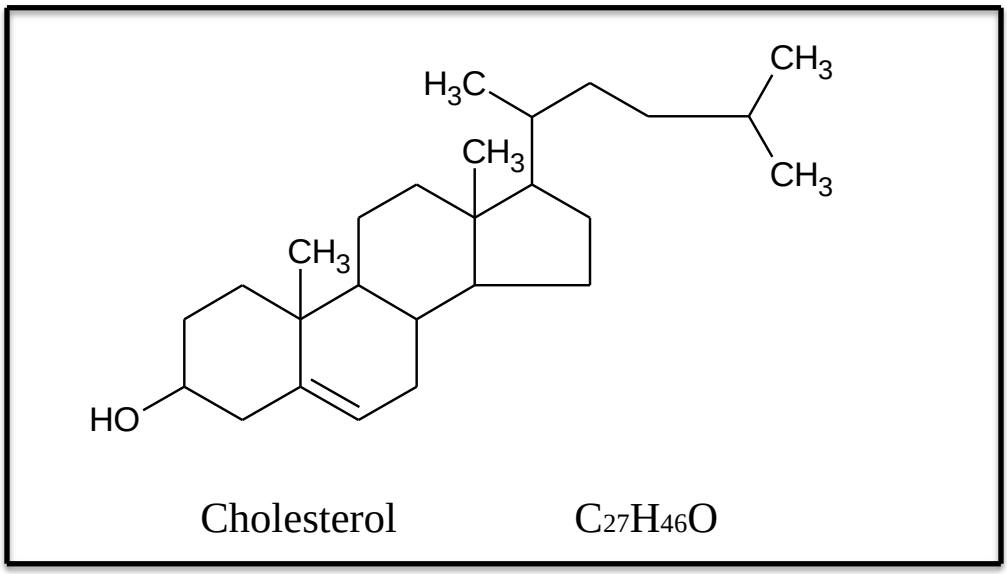
3- Qualitative tests of cholesterol:

Cholesterol is a steroid lipid built from four linked hydrocarbon rings. It is an amphipathic molecule, with a polar head group (the hydroxyl group at C-3) and nonpolar hydrocarbons body (the steroid nucleus and the hydrocarbon side chain at C-17), about as long as a 16- carbon fatty acid in its extended form.



Cholesterol $C_{27}H_{46}O$

When steroids that contain unsaturated bonds are treated in non-aqueous conditions with strong acids, they interact yield distinct color outputs depending on the conditions of the experiment, the resulting colors show significant differences from one compound to another.

There are the tests used to detect cholesterol:

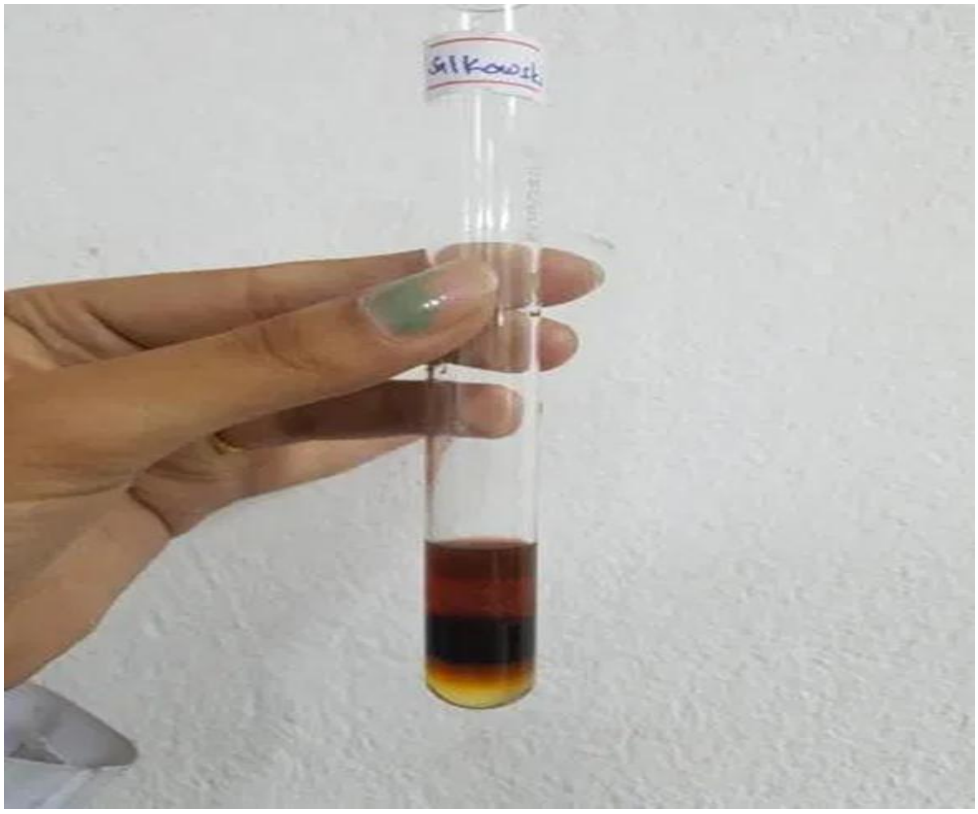
1- *Salkowaki test* :

Salkowski test is used to detect cholesterol in a solution. It is an important test used to detect cholesterol depending on the colors (distinct and clear colors) that yield from the reaction of cholesterol with concentrated sulfuric acid.

Method:

1- One milliliter of cholesterol is added to a test tube and then the same volume of H_2SO_4 is added and shaken well.

2- The tube is allowed to stand until the mixture separates sharply into two layers; the top layer is red and the bottom layer is green.



Caution: In order for this test to be successful, the tubes must be dry and the solutions should be non-aqueous.

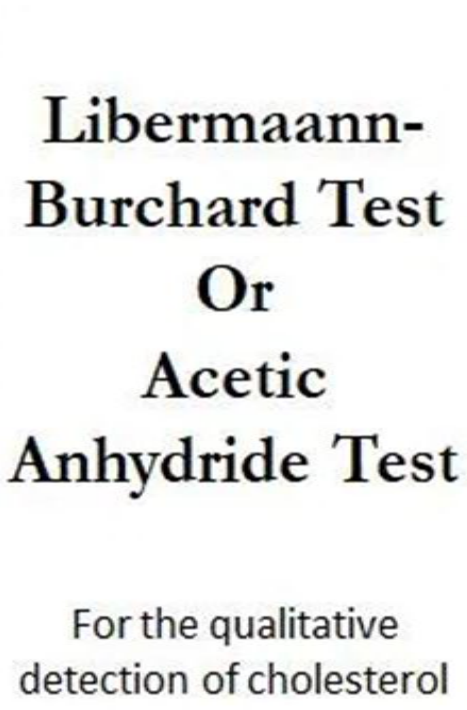
2 - *Liberman – Burchards test :*

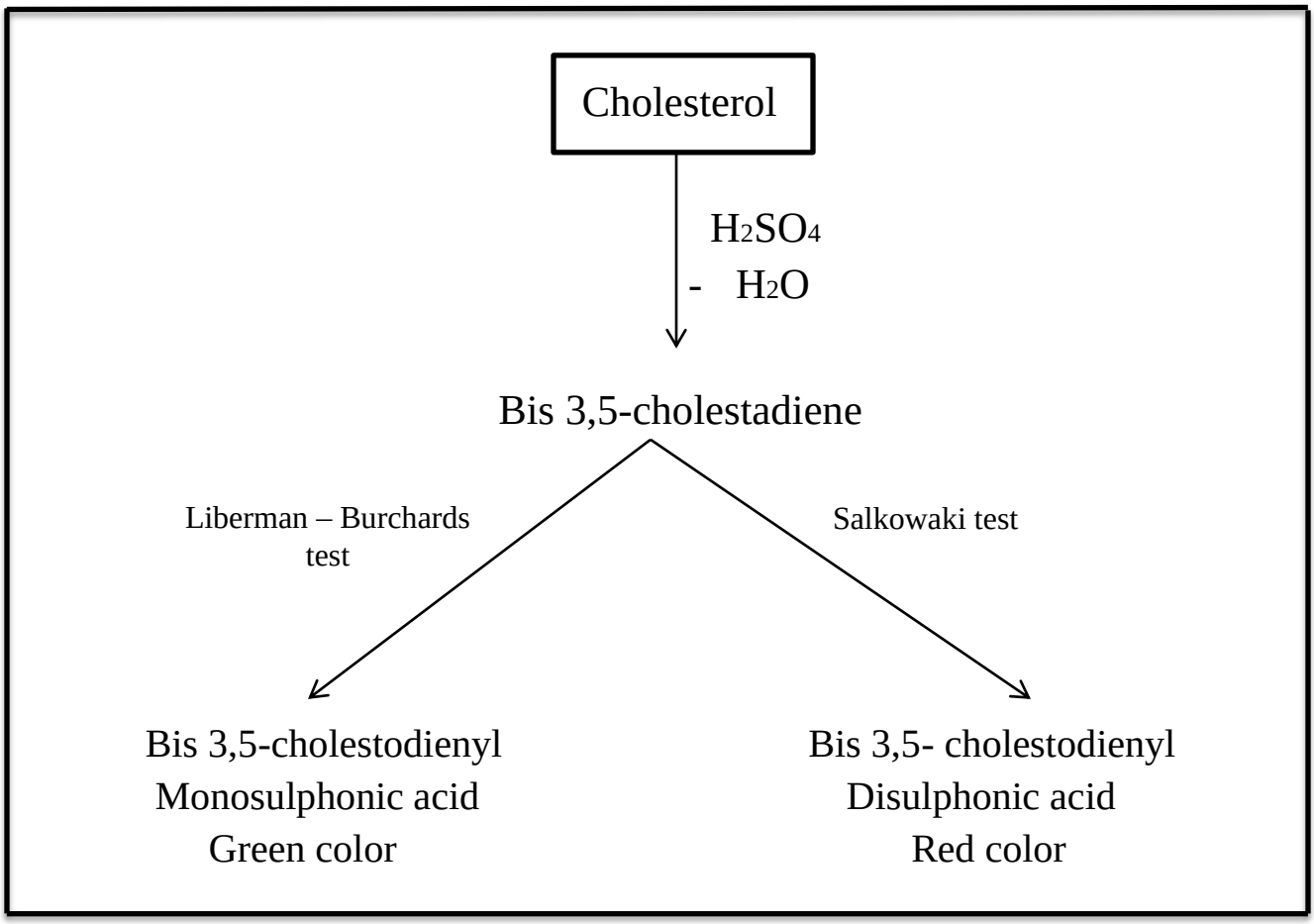
Liebermann – Burchard test is a chemical estimation of cholesterol. The cholesterol is react as a typical alcohol with a strong concentrated acids to give colored substances. In this test acetic anhydride are used as solvent and dehydrating agents, and the sulfuric acid is used as dehydrating and oxidizing agent. After adding these acids to the cholesterol solution a result is observed when the solution becomes red or pink, then purple, blue and finally bluish – green colour.

Method :

1- In a clean and dry test tube, 1 ml of 5% cholesterol dissolved in chloroform is added to 1 ml of acetic anhydride., then 2 drops of H_2SO_4 are added to the same tube and shaken well.

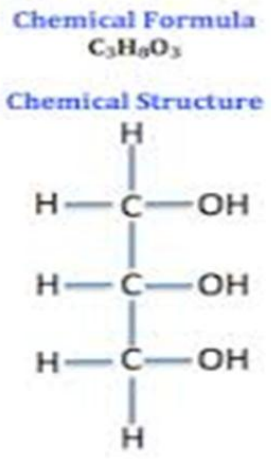
2- The color change is noticed (Observe the appearance of pink color which gradually turns into deep green).





4-Acrolin test:

Acrolin test is a general test for the presence of glycerin or fats. It is an important test for glycerol where in the presence of a dehydrating agent potassium bisulfate ($KHSO_4$); the glycerol part of the molecule is dehydrated and formed a volatile substance with a smell that is similar to the smell of burned fat (unsaturated aldehyde) that called Acrolin. This test is distinctive for glycerol whether it is free or combined with fatty acids.



Method :

1- In a clean and dry test tube, 0.5 ml of glycerol is added with 0.5ml of $KHSO_4$.
2- The tube is heated and the change of the solution in the tube is noticed.