



## Clinical Biochemistry

### Lab. 9

### Measurement of Cholesterol

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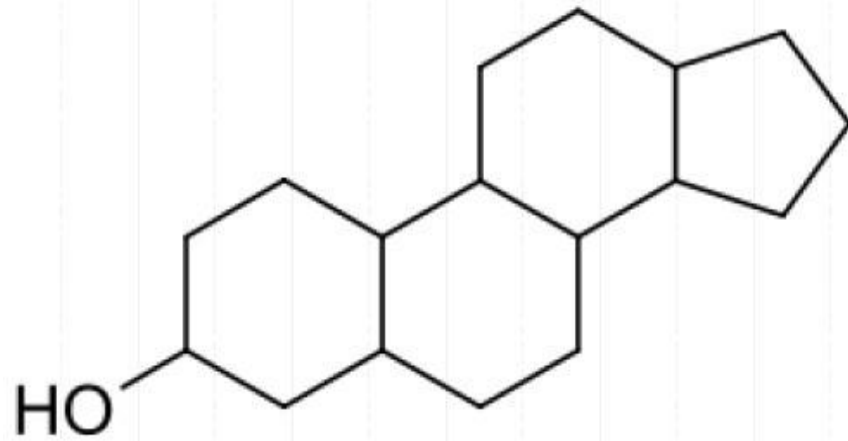
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# Introduction



## Sterol:



Cyclopentanoperhydrophenanthrene ring

## Sterols

📌 Phytosterols

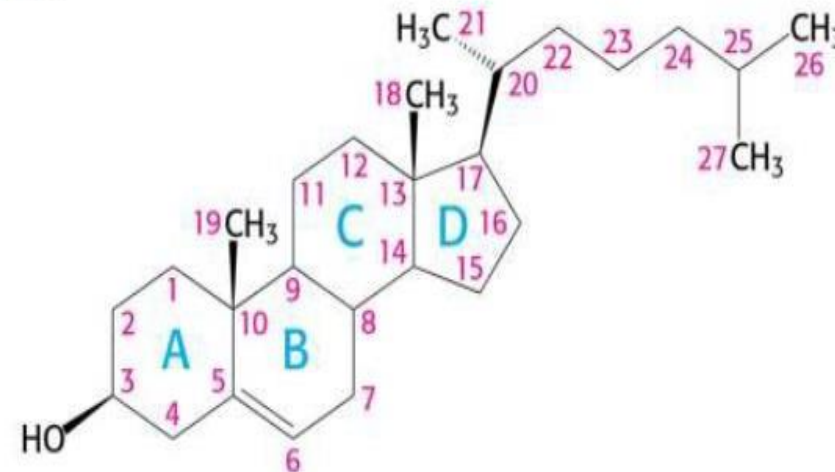
- Sitosterol
- Stigmasterol

📌 Zoosterols

**Cholesterol**

📌 Ergosterol

- Fungi
- Protozoa



# Cholesterol Imbalance



## • Hypercholesterolemia

1. Hypothyroidism
2. Nephrotic syndrome
3. Cholestasis
4. Familial hypercholesterolaemia

## • Hypocholesterolemia

1. Cancer
2. Hyperthyroidism

# Measurement of Cholesterol in diagnosis

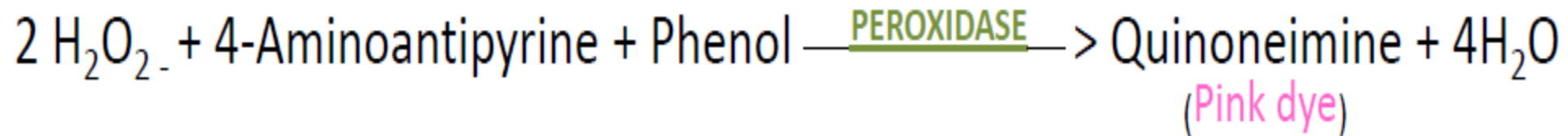


- Cholesterol testing evaluates the risk for:
  - ✓ **arthrosclerosis**
  - ✓ **coronary heart disease (CHD)**
- Cholesterol determinations are also frequently a part of :
  - **thyroid function**
  - **liver function**
  - **diabetes mellitus studies.**
- It is also used to monitor effectiveness of **diet, medications, lifestyle changes (e.g., exercise), and stress management.**

# Principle of Total Cholesterol Measurement



The enzymatic reaction sequence employed in the assay of cholesterol is as follows:



# Principle



- **Cholesterol Esters** are hydrolyzed to produce **cholesterol**,
- **Hydrogen peroxide** is then produced from the oxidation of cholesterol by **cholesterol oxidase**.
- In a coupled reaction catalyzed by **peroxidase**, quinoneimine pink colored dye is formed from **4-aminoantipyrine, phenol and hydrogen peroxide**.
- The absorption of **light at 505 nm** of the solution of pink dye is **proportional to the concentration of cholesterol in the sample**.

# Introduction



## REAGENTS

R1	CHOLESTEROL CHOD PAP	Buffer
Phosphate buffer	100	mmol/L
Chloro-4-phenol	5	mmol/L
Sodium Cholate	2.3	mmol/L
Preservative		

According to 1272/2008 regulation, this reagent is not classified as dangerous

R2	CHOLESTEROL CHOD PAP	Enzymes
Cholesterol oxidase (CO)	$\geq 100$	IU/L
Cholesterol esterase (CE)	$\geq 170$	IU/L
Peroxidase (POD)	$\geq 1200$	IU/L
4 - Amino – antipyrine (PAP)	0.25	mmol/L
PEG 6000	167	$\mu\text{mol/L}$

According to 1272/2008 regulation, this reagent is not classified as dangerous

R3	CHOLESTEROL CHOD PAP	Standard
Cholesterol	200 mg/dL (5.17 mmol/L)	

Attention Danger

## REAGENTS PREPARATION

Use a non-sharp instrument to remove aluminum cap.

Add promptly the content of vial R2 into vial R1.

Mix gently until complete dissolution.

Vial R3: Ready to use

# Procedure



## PROCEDURE

### Manual method:

Let stand reagent and specimens at room temperature.

Reagent	1000 $\mu$ L
Blank, Standard, Control or specimen	10 $\mu$ L

Mix. Let stand for 10 minutes at room temperature or 5 minutes at 37°C.  
Record absorbances at 500 nm (480-520) against reagent blank.  
Color is stable for 1 hour.

- 1- Performances with manual procedure should be validated by user.
- 2- Kenza applications and other applications proposal are available on request.

## CALCULATION

### Manual Procedure:

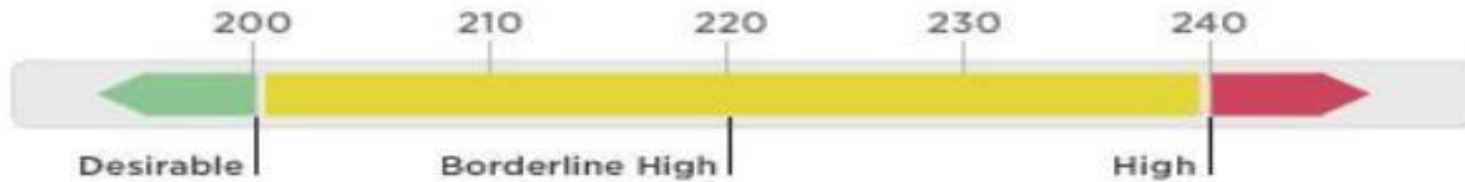
$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{Standard concentration}$$



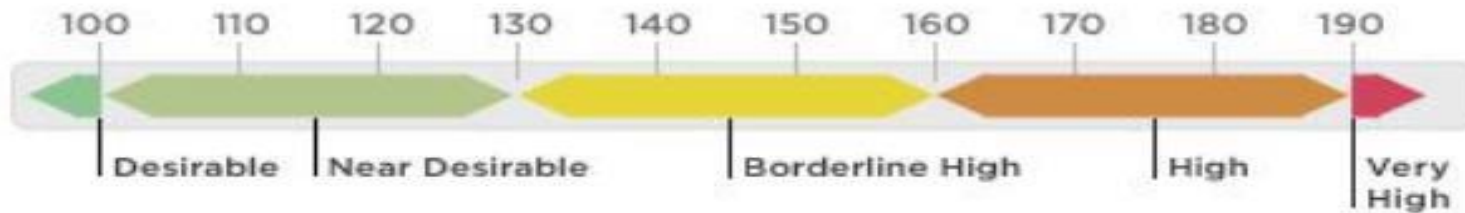
# Expected Values



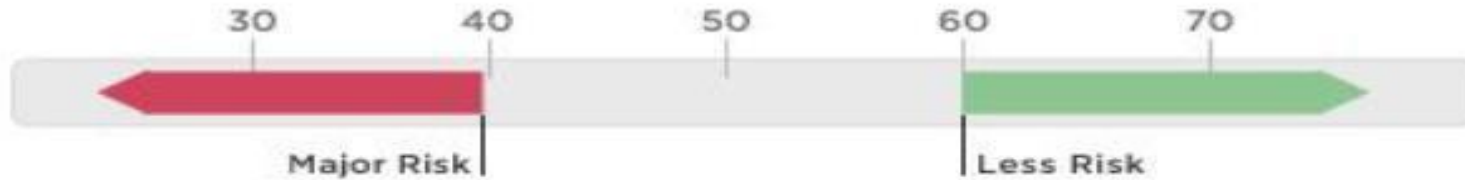
## TOTAL CHOLESTEROL LEVEL (in mg/dl)



## LDL CHOLESTEROL LEVEL (in mg/dl)



## HDL CHOLESTEROL LEVEL (in mg/dl)



Values for adults, in term of risk for atherosclerotic diseases:

Total cholesterol	mg/dL	[ mmol/L ]
Recommended values	< 200	[ < 5.18 ]
Low risk	200-239	[ 5.18-6.19 ]
High risk	≥ 240	[ ≥ 6.22 ]