

Human Physiology (Sem IV)

Aim: Determination of iron and TIBC content in serum sample.

Materials required: microcentrifuge tubes (1.5ml), microcentrifuge tubes stand, autopipettes and tips.

Iron reagents: Iron buffer reagent, iron colour reagent, iron standard (100 μ g/dl) (from kit).

TIBC reagents: TIBC saturating reagent, TIBC precipitating reagent (from kit).

Theory:

Iron found in blood is mainly present in the hemoglobin of the RBCs. Its chief role in the body is transportation of oxygen and cellular oxidation. Iron is absorbed in the small intestine, and bound to a globulin protein in the plasma called transferrin and transported to the bone marrow for the formation of hemoglobin. Increased serum levels are found in hemolytic anemia, hepatitis, lead and iron poisoning. Decreased serum levels are found in anemia caused by iron deficiency due to insufficient intake or absorption of iron, chronic blood loss, late pregnancy and cancer. Increase TIBC concentration is found in iron deficient anemia and pregnancy. Decreased concentration of TIBC is found in hypoproteinemia, hemolytic/ pernicious/ sickle cell anemia, inflammatory diseases and cirrhosis.

Principle

Iron, bound to transferrin, is released in an acidic medium and the Fe^{2+} ions react with Ferrozine to form a violet coloured complex. Intensity of the complex formed is directly proportional to the amount of iron present in the sample. For TIBC, serum is mixed with TIBC saturating reagent containing Fe^{2+} and sodium ascorbate, to saturate serum transferrin. Unsaturated sites on transferrin become saturated by oxidation of Fe^{2+} to Fe^{3+} (by sodium ascorbate). Excess Fe^{2+} is precipitated and iron bound to transferrin in the supernatant is dissociated at acidic pH and reacted with ferrozine. Fe-ferrozine is measured spectrophotometrically at 570nm.

TIBC is the index of transferrin present in circulating blood. TIBC test is a blood test to see if a person has too much or too little iron in his/her blood. Iron moves through the blood attached to a protein called transferrin and therefore this test helps us to know the carrying capacity of the transferrin protein for iron.

TIBC represents the sum of UIBC (unsaturated iron binding capacity) and plasma iron.

Normal reference values

The normal value for TIBC lies in the range of 250-400 μ g/dl and for UIBC is 160-360 μ g/dl.

For iron, the normal reference values are 60-160 $\mu\text{g/dl}$ (males), 35-145 $\mu\text{g/dl}$ (females) and 150-200 $\mu\text{g/dl}$ (neonates).

Decrease levels of iron can occur during iron deficiency, anemia or late pregnancy.

Lower than normal TIBC may occur under following conditions: Hemolytic anemia, Inflammation, liver disease, malnutrition, Pernicious anemia or sickle cell anemia.

Procedure:

1. 0.5 ml of serum was taken in a microcentrifuge tube and 1 ml of TIBC saturating reagent was added to it.
2. It was mixed well and allowed to stand at room temperature for 10 minutes.
3. TIBC (~50 mg) precipitating reagent was added to the above, mixed gently and incubated for 10 minutes at room temperature.
4. The sample was centrifuged at 2000 - 3000 rpm for 10 minutes.
5. Pellet was discarded and supernatant (marked as T2) was used for the determination of iron content.

For estimation of iron and TIBC content in the given serum sample:

1. Five microcentrifuge tubes were labeled as blank, standard, standard blank, T1 and T2.
2. 1 ml of Iron buffer reagent was added in tubes of standard, blank, T1 & T2 and 1.05 ml in standard blank.
3. 0.2 ml of distilled water was added to the blank tube.
4. 0.2 ml of iron standard sample was added to standard, standard blank, T1 and T2.
5. 0.05 ml of iron colour reagent was added to blank, standard and T1 and T2.
6. All the microcentrifuge tubes were mixed well and incubated for 5 mins at room temperature.
7. The absorbance was then measured at 570 nm.

Result:

Discussion:

Precautions:

1. Autopipettes should be handled with care.
2. For different reagents, separate tips must be used.
3. The spectrophotometer must be switched on 15 mins before the readings to be taken.