Anticoagulants

It is a substance that prevents [coagulation](http://en.wikipedia.org/wiki/Blood_coagulation) (clotting) of blood. Some anticoagulants are used in medical equipment, such as [test tubes](http://en.wikipedia.org/wiki/Test_tube), [blood transfusion](http://en.wikipedia.org/wiki/Blood_transfusion) bags, and [renal dialysis](http://en.wikipedia.org/wiki/Renal_dialysis) equipment.

There are different types of anticoagulants are.

1- Ethylene diamine tetra-acetic acid (EDTA)

EDTA is the anticoagulant of choice for most hematological analyses. It prevents clotting by chelates calcium ions to form a soluble complex. This anticoagulant causes a minimum of distortion to the cells and platelets.  
2- Double oxalate

Oxalates are able to precipitate calcium from the blood.  The most commonly used oxalate is potassium oxalate, which is often used to provide plasma for glucose testing.

3- Trisodium citrate

Citrates are able to prevent coagulation by binding calcium and forming a soluble calcium citrate complex.  Sodium citrate is used for coagulation tests because it is very effective at preserving the coagulation factors of the blood, which are performed on plasma.

4- Heparin

Heparin does not alter the size of cellular components. It is prevents coagulation by stops the formation of thrombin from prothrombin therefore stopping formation of fibrin from fibrinogen. It's more expensive and not readily dissolves.

5- Sodium fluoride

Sodium citrate is the anticoagulant of choice for coagulation studies. It is used in a concentration of 1 part sodium citrate to 9 parts whole blood. It prevents coagulation by binding the calcium of the blood in a soluble complex.

6- Acid citrate dextrose (ACD)  
  
Hematological tests

1- Hemoglobin estimation

Hemoglobin is a protein in [red blood cells (RBCs)](http://lungcancer.about.com/od/glossary/g/redbloodcells.htm) that carries oxygen from the lungs to the tissues in the body. The pigment in hemoglobin is responsible for the red color of blood.

Methods of hemoglobin estimation

1- Gasometric methods (estimation of oxygen binds to hemoglobin).

2- Chemical methods (estimation of iron contents of Hb)

3- Colorimetric methods

a. Visual (Sahli's Method)   
b. Cyanomethmoglobin ( Drabkin’s method)

### Hemoglobin estimation by Sahli's method

Principle

The Sahli method is based on converting hemoglobin to acid hematin and then visually matching its color against a solid glass standard. Dilute hydrochloric acid is added to a graduated cylinder containing a blood sample until the color of the diluted blood sample matches that of the glass standard. The quantity of dilute acid added will be determined by the hemoglobin level of the blood sample.

Materials used

1- Sahli hemoglobinometer (solid glass standard and a calibrated graduated cylinder)

2- Sahli blood pipette (calibrated to 0.02 ml)

3- Small glass rod for stirring or wooden applicator sticks if glass rods are not available

4- Dropper for adding the hydrochloric acid

5- Dilute 0.1 M hydrochloric acid (0.1 N)

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Test Procedures

1. Fill the Sahli graduated cylinder to the 2 mark with dilute 0.1 M hydrochloric acid (approximately 0.15 ml).

2. Clean the fingertip with cotton wool soaked with 70% alcohol. Allow alcohol to dry. Obtain a drop of blood by puncturing the fingertip with a sterile lancet. Wipe away the first drop of blood.

3. Draw the blood to the 0.02 ml mark using the Sahli blood pipette. Do not pipette by mouth .

4. Wipe any residual blood from the exterior of the pipette. Recheck that the blood still reaches the 0.02 ml mark.

5. Add the blood to the dilute acid. Mix the blood and acid thoroughly by flushing the pipette several times .

6. Allow the acid/blood mixture to stand for five minutes.

7. Place the tube into the tube holder of the colored scale.

8. Hold the scale up to the light.

9. If the color of the solution is the same or lighter than that of the colored standard, the hemoglobin level is 4 g/dl or less.

**If the color of the solution is darker than the colored standard, continue to add dilute acid drop by drop. Stir the solution with the glass rod after each drop is added, and compare the solution to the colored glass standard.**

10. Keep adding the acid until the color of the solution matches the color of the glass standard (*Figure 35*). Hold the scale up to a window when assessing the color match.

11. Once the colors match, hold the instrument at eye level and record the value of percent hemoglobin indicated on the side of the tube by the level of fluid.

Advantages

• Instrument and reagents are inexpensive.

• Test is easy to perform.

• Electricity is not required.

Disadvantages

• Color matching is subjective.

• The color of the glass standard is not a true match for the color of diluted blood.

• Color of the standard will fade with time and needs periodic calibration to determine the correction factor.

• It is easy to add too much dilute hydrochloric acid during the color comparison process.

• Acid hematin is not a stable compound and readings must be taken within the recommended time interval.

• After prolonged use, the numbers on the graduated cylinder fade and are difficult to read.

### Hemoglobin estimation by Drabkin’s method

### Principle

In an alkaline medium, potassium ferricyanide oxidizes hemoglobin and its derivatives to methemoglobin. Methemoglobin reacts with potassium cyanide to form cyanmethemoglobin, which has maximum absorption at 540 nm. The color intensity measured at 540 nm is proportional to the total hemoglobin concentration.

Drabkin’s solution

Sodium bicarbonate - 1 gm

Potassium cyanide - 0.2 gm

Potassium ferricyanide - 0.2 gm

Distilled water (made upto 100 ml)

Procedure (Manual)

1. Dispense 5.0 ml of total hemoglobin reagent (Drabkin’s solution) into test tubes labeled “blank”, “Standard”, “patient”, etc.

2. Place 0.02 ml (20µl) of standard, control and sample into respective tubes, then mix

3. Allow all tubes to stand for 10 minutes at room temperature.

4. Set spectrophotometer to 540 nm (520-560 nm) and zero with the reagent blank.

5. Read and record absorbance values of all tubes.

6. See “Calculations” to obtain values.

Calculations

Abs. = Absorbance

Abs. of Unknown × Concentration of = Hemoglobin (g/dl)

Abs. of Standard Standard (g/dl)

Example: If a 15 g/dl standard has an absorbance of 0.406 and the absorbance of the unknown is 0.350 then:

0.350 x 15.0 = 12.9 g/dl

0.406

Normal levels are:

Women: 11.5 - 16.5 g/dl

Men: 13.0 - 18.0 g/dl

Children: 11 - 16 g/dl

Pregnant women: 11 - 12 g/dl