

WELCOME

TO



FDSPharmacy

Learn and Educate

This is an Education Platform

We provide Free PDF Notes and Videos Classes for Pharmacy Students

Web Site <http://www.fdspharmacy.in/>

You tube <https://www.youtube.com/channel/UC77iEsiuZolU4pB8WAJIR5Q>

What app <https://chat.whatsapp.com/IzSgXtFEvhS4LN5xhUgq5z>

Telegram <https://t.me/+cvxm17xSloA4MjVI>

Face book <https://www.facebook.com/Fdspharmacy-105764311994440/>

E-mail fdspharmacyinfo@gmail.com

Diploma in Pharmacy 2nd Year

Biochemistry & Clinical Pathology

Experiment

To determine the cholesterol in blood/serum.

Aim:

To determine the cholesterol in blood/serum.

Reference :

‘ Dr. Gupta G.D. , Dr. Sharma Shailesh, Kaur Manpreet, “Practical Manual of Biochemistry & Clinical Pathology” Published by Nirali Prakashan, Page no 44 – 48

Materials Required

Absolute alcohol, Diethyl ether, Chloroform, Acetic anhydride, Standard solution of cholesterol (100 mg%), test tube and stir rod.

Theory :

The blood contains both esters and free cholesterol. Normally, the ester fraction forms 70% of the total cholesterol while free cholesterol forms roughly 30%. An alcohol-ether mixture is used to extract the cholesterol and cholesterol esters from the serum (Alcohol precipitates the proteins or either solubilises the cholesterol part). The contents are centrifuged. The protein free extract is dried using evaporation. Using the Liebermann-Burchard procedure, the cholesterol residue is dissolved in chloroform and colorimetrically quantified.

Reagent Preparation

100 mg of cholesterol should be dissolved in some amount of alcohol. It should be slightly warmed (in water bath), if needed. The volume should be maintained to 100 ml. with alcohol.

Procedure

1) Test:

- i. 8mL of alcohol and 2ml. of ether should be taken in a centrifuge tube and then mixed.
- ii. 0.2mL of blood should be added and mixed. It should be kept in slanting position for half an hour and centrifuged
- iii. The supernatant should be collected (which contains cholesterol) in another tube. This test tube should be kept in boiling water for the evaporation of solvent and the residue, ie, leaving behind a residue of cholesterol that adheres to the bottom of the flask.
- iv. The best quality chloroform, acetic anhydride, and sulphuric acid should be used. Chloroform needs to be extremely anhydrous. The colour produced by regular chloroform or outdated stock will be weak and unpredictable.

2) Standard:

- i. 0.2, 0.4, 0.6 and 0.8ml. of standard cholesterol solution should be added to the labelled tubes, i.e., S₁, S₂, S₃, and S₄
- ii. All test tubes should be stored in a hot water bath to allow the solvent to evaporate and leave any residue behind.
- iii. The blank test tube should be cleaned and dried.
- iv. The following reagents should now be added in test, different standard, and blank.

Reagent	Blank	Standard				Test
		S ₁	S ₂	S ₃	S ₄	
Standard cholesterol solution	-	0.2mL	0.4mL	0.6mL	0.8mL	Test sample
CHCl ₃	5mL	5mL	5mL	5mL	5mL	5mL
Acetic anhydride	2mL	2mL	2mL	2mL	2mL	2mL
Concentrated H ₂ SO ₄	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL

- v. It should be mixed well and kept in dark for 10 minutes.
- vi. The optical density should be read at 610nm.

Calculations

Optical density of test =

- 1) Optical density of standard S_1 = concentration = 0.02 mg
- 2) Optical density of standard S_1 = concentration = 0.04 mg
- 3) Optical density of standard S_1 = concentration = 0.06 mg
- 4) Optical density of standard S_1 , concentration = 0.08 mg

$$\text{The concentration of cholesterol} = \frac{\text{Optical density of test}}{\text{Optical density of std.}} \times \frac{\text{Conc of std.}}{\text{Vol. of blood/serum used}} \times 100$$

Interpretation

- 1) Normal serum cholesterol level is 150-250 mg/dl.
- 2) The normal level of serum cholesterol is 150-250 mg/dl.
- 3) Following conditions are caused due to hypercholesterolemia:
 - i) Diabetes mellitus
 - ii) Obstructive jaundice
 - iii) Nephrotic syndrome.
 - iv) Cirrhosis of liver
 - v) Hypoparathyroidism.
 - vi) Xanthomatosis.
- 4) Following conditions are caused due to hypocholesterolemia:
 - i) Hyperthyroidism
 - ii) Pernicious anaemia
 - iii) Haemolytic anaemia
 - iv) Malabsorption syndrome

Result :

Cholesterol concentration in blood /serum was determined.

Hello

Friends

**If you Get Any Help From This Notes /
Videos**

Next You Turn To Help Us

Please Contribute Some Amount

To Our

FDSPharmacy Team

Phone Pe 6398439940

Paytm 6398439940

Google Pay 6398439940



Amir Khan

Thank You

Keep Supporting