Experiment : Quantitative determination of glucose 6-phosphat dehydrogenase deficiency hemolysed RBC sample

Experiment : Qualitative determination of hemoglobin S (HbS) in blood.

Experiment : Quantitative determination of iron in serum

Experiment :

Quantitative determination of glucose 6-phosphate dehydrogenase deficiency hemolysed RBC sample

Objective:

Quantitative determination of glucose 6-phosphate dehydrogenase (G6P-DH) activity in erythrocytes (hemolysate).

Introduction:

- G6PD is the enzyme responsible for the initial deviation of glucose into pentose phosphate pathway to form 6-phosphogluconate.
- Most of the interest of G6P-DH on its role in the erythrocyte. it functions to maintain NADPH in its reduced form. An adequate concentration of NADPH is required to regenerate sulfhydryl-containing proteins, such as glutathione, form the oxidized to the reduced state. Glutathione in the reduced form, in turn, protects hemoglobin from oxidation by agents that may be present in the cell.

- deficiency of G6P-DH consequently results in an inadequate supply of NADPH and, ultimately, in the inability to maintain reduced glutathione levels. When erythrocytes are exposed to oxidizing agents, **hemolysis occurs because of oxidation of hemoglobin and to damage of the cell membrane.**
- G6P-DH deficiency is an inherited **Sex-linked recessive** trait. Or The disorder can results in several different clinical manifestations, one of which is drug-induced hemolytic anemia. When exposed to an oxidant drug such as anti-malarial drug,
- G6P-DH deficiency is most common in African Americans,

Principle:

The enzyme G6PD is catalyses the dehydrogenation of glucose 6-phosphate as the first step in pentose phosphate pathway. $NADP^+$, the electron acceptor, is reduced to NADPH in the reaction. The pH optimum for the G6P-DH reaction is 8.3 for the enzyme from yeast or blood cells. The rate of formation of NADPH is a measure of the G6P-DH activity and it can be followed by means of the increase in extinction at 340 nm .

Method:

A red cell hemolysate is used to assay for deficiency of the enzyme

By using the kit.

Procesure:

- Pipette into clean and dry test tubes

G6P-DH Buffer	3 ml
NADP reagent	100 µl
Hemolysate	50 µl
Mix and incubate for 5 min at 25°C, the add	
G6P-DH Substrate	50 µl
Mix and after 30 seconds read initial absorbance at 340 nm against distilled water. Repeat absorbance reading every min for 3 min and calculate ΔA/min	

Calculations

G6P-DH Activity in mU/erythrocytes/ml of blood(P)= ΔA/min X 30868=

- If the erythrocytes count per ml of blood is 4.5 X 10^9

Then the G6P-DH activity in mU/ 10^9 cells = P/4.5

Abnormal value= 0- 11 mU/ 10^9 cells.

Expected value 80- 180 mU/ 10^9 cells

Experiment :

Qualitative determination of hemoglobin S (HbS) in blood.

Objective:

Qualitative determination of hemoglobin S (HbS) in blood using a phosphate solubility method.

Introduction:

There are hundreads of hemoglobin variants , and the most common an important are:

- **Hemoglobin A**. This is normal hemoglobin that exists after birth.

It consist of ($\alpha_2\beta_2$). In normal adult 95% of Hb is present as HbA

• **Hemoglobin A2**. This is a minor component of the hemoglobin found in red cells after birth

and consists of ($\alpha_2\delta_2$) , less than 3% of the total red cell hemoglobin.

• **Hemoglobin F.** Hemoglobin F is the predominant hemoglobin during fetal development. ($\alpha_2\gamma_2$).

Clinically Significant Variant Hemoglobins:(abnormal Hb):

- **Hemoglobin S.** The alpha chain is normal. The disease-producing mutation exists in the beta chain, giving the molecule the structure, $\alpha_2\beta^S_2$.
  - Hb S can inherited in the homozygous state (S/S) result in sickel cell anemia or heterozygous state (A/S) is usually benign (sickel cell trail)
  - Hb S will appear in Sickle cell anemia patients and in sickle cell trail(carrier) and also in occure in the presebce of other abnormal Hb i.e HbC , HbD

Method:

By kit , this test is a simple and rapid method for determination of the presence of HbS

Principle:

Erythrocytes are lysed (by saponin) and the released hemoglobin is reduced (by dithionite) in phosphate buffer. Reduced HbS is characterized by ites very low solubility . So that in the presence of HbS or non-S sickling Hb the system become turbid.(formation of nematic liquid crystals “ tactoids”)

Procedure:

	test
Sickling solution	2 ml
Patient sample (whole blopd)	0.02 ml (20 µl)
Mix by inversion and allow stand at room temperature for 5 to 10 min	
Read the test by holding the test tube approximately 3 cm in front of a lined scale on the card.	

HbS was present, the solution is turbid and the lines behind the test tube will not be visible while, if no sickling hemoglobin was present the clear solution will permit the lines to be seen through the test tubes

Experiment :

Quantitative determination of iron in serum

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Quantitative determination of **iron** , unsaturated iron binding capacity **(UIBC)** and total iron binding capacity **(TIBC)** in serum using a colorimetric method.

Objective:

- To determine the normal level of serum iron.
- And use this test in diagnosis of anemia.(iron deficiency)

Introduction:

- **Transferrins** : are iron-binding blood plasma glycoproteins that control the level of free iron in biological fluids (When iron stores become low, transferrin levels will increase. When there is too much iron, transferrin levels are low) . it contains **two** specific high-affinity Fe(III) binding sites. It has high affinity to bind with Fe(III).
- **Total iron-binding capacity** (TIBC) is a medical laboratory test that measures the blood's capacity to bind iron with transferrin , it is measuring the maximum amount of iron that it can carry,(which indirectly measures transferrin since transferrin)

- TIBC calculated by adding serum iron and UIBC .
- Total iron-binding capacity (TIBC) is most frequently used along with a serum iron test to evaluate people suspected of having either iron deficiency anemia or iron overload (hemochromatosis)

Defect in Serum iron

- ✓ Increase serum iron indicate for: increase erythrocyte , destruction decrease erythrocyte formation , increase absorption , defect in iron storage or iron overload (hemochromatosis)
- ✓ Decrease serum iron indicate for :iron deficient

Defect in Iron binding capacity (Saturated and unsaturated)

- ✓ increase in iron deficient anemia
- ✓ Decrease in hemochromatosis , malignant or rheumatic fever .

- **Normal range of serum iron :**

50-160µg/dl

- **Normal range of TIBC :**

250 – 450 µg/dl

Method: By kit

Principle:

- *Serum iron :* the iron dissociated from its Fe-III-transferrin complex by addition of acidic buffer containing hydroxylamine which reduces the Fe(III) to Fe(II) . Then the chromogenic agent (PDTS)form a highly colored Fe(II) complex that is measure spectrophotometric at 565nm .
- *UIBC:* determined by adding Fe(II) to serum so that bind to unsaturated iron binding site on transferrin . The excess Fe(II) are reacted with PDTS to form color complex which is measured spectrphotometric . The difference between the amount of Fe(II) added and the amount of Fe(II) measured represent the UIBC
- *TIBC* is determined by adding serum iron to UIBC value.

PROCEDURE:

	IRON			UIBC		
	BLANK	STD	TEST	BLANK	STD	TEST
IRON BUFFER	2.5 ml	2.5 ml	2.5 ml	-	-	-
UIBC BUFFER	-	-	-	2 ml	2 ml	2 ml
IRON STD	-	0.2 ml	-	-	0.2 ml	0.2 ml
SAMPLE	-	-	0.2 ml	-	-	0.2 ml
WATER	0.2 ml	-	-	0.4 ml	0.2 ml	-
	Mix and read the absorbance of std and test (iron) at 565 nm against blank. Also read the absorbance of std and test (UIBC) against blank					
Iron reagent color	0.05 ml	0.05 ml	0.05 ml	0.05ml	0.05 ml	0.05 ml
	Mix and incubate at 37c for 10 min Mix and read the absorbance of std and test (iron) at 565 nm against blank. Also read the absorbance of std and test (UIBC) against blank					

CALCULATION

- Iron concentration µg/dl= (Abs. of test/Abs. of std) X Std conc. =
- if you know the Std Conc. = 500 µg/dl

SERUM IRON + SERUM UIBC = SERUM TIBC)
(µg/dL) (µg/dL) (µg/dL)

Transferring **saturation %=** serum iron concentration **. 100**
TIBC