patients presenting late after symptom onset. However, LDH is a nonspecific marker, as it is released from many tissues, including the liver, kidneys, red blood cells, and skeletal muscle. Consequently, LDH may also be elevated in hepatitis, hemolytic anemia, renal disease, and muscle injury, reducing its diagnostic specificity for cardiac events.

Although LDH has largely been replaced by cardiac troponins and CK-MB in modern practice, it still holds value in certain contexts, particularly in resource-limited settings where high-sensitivity troponin assays are unavailable (Beigvand *et al*, 2021) [3].

3.3 Aspartate Aminotransferase (AST)

- Method: Coupled enzymatic assays measure oxaloacetate formed during transamination, often linked to NADH oxidation (Israr *et al*, 2025) [11].
- Diagnostic Value: AST was among the first biochemical markers used for the diagnosis of myocardial infarction (MI) in the 1950s and 1960s. Serum levels rise within 6–12 hours after the onset of chest pain, peak at 24–36 hours, and return to baseline within 3–5 days. This temporal profile made AST a useful early tool for confirming myocardial injury.

However, AST is a nonspecific marker, as elevations are also seen in liver disease (e.g., hepatitis, cirrhosis), skeletal muscle injury, hemolysis, and renal disorders. Because of this lack of specificity, AST has been largely replaced by more cardiac-specific biomarkers such as CK-MB and cardiac troponins in contemporary clinical practice. Nonetheless, AST may still provide supportive information in resource-limited settings or when evaluated alongside other enzymes in a broader diagnostic panel (Lala *et al,* 2023) [13].

3.4 Cardiac Troponins (cTnI, cTnT)

- Method: High-sensitivity immunoassays detect troponin subunits released into circulation following cardiac injury (McCarthy *et al*, 2019) [16].
- Diagnostic Value: Troponins are considered the gold standard biomarkers for the diagnosis of acute myocardial infarction (AMI). Following myocardial injury, troponin levels begin to rise within 3–6 hours, peak at 12–24 hours, and remain elevated for up to 7–10 days, providing both early and late diagnostic windows. Compared to traditional markers (AST, LDH, CK-MB), troponins offer superior sensitivity and specificity, particularly for distinguishing cardiac injury from non-cardiac sources (Aydin *et al,* 2019) [2].

In addition to MI diagnosis, troponins are also clinically useful in detecting myocardial injury due to myocarditis, heart failure, pulmonary embolism, renal failure, and sepsis. Persistently elevated troponins are associated with adverse prognosis, and serial measurements are integral to risk stratification in acute coronary syndrome (ACS).

Despite their advantages, troponin testing has limitations: elevated values may be seen in non-ischemic cardiac injury or chronic disease states, and high-sensitivity assays may generate false-positive results if not interpreted in the appropriate clinical context. Nonetheless, troponins remain the central biomarker in current guidelines for MI diagnosis and management (Fathil *et al,* 2015) [7].

4. Strengths and Limitations of Biochemical Tests

4.1 Strengths

- **Early Detection**: Troponins and CK-MB enable early diagnosis of myocardial damage (Al-Hadi *et al,* 2009) [1].
- **Risk Stratification**: Lipid profile helps predict long-term cardiovascular risk (Marschner *et al*, 2001 [14]; Devarajan *et al,* 2020).
- **Quantitative Monitoring**: Enzymatic assays allow dynamic monitoring of disease progression and therapeutic response (Sawka *et al,* 2003) [24].

4.2 Limitations

- **Specificity Issues**: AST and LDH are elevated in non-cardiac conditions. CK-MB can be elevated in skeletal muscle injury.
- **Biological Variability**: Lipid levels fluctuate with diet, stress, and metabolic state.
- **Analytical Constraints**: Friedewald equation loses accuracy in hypertriglyceridemia. Immunoassays for troponins may show cross-reactivity.
- **Cost and Availability**: High-sensitivity assays may not be readily available in resource-limited settings.

Table 3 outlines the comparative strengths and limitations of major biochemical assays used in cardiovascular diagnosis. The lipid profile is quantitative, widely available, and useful for assessing long-term cardiovascular risk; however, results may be influenced by diet, fasting status, and individual biological variability. CK-MB is valuable for detecting early myocardial injury but lacks specificity as it can also rise in skeletal muscle damage. LDH retains utility for diagnosing late-presenting myocardial infarction, yet its lack of specificity—being elevated in liver disease and hemolysis—limits its reliability. AST, once used historically, is nonspecific and largely replaced by troponin assays. Troponins (cTnI, cTnT) stand out as highly sensitive and specific markers with prolonged elevation after myocardial infarction, but their use can be limited by higher cost and reduced accessibility in resource-constrained healthcare settings.

Table 3: Strengths and limitations of biochemical assays in cardiovascular diagnosis

Category	Strengths	Limitations
Lipid Profile	Quantitative, widely available, indicates long-term risk	Affected by diet, fasting state, and biological variability
CK-MB	Detects early myocardial damage	Elevated in skeletal muscle injury
LDH	Useful in delayed MI diagnosis	Non-specific (liver, hemolysis)
AST	Historically useful	Nonspecific; replaced by troponin
Troponins	Highly sensitive and specific, prolonged elevation	Costly; may not be available in resource-limited settings

5. Conclusion

The biochemical evaluation of serum lipids and cardiac enzymes remains indispensable in cardiovascular medicine. Lipid profile estimation provides long-term risk assessment, while enzymatic assays for cardiac markers, particularly troponins, facilitate accurate and timely diagnosis of acute myocardial infarction. Despite inherent limitations, the integration of these tests with clinical evaluation and imaging enhances diagnostic accuracy and improves patient outcomes. Continued refinement of biochemical methods promises even greater precision and accessibility in cardiovascular diagnostics.

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