**Method**

**Separation of Main Proteins in Plasma and Serum:**

***a) Plasma:***

5 ml Plasma + 5 ml saturated NaCl solution

Centrifuge al 3500 rpm /10 min

Fibrinogen Precipitate

Dissolve in 2ml 0.9% saline

Clotting Test (Tube B)

Biuret test (Tube A)

1 ml Fibrinogen + 1ml Serum

Incubate at water bath at 37 °C / 10 min

 🡪 Clotting occurs (because serum contains

 active thrombin which converts fibrinogen

 to insoluble fibrin)

1 ml Fibrinogen + 1ml Biuret Reagent

Mix well, allow to stand in water bath at 37 °C/10 min

 🡪Blue color (confirms the presence of protein “fibrinogen”)

***To Filtrate***

Filtrate + Few drops of 5 % CaCl2

Incubate at 37 °C / 10 min

No clotting occurs (although calcium ions are required in the clotting process, no clotting occurs because of the absence of the fibrinogen in the solution)

***b)* serum proteins**

in Two dry centrifuge tubes labeled P (total protein) and G (globulin)

|  |  |  |
| --- | --- | --- |
|  | P | G |
| Saline | 3.8 ml |  |
| saturated ammoniumsulphate |  | 3.8 ml |
| Serum | 0.2 ml | 0.2 ml |

-Mix G by inverting the tube a couple of times, then filter immediately to separate

 globulins ( a can be used)

Globulin Precipitate

Dissolve in 2ml 0.9% saline

Biuret test (Tube A)

Heat Coagulation Test (Tube B)

(Denaturation by heat)

1 ml Globulin + drops of 2M acetic acid

Heat the content of the tube at 70 °C/10 min

🡪Cloudiness (confirms the presence of

protein “globulin”)

 In part 3 test

***To Filtrate (Albumin)***

Divide the filtrate into 2 tubes

Add solid ammonium sulphate until albumin is precipitated

🡪(Confirms the presence of protein “albumin”)

Add drops of 2M acetic acid

Heat the content of the tube at 70 °C/10 min

🡪Cloudiness (confirms the presence of protein “albumin”)

Heat Coagulation Test (Tube B)

Salting out (Tube A)

Part 3

Label 4 test tubes as T (total), A (albumin), B (blank), and S (standard)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | T | A | B | S |
| saline | - | - | 1ml | - |
| BSA stander (5mg/dl) | - | - | - | 1ml |
| Solution fromP | 1ml | - | - | - |
| Filtrate of G | - | 1ml | - | - |
| Biuret reagent | 5ml | 5ml | 5ml | 5ml |
| Mix and keep standing for 1 minutes, read the absorbance using a spectrophotometer at 540 nm |
| absorbance |  |  |  |  |