How is blood plasma different from serum?

- Plasma is the fluid portion of whole blood
- Plasma is the fluid obtained when whole blood containing anti-coagulant is centrifuged
- Serum is the liquid portion of clotted blood
- Serum does not contain clotting factors that are normally present in plasma
- Serum is the fluid portion obtained after centrifuging clotted blood

What are some of the proteins in blood plasma or serum?

- Plasma contains a variety of proteins with different functions
- Plasma also contains some proteins of unknown functions
- “Total Protein” in plasma is made up of Albumin and Globulins
- Clinical Biochemistry laboratory routinely measures “Total Protein” and “Albumin” concentrations, usually in a serum specimen, and reports the “Globulin” fraction as the difference between total protein and albumin
- Other plasma proteins (e.g., Immunoglobulins) are measured as Classes
- Immunochemical methods are available for measuring specific plasma proteins and hormones
- Enzymes in serum or plasma are measured both by determining their activity and by immunochemical methods to assess their concentration and mass
- Routine Electrophoresis to separate protein components in Serum or Plasma gives 5 major bands, which can be useful in demonstrating the presence of Paraproteins

What are some of the functions of proteins in blood plasma?

- Blood clotting factors: Blood clotting factors in coagulation cascade,
- Immune defense: Immunoglobulins, Complement proteins,
- Involved in inflammatory responses: Acute phase response proteins: C-reactive protein, alpha-acid glycoprotein (Orosomucoid)
- Transport or binding proteins: Albumin, Ceruloplasmin, Haptoglobin, Retinol Binding Protein, Sex Hormone Binding Globulin, Thyroid Hormone Binding Protein, Transferrin
- Anti-proteases: Anti-Chymotrypsin, Antithrombin, α2-Macroglobulin,

What are some of the diagnostic significance of Total Protein?

- Total Protein in plasma is about 7 – 7.5 g/dL
- Proteins make up the major part of the solids of plasma
- Proteins in plasma are a complex mixture that includes Simple Proteins, Mixed or Conjugated Proteins, such as Glycoproteins and various types of Lipoproteins
- Changes in Total Protein concentration in plasma are common in some disease conditions
- Elevated Total Protein in plasma may indicate the presence of Paraproteins
- Decrease Total Protein in plasma may indicate low Albumin concentration

What are some of the functions of Albumin?

- Albumin is the major plasma protein and is synthesized and secreted by the Liver
- Albumin has a biological half-life in plasma of about 20 days
- A significant decrease in Albumin concentration in plasma is usually slow to occur if it is due to reduction in the biosynthesis of Albumin
Albumin makes the biggest contribution to plasma Oncotic Pressure
Edema usually occurs when Albumin concentration falls very low

What are some of the possible causes of Hypoalbuminemia?
Three main reasons for the occurrence of Hypoalbuminemia:
- **Decreased synthesis:**
  - May be due to malnutrition or mal-absorption
  - May be a feature of advanced liver disease

- **Abnormal distribution or dilution:** May be induced by overhydration or if there is increased capillary permeability as occurs in septicemia

- **Abnormal excretion or degradation:**
  - May be causes by any of the following: Nephritic Syndrome, Protein-losing Enteropathies, Burns, Hemorrhage, Catabolic states

How significance is some of the Specific Serum/Plasma Proteins?
Measurement of a number of specific proteins gives useful information for diagnosis and management of some diseases: Examples include: Transferrin, Thyroid Binding Globulin, Sex Hormone Binding Globulin, Haptoglobin, Albumin, Globulins, C-reactive protein, Immunoglobulins; etc.

Characteristic changes in the concentration of certain plasma proteins are seen following Surgery or Trauma, or during Infection or Tumor growth
- Proteins involved are called **Acute Phase Proteins**
  - Acute Phase Protein response leads to greatly increased De Novo biosynthesis (mainly in the liver) of a number of plasma proteins along with decease in the plasma concentration of others
  - Response is stimulated by release of Cytokines: **Interleukin-1**, **Interleukin-6** and **Tumor necrosis factor (TNF)** and increased concentrations of the hormones **Cortisol** and **Glucagon**

Acute Phase Protein response is an adaptive response to disease,
Example:
- Increases in C-reactive protein (CRP) and Complement will contain and eliminate infection
- Increased Coagulation Factors will aid and prevent excess blood loss
- Protease Inhibitors will prevent the spread of tissues necrosis when damaged cells at the site of injury release Lysosomal enzymes

Clinically some of these Acute Phase proteins may be used to monitor progress of some disease condition or its response to treatment

How useful is Electrophoresis of serum/plasma proteins (pH 8.6)?
- Electrophoresis may be carried out to study protein abnormalities
- Serum is a better choice for Electrophoresis, because the Fibrinogen of Plasma gives a discrete band, which can easily be mistaken for a Paraprotein
- General pattern of electrophoresis result is shown in **Fig. 4**,
  - It shows the order of migration along the Horizontal Axis with proteins of highest mobility closest to the Anode
  - Height of the band along the Vertical Axis shows the protein concentration
  - Location of some major proteins are indicated underneath their Electrophoretic mobility peaks
- Electrophoresis can show gross deficiency or excess of Immunoglobulins and whether Paraproteins are present.
- Quantitative measure of each protein class may be obtained by scanning Electrophoretic strip.

Electrophoresis of serum proteins. (a) Normal pattern; (b) and (c) sera with paraprotein bands.

Scan of an electrophoresis strip.
What are Immunoglobulins?
- Immunoglobulins are a group of structurally related proteins that function as Antibodies
- Immunoglobulins are produced by cells of the Lympho-reticular System
  - Immunoglobulins are also produced by Plasma Cells, which are B-lymphocytes transformed after exposure to a foreign (or occasionally an endogenous) Antigen

What is an Immunogen?
- IMMUNOGEN is a molecule that can generate an Immune response (cellular or humoral)

What is Antigen?
- ANTIGEN is a molecule that reacts with Antigen Receptors, irrespective of its ability to generate an Immune Response
- Antigen may, or may not be an Immunogen

What is Hapten?
- HAPTEN is a small molecule that is able to react with preformed Antibodies, i.e., has Antigenicity, but is not capable, by itself, of stimulating a specific Immune Response, i.e., is not Immunogenic
- Haptens are only Immunogenic when coupled to a large protein called a carrier
  - All Immunogens are therefore Antigenic but not all Antigens are Immunogenic

What are Epitopes?
- Epitopes or Antigenic determinants
  - Antigen Receptors on Lymphocytes recognize discrete sites on an Antigen called Epitopes or Antigenic Determinants
  - Antigen recognition by B cells and T cells is fundamentally different and does not involve the same Epitopes

What is the basic structure of Immunoglobulins (Fig 6)?
- Basic structure of Immunoglobulin (Ig) molecule is composed of:
  - 2 Identical “Heavy” Polypeptide Chains and
  - 2 Identical “Light” Polypeptide Chains
  - Both Chains have Inter-chain and Intra-chain Disulfide (S-S) Bonds and Non-covalent Interactions

  - Two types of “Light” Polypeptide Chains:
    - Kappa “Light” Chains
    - Lambda “Light” Chains

  - Five principal types of “Heavy” Polypeptide Chains:
    - Alpha, Gamma, Delta, Epsilon and Mu

What are the classes of Immunoglobulins?
- Immunoglobulins are named and classified by their heavy chain type
- Five types of Heavy chains gives Five Classes of Immunoglobulins:
  - IgA, IgG, IgD, IgE, IgM
What products are obtained when IgG is hydrolyzed by (a) Papain and (b) Pepsin?

**Action of Papain on IgG:**
- Papain is a protease enzyme that acts on the Hinge region (in front of the inter chain S-S bonds) in IgG
- Hydrolysis of IgG by Papain produces 3 components:
  - 2 identical Fab (Fragment antigen-binding) fragments, and
  - 1 Fc (Fragment-crystalizable) fragment

**Action of Pepsin on IgG:**
- Pepsin is a protease enzyme that acts on the Hinge region (behind the inter chain S-S bonds) in IgG
- Hydrolysis of IgG by the enzyme Pepsin produces:
  - A single divalent F(ab’)_2 and
  - A pFc’ fragment

**What are the regions in the structure of an Immunoglobulin?**
- **Amino-terminal portions** of the “Heavy” and “Light” chains show considerable variability in Amino Acid composition (V region).
- Remaining parts of both Heavy and Light chains are relatively Constant in terms of Amino Acid composition (C region).
- Three areas in the variable regions (V regions) of the Light and Heavy chains showed remarkably diverse amino acid sequences (Hyper-Variable Regions or Complementarity-Determining Regions).
- Light chains contain One Variable Domain (VL) and One Constant Domain (CL).
- Heavy chains contain One Variable Domain (VH) and 3 or 4 Constant Domains designated CH 1 – 3 or CH 1 – 4) accordingly.

**Take Note:**
Immunoglobulin molecules contain two functional areas:
- Fab, or Variable end – is the area that recognizes and binds to Antigens
- Fc end – is responsible for interaction with other components of the Immune system, e.g., Complement and T helper cells
- Hyper-variable Loops form the Antigen-Binding Site of an Immunoglobulin molecule, i.e.,
  - Each Hyper-variable Loop contributes to the Antigenic Specific or Complementarity of the binding site for Antigen
- Various Classes/Types of Immunoglobulins have different Tertiary structure and Functions
- Major Immunoglobulins in plasma are:
  - IgG (it neutralizes toxins, activates complement, capable of crossing Feto-placental barrier),
  - IgA (usually contains J chain and secretary component, part of defense against local viral and bacterial infections),
  - IgM (usually first to be made in immune response, contains J chain, in presence of complement are very effective in producing Lysis of cells)

**What is the significance of an increase in Immunoglobulin level in serum?**
- Immunoglobulins may be increased non-specifically in a wide variety of Infections and also in Autoimmune diseases
- Increase biosynthesis of Ig may be cause by several Cell Lines, each producing specific type of Immunoglobulins (Hyper-Gamma-Globulinemia)
Such response is said to be “Polyclonal” and results in diffuse increase in protein mass throughout the Gamma Globulin region. Appears as broad band during Electrophoresis of Serum protein.

Increase biosynthesis of Ig may be cause by a Single Clone of cells making Identical Immunoglobulin. Such is said to be “Monoclonal”.

Immunoglobulin production may increase, becomes large enough to be observed as a single discrete band on electrophoresis of the serum. Such single discrete band may be due to increase in Intact Immunoglobulin or fragments called Paraproteins.

What are Paraproteins?

Paraproteins (also called Monoclonal components) are discrete Immunoglobulin bands, seen on electrophoresis of Serum. Paraproteins are due to production of a Single type of Immunoglobulin or Immunoglobulin fragments (Light-chain or Heavy-chain fragments) by a Single clone of B cells. Paraproteins may arise from any of the Immunoglobulin classes. Detection of Paraprotein in blood or urine requires further investigation to determine if the Paraproteinemia is caused by Benign or Malignant condition.

Benign Paraproteinemia may occur transiently during acute infection and in autoimmune disease due to Antigen stimulation. Paraproteins are found in malignant conditions such as, Multiple Myeloma, Macroglobulinemia, in Heavy chain diseases, etc. Monoclonal Light Chains are produced in excess of Heavy chains in about 50% of cases of Myeloma, and in about 15% of cases only Light chains are found.

- These light chains are small enough to spill into the urine where they are known as Bence-Jones Protein.
- Serum electrophoresis may not show the presence of light chains, and Urine electrophoresis after concentration may be required to demonstrate the Paraproteins.

Take Note:

- Myeloma is characterized by Bony Metastases.
  - Bone pain is often the presenting symptom.
  - In the face of increasing synthesis of abnormal Immunoglobulins, other bone marrow function is reduced, and there is a decline in Red and White cell and Platelet formation and decreased production of normal Immunoglobulins.
  - Anemia and susceptibility to infection are the usual consequences.
Simplified model for an IgG1 (κ) human antibody molecule showing the basic four-chain structure and domains (VH, CH1, etc). V indicates the variable region. C indicates the constant region. Sites of enzyme cleavage by pepsin and papain are shown. Note positions of inter- and intrachain disulfide bonds. (Reproduced, with permission, from Stites DP, Terr AI, Parslow TG [editors]: Basic & Clinical Immunology, 8th ed. Appleton & Lange, 1994.)