Separation of Main Proteins in Plasma and Serum

First, working on Plasma:

Add equal volumes of **Plasma** and saturated NaCl solution.

This Separation by …… Method.

Centrifuge at 3500 RPM/10 min

Transfer the supernatant, into another empty test tube.

Take the precipetate (Fibrinogen), and dissolve it in 2 ml 0.9% saline. (then devide it to A and B tube)

(Tube A)

1 ml for Biuret test

1 ml Fibrinogen + 1ml Biuret Reagent Mix well, allow to stand in water bath at 37 °C/10 min a purple color (confirms the presence of protein "fibrinogen")

(Tube B)

1 ml for Clotting Test

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)

For Supernatant:

Supernatant + Few drops of 5 % CaCl2

The objective is to…….

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)

Second, working on Serum:

Add equal volumes of **serum** and saturated ammonium sulphate solution

Aim to separate ……

Centrifuge at 3500 rpm /10 min

Globulin Precipitate

Transfer the supernatant into another empty test tube.

Dissolve the precipitate in 2ml 0.9% saline. (then divide it to A and B tube)

Biuret test (Tube A)

1 ml Globulin + 1ml Biuret Reagent
Mix well, allow to stand in water bath at 37 °C/10 min
→purple color (confirms the presence of protein "mainly globulin")

Heat Coagulation Test (Tube B)

1 ml Globulin + drops of 2M acetic acid
Heat the content of the tube at 80 °C/10 min
→Cloudiness (confirms the presence of protein "mainly globulin")

For Supernatant:*(Mainly Albumin)*

Divide the **Supernatant** into 2 tubes.

Salting out of the supernatant (Tube A)

Add solid ammonium sulphate until albumin is precipitated.
→(Confirms the presence of protein "albumin")

Heat Coagulation Test (Tube B)

Add drops of 2M acetic acid

Heat the content of the tube at 80 °C/10 min
→Cloudiness (confirms the presence of protein "albumin")