DETERMINATION OF PLASMA PROTEINS

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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
α_1–Globulin	0.06–0.39
α_2–Globulin	0.28–0.79
β–Globulin	0.69–1.25
γ–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: 1 ml Plasma + 24 ml of Saline
25 dilution factor

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

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

NORMAL RANGE:

Serum Total Proteins = 6.3–7.8gm/dl.

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, Diarrohea, 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
- γ - globulins are increased.
- Addison's Disease,
- Infections.

EXPERIMENT No: 08 Date: 14 March 2012

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

CALCULATIONS:

Concentration of standard solution of Protein = C_1 = 8mg / 2.5ml = 3.2
Concentration of unknown solution = C_2 = ?
Optical density of standard solution = D_1 = 16
Optical density of unknown solution = D_2 = 8

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

$$C_2 = \frac{D_2 \times C_1}{D_1} \times 25$$

$$C_2 = \frac{8 \times 8}{16} \times 25$$

$$C_2 = 100$$

Normal Range = 6.3 – 7.8 gm/dl.

2.5 ml of standard protein solution contains = 100
1 ml of " " contains = $\frac{100}{2.5} \times 1$
= 40

100 ml of standard protein solution contains = 40 × 100
= 4000mg/dl

∵ 1g contains = 1000mg
therefore 4000mg contains = $\frac{4000}{1000}$
= 4g/dl

RESULT: 4g/dl

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