Using a Plate Reader for the Cholesterol Oxidase Assay

Prep Notes

Cholesterol (µg/well)	Cholesterol Stock (µl)	H_2O (µl)	Final volume	Patient Samples	Absorbance at 500 nm (y-axis)	Cholesterol Concentration (µg/mL)
30	24	56	80 µl			
25	20	60	80 µl			
20	16	64	80 µl			
15	12	68	80 µl			
10	8	72	80 µl			
5	4	76	80 µl			
2.5	2	78	80 µl			
0	0	80	80 µl			
				Patient Sample I		
				Patient Sample 2		
				Patient Sample 3		

Patient sample prep:
20 µL sample + 60 µL H_2O (use 20 µL per well)

Reaction mixture:
For 180 µL/well * 75 wells (slightly more than what is needed for 2 plates).

7.5 mL enzyme
1.5 mL KI
1.5 mL enhancer
1.5 mL developer
1.5 mL H_2O

Student Handout

Cholesterol is a complex lipid essential to all animal cells. Its primary function is as a stabilizing, structural component of cell plasma and organelle membranes. It is also a precursor for steroid hormones. The structure of cholesterol and 2 of its derivatives, shown in Figure 1, demonstrates the four ring backbone found in steroids such as testosterone and progesterone. Cholesterol is also a precursor for vitamin D and for bile salts, which facilitate the digestion of lipids in the intestine.

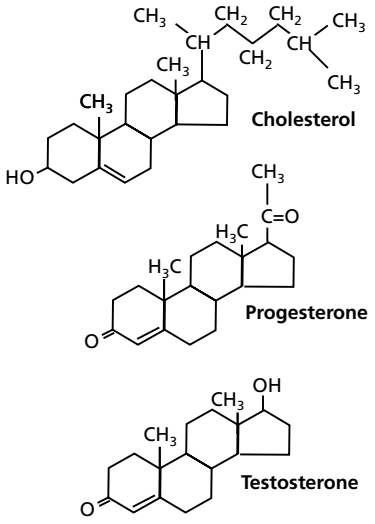

Figure 1: Chemical structure of cholesterol.

Cholesterol is synthesized in the liver and is absorbed from dietary sources. It is circulated in body fluids in spherical bodies known as lipoprotein particles. These lipoproteins are classified according to their density during centrifugation. Cholesterol is processed by the liver and packaged into particles known as very low-density lipoproteins, which are then processed in the circulation to form low-density lipoproteins (LDL) (Figure 2). Most circulating cholesterol is found in LDL and is destined for the periphery. It is often referred to as "bad cholesterol" (LDL: L = lousy). High-density lipoproteins (HDL) take up cholesterol from LDL and peripheral tissues and transport it back to the liver for repackaging or excretion (Figure 2). Because HDL takes cholesterol out of the circulation, cholesterol found in these particles is often referred to as "good cholesterol" (HDL: H = healthy).

While cholesterol is essential for life, excess serum cholesterol can have serious negative consequences. The role of elevated blood cholesterol in cardiovascular disease is well established. Arterial cholesterol accumulation in occlusions known as "plaques" eventually leads to blood flow blockage, resulting in heart attack or stroke Coronary heart disease is the leading cause of death in the United States and claims 512,000 lives every year. Stroke is the third leading cause of death at 150,000 per year. Over 30 billion dollars in medical costs are spent on stroke alone.

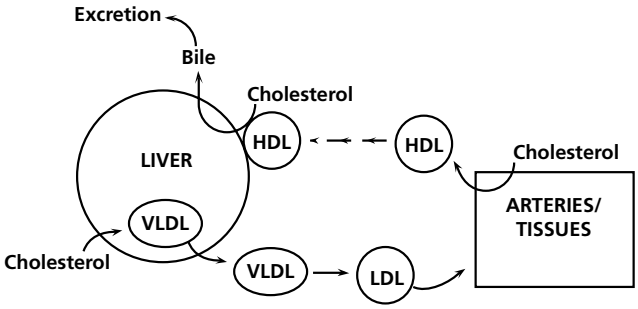

Figure 2: Cholesterol metabolism.

Due to the correlation between cardiovascular disease and elevated blood cholesterol, serum cholesterol levels are now determined routinely, both in clinical laboratory tests and, more recently, in home tests. Elevated levels indicate the need for cholesterol reduction either by diet, or, if necessary, medication. To measure blood cholesterol levels, a chemical and an enzymatic assay have been used. The chemical method, which is quite laborious, has largely been replaced by the enzymatic method, which is quite rapid and simple.

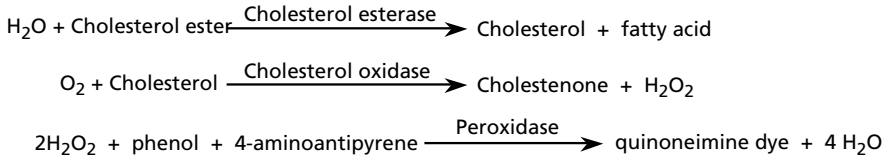

Figure 3: Cholesterol oxidase assay.

In this assay, outlined in Fig. 3, cholesterol esterase is used to reduce cholesterol ester to cholesterol. Cholesterol is then oxidized by cholesterol oxidase to produce cholestenone plus hydrogen peroxide. The hydrogen peroxide then acts as a substrate for peroxidase to produce water plus oxygen, which reacts with the detection reagents to form a color compound. By using density gradient centrifugation, the fractions of total cholesterol contained in LDL ("bad") or HDL ("good") can be determined. Total cholesterol levels greater than 200 mg/100 mL or LDL levels greater than 130 mg/100 mL are considered high risk factors for the development of cardiovascular disease. The levels of another lipid, known as triglyceride, which has also been associated with increased risk of cardiovascular disease, are also usually determined.

The goal of this lab is to utilize the cholesterol oxidase assay to determine the total cholesterol levels in 3 patient samples.

Student Protocol

1. Prepare cholesterol standards from the tube marked "std". The cholesterol standard solution is supplied at a concentration of 5.0 µg/µL.
2. Dilute the standard to the concentrations listed in the table below
3. Pipet 20 µL of each patient sample or standard into the corresponding wells in a 96 well plate (see layout).
4. Add 180 µL of assay solution to each well using a multichannel pipettor
5. Cover the plates with a piece of clear film and Incubate for 15 minutes at 37°C
6. Remove samples from the incubator, remove the film, and measure absorbance at 500 nm in the plate reader.
7. Using the known cholesterol concentrations as x values and absorbance as y values use Google sheets or Excel to create a graph.
8. Generate a trendline for the standard curve (it should go through the intercept at 0), making sure to include the equation and the R2 value on the graph. Use the equation of the line along with the absorbance values of the unknowns to calculate the cholesterol concentration of each patient sample.
9. Complete the table and page 4 and submit a copy of your graph and your table along with the answer to the following questions on Nexus:
    a. Cholesterol is typically reported in mg/100 mL (or mg/dL). What are the concentrations of cholesterol in your patients in mg/dL? Are any of your patients suffering from hypercholesterolemia?
    b. Describe the differences between LDL and HDL.
    c. Does it make any difference (to one's health) whether cholesterol is found in LDL or HDL? Does this assay distinguish between LDL and HDL?
    d. Why is it important to avoid having high blood cholesterol levels? What are some possible causes of high cholesterol levels?

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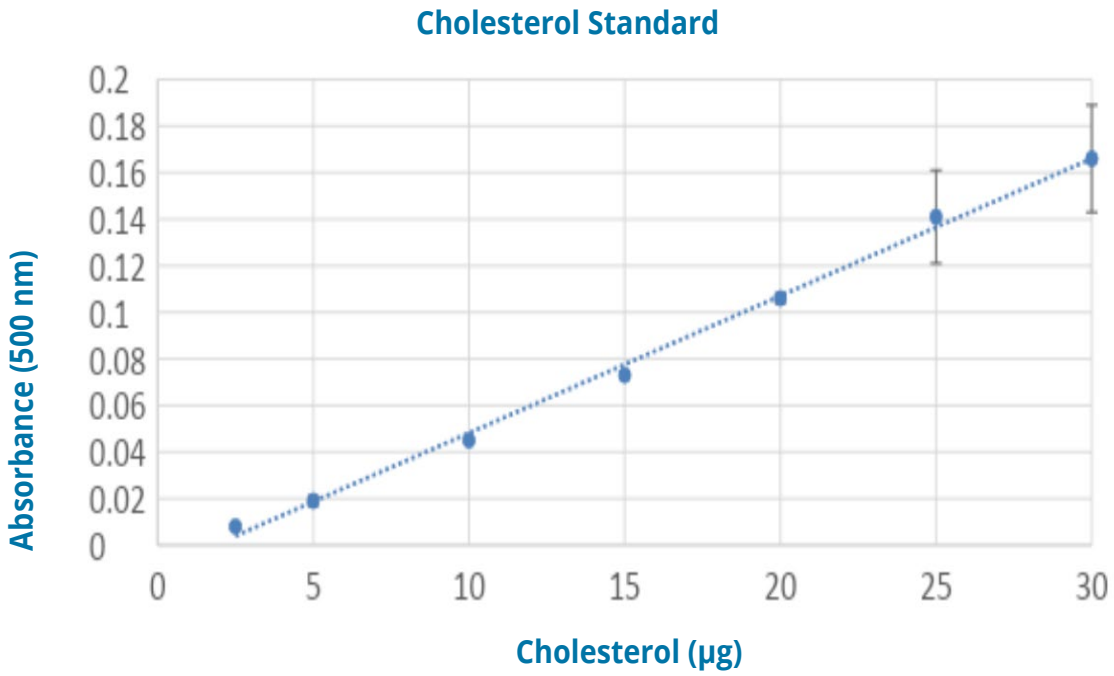
				Patient Sample I Patient Sample 2 Patient Sample 3		

Sample Data

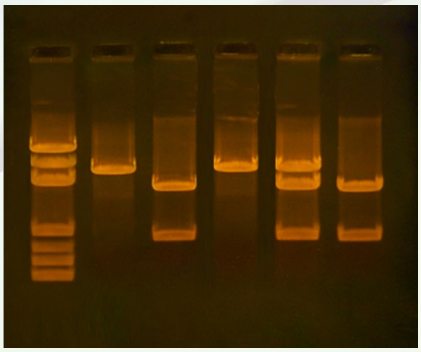


Patient Sample	Cholesterol (µg/5 µL)	Std.Dev.	cholesterol (mg/dL)
1	3.02	0.28	60.49
2	10.06	0.39	201.23
3	21.30	2.18	425.93

RELATED EXPERIMENT

Cat. #316

In Search of the Cholesterol Gene



For 10 Lab Groups. Coronary heart disease and stroke are major causes of death in the Western world. Elevated blood cholesterol levels are a serious risk factor for both conditions. The genetic disease familial hypercholesterolemia (FH) causes an increase in blood levels of the "bad" form of cholesterol, low-density lipoprotein (LDL). In untreated patients with the mutant FH gene, the condition can cause premature death. This experiment includes reagents for the colorimetric enzymatic reaction which is the basis of the clinical cholesterol test. In addition, using agarose gel electrophoresis, students will analyze a simulated genetic screening for a disease. CLICK HERE FOR MORE INFO.