Hemoglobin: ´ is the protein molecule in red blood cells that carries oxygen from the lungs to the body's tissues and returns carbon dioxide from the tissues back to the lungs.Structure ofHb: ´ Each hemoglobin molecule is a tetramer made of four polypeptide globin chains. ´ Each globin subunit contains a heme moiety formed of an organic porphyrin ring and a central iron ion in the ferrous state (Fe2+).´ 4 globin polypeptide chains composed of amino acids (a.a). ´ Alpha , beta chains with gamma and delta being less often. ´ Each polypeptide chain is composed of 141–146 amino acids. ´ The absence, replacement or addition of only one a.a modifies the property of the hemoglobin. ´ The normal adult hemoglobin molecule contains two alpha–globin chains and two beta–globin chains. ´ In fetuses and infants, beta chains are not common and the hemoglobin molecule is made up of two alpha chains and two gamma chains. ´ As the infant grows, the gamma chains are gradually replaced by beta chains, forming the adult hemoglobin structure.Types ofHemoglobin: ´ Adult haemoglobin: ´ Hb A(a b) : the two types of polypeptide chain are called (alpha) chains each of which contains 141 amino acid residues and the beta chains each of which contains 146 amino acid resides. 2 2 ´ Not all hemoglobin in the blood of normal adult is hemoglobin A. ´ Hb A2(a d) : consists of two alpha and two delta chains and is found at low levels in normal human blood. 2 2 ´ About 2.5% of the hemoglobin is hemoglobin A2. ´ In which beta chain are replaced by delta chain. ´ the delta chains also contain 146 amino acids residues but 10 individual residues differ from those in the beta chains. ´ Hemoglobin A2 may be increased in beta thalassemia and in people with Sickle–cell diseaseHemoglobinA1c(HbA1c): ´ Glycated hemoglobin test, or glycohemoglobin, is an important blood test that provides an average of blood sugar control over the past 2 to 3 months. ´When diabetes is not controlled (meaning that sugar is too high), sugar builds up in blood and combines with hemoglobin, becoming "glycated. Has a glucose attached to terminal valine in each beta chain and is of special interest because the quantity in the blood increases in poorly controlled diabetes mellitus, hemoglobin A1c test will be higher.HemoglobinA1c(HbA1c): ´ Normal range for the hemoglobin A1c test is between 4% and 5.6%. ´ Hemoglobin A1c levels between 5.7% and 6.4% indicate increased risk of diabetes. ´ Levels of 6.5% or higher indicate diabetes.Fetal hemoglobin HbF (a g2)2 ´ Its structure is similar to that of hemoglobin A except that beta chains are replaced by gamma chains. ´ The gamma chains also contain 146 a.a residues but have 37 that differ from those in the beta chain. ´ Fetal hemoglobin is normally replaced by adult hemoglobin soon after birth.Hemoglobin S: ´ The alpha chain are normal but the beta chains are abnormal because among the 146 a.a residues in each beta polypeptide chain ,one glutamic acid residue has been replaced by a valine residue. ´ Hemoglobin S has a slight change in the coding for the beta chain in adult haemoglobin and this causes sickle cell anaemia.Reactions ofhemoglobin : ´ Oxyhemoglobin that is carrying oxygen ,it is bright red. ´ Hemoglobin binds to oxygen to form oxyhemoglobin ,oxygen bind to Fe+2 in the heme. ´When Hb with CO2 give carbaminohemoglobin. ´CO reacts with Hb to form carboxyhemoglobin (Hbco). ´ The affinity of hemoglobin for O2 is much lower than its affinity for CO, which consequently displaces O2 on Hb and reducing oxygen carrying capacity of blood.Carbonemonoxide (Co) poisoning ´ Is often listed as form of anemic hypoxia because the amount ofHb that carry oxygen is reduced but the total Hb content of the blood is unaffected by Co. ´ The affinity of Hb for Co is 210 times its affinity for O2 and Carboxy hemoglobin (CoHb) liberate Co very slowly. ´ The chery–red color of CoHb is visible in skin , nail and

mucous membrane.Methemoglobin: ´ Can be genetic or when blood is exposed to various drugs and other oxidizing agents in vitro and in vivo. ´ The ferrous iron Fe+2 that is normally in the molecule is converted to ferric Fe+3 forming methemoglobin. ´ methemoglobin is dark bluish colored and when it is present in large quantities in the circulation it cause a dusky discoloration of the skin resembling cyanosis.The normal concentration ofHb in the blood: ´ 12–15.5 gm / dL in women. ´ 13.5–17.5 gm/ dL in men.Decreased levels ofhaemoglobin are found in: ´ Anemia. ´ After severe hemorrhage. ´ Cancers that affect the bone marrow, such as leukemia ´ Kidney disease. ´ Liver disease. ´ Hypothyroidism. ´ Thalassemia — a genetic disorder that causes low levels of hemoglobin and red blood cells.Increased levels ofhaemoglobin are found in: ´ Polycythemia vera — a blood disorder in which your bone marrow makes too many red blood cells ´ Lung disease . ´ Dehydration ´ Living at a high altitude ´ Heavy smoking. ´ Burns. ´ Excessive vomiting.The method for the determination of the hemoglobin by: 1–Sahli method ´ This test requires dilution of blood and visual color match. ´ Principle: ´ Haemoglobin is converted into acid haematin by addition of 0.1 N HCl. The resultant solution is then matched against a reference solution in a colorimeter or colored strip (SAHLI'S Haemoglobinometer).Reagents and Equipment: ´ 1– Sahli's haemoglobinometer. ´ 2– Sahli's pipette. ´ 3– 0.1 N HCl. ´ 4– Dropper, ´ 5– Stirring rod.Procedure: ´Fill the calibrated tube to the '20' mark with 0.1N HCl. ´Clean the fingertip with surgical spirit and prick with a sterile lancet. ´Using the micropipette, suck blood upto the 20 mark. Do not allow air bubbles to enter. ´Wipe the outside of the pipette with absorbant paper ´–Blow the blood from the pipette into the calibrated tube containing the acid solution. –´ Rinse the pipette by drawing in and blowing out the acid solution 3 times. ´ The mixture of blood and acid will now give a brownish colour ´ Allow it to stand for 10 minutes, so that haemoglobin gets converted into acid haematin. ´ Compare the color of the solution in the graduated tube with that of reference strip on either side of haemoglobinometer. ´ If the color of the graduated tube is darker, add drop by drop distilled water by the dropping pipette and mix with glass rod, until the color matches with the reference strips. ´ Note the reading on the graduated tube. This is the haemoglobin level in g/dl. Some tubes also give level in percentage. ´ To convert into g/dl, multiply the percentage with 0.146 (Example:10% X 0.146=14.6 g/dL)Advantages: ´ 1–Instruments and reagents are inexpensive ´ 2– Test is easy to perform ´ 3– Electricity is not required. ´ Disadvantages: ´ 1–Color matching is subjective. ´ 2– The color of the glass standard is not a true match for the color of diluted blood. ´ 3–Graduated tubes must be cleaned before use. ´ 4–Acid hematin is not a stable compound and readings must be taken within the recommended time interval. ´ 5–sample can only be measured one at a time. ´ 6– after prolonged use the numbers on the graduated cylinder fade and are difficult to read.2–Cyanmethaemoglobin method: ´This method is the most accurate and most commonly used method ´Principle: ´ The blood is diluted in a solution containing Potassium Cyanide and Potassium Ferricyanide (Drabkin's solution). ´It converts haemoglobin (Hb) and methaemoglobin (Hi) to cyanmethaemoglobin (HiCN), which is a stable compound. The absorbance of the solution is measured in photoelectric calori–meter at a wavelength of540nm