

### DETERMINATION OF PLASMA PROTEINS

Plasma contains more than 100 different proteins. The liver synthesizes most of these proteins, whereas the lymph nodes, spleen and plasma cells also synthesize gamma globulins, in addition to liver.

Different methods such as salting-out, alcoholic precipitation, centrifugation, electrophoresis and radio-immuno-assay have been used to separate the individual proteins from the plasma.

Laboratory values of the major protein components in the normal human plasma expressed as gm/dl are :-

PLASMA PROTEINS	NORMAL VALUES (gm/dl)
Total Proteins.	6.3-7.8
Albumin -	3.2-5.1
Globulin	1.5-3.2
$\alpha_1$ -Globulin	0.06-0.39
$\alpha_2$ -Globulin	0.28-0.79
$\beta$ -Globulin	0.69-1.25
$\gamma$ -Globulin	0.8-2.0
Fibrinogen	0.2-0.4
Heptoglobulin	0.03-0.19

#### METHOD:

Biuret Method

#### PRINCIPLE:

The proteins on reaction with alkaline copper sulphate solution (Biuret Reagent) give a purple colour due to the formation of a coordination complex between peptide nitrogen and cupric ions. The intensity of the colour produced is compared with that of a standard protein solution treated similarly.

#### REAGENTS:

1. Biuret Reagent ( Benedict's Qualitative Reagent )
  - i - Copper Sulphate,
  - ii - Sodium Citrate,
  - iii - Sodium Carbonate.
2. 6% NaOH,
3. 0.9% NaCl.
4. Standard Protein Solution (8mg/2.5ml)

PROCEDURE:

Label THREE test tubes as Unknown (U), Standard (S) and Blank (B).

Dilute plasma/serum 1:25 times and then pipette 2.5ml of the diluted plasma/serum into tube 'U', 2.5ml Standard Protein solution into tube 'S' and 2.5ml distilled water into tube 'B' respectively.

To each tube add 2.5ml 6% NaOH and 1ml Biuret Reagent. Mix thoroughly and let it stand for 15-20 minutes at room temperature.

Record the optical density in a photoelectric colorimeter at 545nm wavelength.

No.	REAGENTS.	TUBES.		
		U	S	B
1.	Diluted Plasma/Serum (1:25)	2.5ml	-	-
2.	Standard Protein Solution.	-	2.5ml	-
3.	Distilled Water.	-	-	2.5ml
4.	6% NaOH.	2.5ml	2.5ml	2.5ml
5.	Biuret Reagent.	1ml	1ml	1ml
6.	Mix and allow to stand for 15 - 20 minutes at room temperature .			
7.	Record the Optical Density at 545nm.			

CALCULATIONS:

Optical Density of Unknown (U) =  $OD_U$   
 Optical Density of Standard (S) =  $OD_S$   
 Concentration of Standard Solution =  $C_S$   
 Dilution Factor =  $D$   
 Volume of diluted plasma/serum used. =  $V$

$$\frac{C_1}{C_2} = \frac{D_1}{D_2}$$

$$\Rightarrow C_2 = \frac{C_1 \times D_2}{D_1}$$

$$\text{PLASMA TOTAL PROTEINS (gm/dl)} = \frac{OD_U \times C_S}{OD_S} \times \frac{D}{V} \times 100$$

$$\frac{C_U}{C_S} = \frac{A_U}{A_S}$$

$$C_U = \frac{A_U \times C_S}{A_S}$$

NORMAL RANGE:

7.5 ml Plasma contains 132.5mg of protein  
 1 ml =  $\frac{132.5}{7.5} = 17.67$   
 1 ml = 53 mg/dl  
 100 ml =  $\frac{5300}{100} = 53$   
 Serum Total Proteins = 6.3-7.8 gm/dl  
 = 6.39/dl  
 - 132.5 + 19

(6.4 - 8.5)  
 normal =  $\frac{12 \times 8}{5.3 \times 25}$  (WF)

EXPERIMENT No: 9

Date: \_\_\_\_\_

TO ESTIMATE THE AMOUNT OF TOTAL PROTEINS IN THE PLASMA /  
SERUM.

CALCULATIONS:

Absorbance of unknown =  $A_2 = 30$   
Absorbance of standard solution =  $A_1 = 40$   
Concentration of standard solution =  $C_1 = 8 \text{ mg} / 2.5 \text{ ml}$   
Concentration of unknown solution =  $C_2 = ?$   
Concentration of plasma protein =  $C_2 = \frac{A_2}{A_1} \times C_1 \times D_F$   
$$= \frac{30}{40} \times \frac{8}{2.5} \times 2.5$$
$$= 150 \text{ mg} / 2.5 \text{ ml}$$

2.5 ml of plasma protein contain = 150 mg  
1 ml of plasma protein contain = 60 mg  
100 ml " " " " = 600 mg

$$C_2 = 600 \text{ mg} / 100 \text{ ml}$$
$$= 6 \text{ g} / 100 \text{ ml} / \text{dl}$$

*Grind*  
15/4/19

RESULT: Amount of total protein in plasma is 6g/100ml

✓  
Interpretations:

HYPOPROTEINAEMIA:

- Relative Hypoproteinaemia:
  - a - Pregnancy,
  - b - Over hydration,
  - c - Malabsorption,
  - d - Hypoxaemia,
- Nephrotic syndrome,
- Glomerulonephritis,
- Liver diseases,
- Protein losing enteropathies,
- Malnutrition,
- Impaired synthesis in malnutrition, Vitamin deficiencies,
- Albuminuria,
- ✓ Loss of plasma by extravasation, Haemorrhage, trauma,
- Increased capillary permeability,
- Catabolic states-severe sepsis, fever, malignant diseases,
- Chloroform or phosphorous poisoning.

HYPERPROTEINAEMIA:

Relative hyperproteinaemia:

Vomiting, Diarrhoea, Dehydration,

Due to absolute increase in globulin content as in various anaphylactic conditions, malignancy, liver cirrhosis and certain chronic conditions

- ✓ Surgical or traumatic shock, excessive burns,
- Neoplastic diseases e.g., Multiple myeloma in which
- $\gamma$  - globulins are increased.
- Addison's Disease,
- Infections.