SPECIFIC PROTEIN MARKERS

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What is a protein?

- **Proteins** - large biological molecules, or macromolecules, consisting of one or more long chains of amino acid residues.

- Perform a vast array of functions within living organisms, including:
  - Catalyzing metabolic reactions (enzymes)
  - Replicating DNA
  - Responding to stimuli
  - Transporting molecules from one location to another

- Proteins differ from one another primarily in their sequence of amino acids.
Protein Synthesis and Function

- Within the cell, they carry out ‘duties’ specified by the information encoded in their respective genes.
- Best known role of proteins: enzymes – catalyse chemical reactions
- Many involved in cell signalling and signal transduction.
- Antibodies are protein components of the adaptive immune system – main function to bind antigens or foreign substances in the body and target them for destruction.
- Structural proteins confer stiffness and rigidity to otherwise fluid biological components.
- Hepatocytes synthesis many plasma proteins, complement proteins also synthesised by macrophages, immunoglobulins mainly derived from β-lymphocytes of the immune system.
Proteins measured in the lab

- **Enzymes** - ALT, AST, CK, Lipase, Amylase etc...
- **Acute phase proteins** – C-reactive Protein (CRP)
- **Transport proteins** – Caeruloplasmin, transferrin, SHBG, Albumin
- **Immune system components** - Immunoglobulins (Ig A, G, M, D and E), complement, β₂ Microglobulin (B₂M), cryoglobulin
- **Protease Inhibitors** – Alpha-1-Antitrypsin
- **Hormones**
- And many more...

- **Variety of different sample matrices:**
  - Serum, plasma, urine, CSF, other fluids
Acute Phase Response (APR)

- The acute phase response is a complex systemic early-defence system activated by trauma, infection, stress, neoplasia, and inflammation.

- Series of cellular and humoral responses that act together to **initiate and control the inflammatory reaction** and remove damaged tissues/foreign substances

- Defense mechanism divided into 2 main groups:
  - **The inflammatory response**
    - Non-specific, change in permeability of cell membranes
    - Dependent on 1. humoral factors e.g. acute phase reactants, complement or cytokines released in response to inflammation and 2. phagocytic cellular response
  - **The immune response...**
The Immune response

- **Immunoglobulins (Igs)**, or antibodies, are glycoprotein molecules produced by plasma cells (white blood cells).

- Act as a critical part of the immune response (+ complement proteins and acute phase reactants e.g. CRP) by specifically recognizing and binding to particular antigens, such as bacteria or viruses and aiding in their destruction.

- **Structure:**
  - Y-shaped molecules composed of two different kinds of polypeptide chain; heavy chain (x2) and light chain (x2) joined by disulfide bonds
  - Each **heavy chain** is linked to a **light chain** and the two heavy chains are linked together.
  - The 2 heavy chains and the 2 light chains are identical, giving an **antibody** molecule two identical antigen-binding sites and the ability to bind simultaneously to 2 identical structures.

- Classified by isotype that differ in function and antigen responses primarily due to structure variability...
Immunoglobulins

Five major isotypes:

- **IgA** - Neutralizing antibody - preventing invading pathogens by attaching and penetrating epithelial surfaces (exists as monomer or dimer).
- **IgG** - Key player in the humoral immune response. Can activate the complement system. Phagocytosis of microorganisms.
- **IgM** - The first antibody built during an immune response and is responsible for agglutination and cytolytic reactions (exists as monomer or pentamer).
- **IgD** – Functions to signal the B cells to be activated. By being activated, they are ready to take part in the defense of the body in the immune system.
- **IgE** - Allergic reactions, parasitic infections, and hypersensitivity reactions.
Acute Phase Proteins

- Pronounced changes in concentrations of plasma proteins in response to e.g. infection, tissue injury, inflammation.
  - Due to increased protein synthesis in liver in response to cytokines.
- Increased concentration of **positive acute phase proteins**:
  - C-Reactive protein (CRP)
  - Fibrinogen
  - Serum amyloid A. Also...
  - Caeruloplasmin
  - $\alpha_1$-Antitrypsin
  - Haptoglobin
  - Ferritin
- Decreased concentration of **negative acute phase proteins**:
  - Albumin
  - Transferrin
  - C3
C-Reactive Protein (CRP)

- So called because it reacts with C-polysaccharide of pneumococci.
- Combines with bacterial polysaccharides or phospholipids released from damaged tissue to become an activator of the complement pathway.
- Plasma concentrations rise rapidly in response to acute inflammation (6h after insult)
  - Conc. related to extent and severity of inflammation
- Clinical uses of this test:
  - **To detect infection** (useful in early detection of acute infection, mostly bacterial) – more specific than ESR
  - **Guide to severity of connective tissue disease** activity e.g. rheumatoid arthritis
  - **Diagnosis/monitoring of IBD** – Crohn’s/Ulcerative Colitis
  - **Cardiovascular risk indicator** – hsCRP (sub-clinical inflammation).
Complement

- Macrophages and hepatocytes synthesis a group of proteins called the **complement system**, as part of the innate immune system.

- Activation of complement system results in attraction of phagocytes to area of inflammation (chemotaxis)
  - Increases capillary wall permeability to cellular and chemical components, allowing them to reach affected cells.

- Results in lysis of foreign cell surfaces and, together with immunoglobulins, some complement proteins enhance phagocytosis.

- Circulate in inactive form (due to inhibitors).

- **Clinically most important complement protein = C3.**

- **Two main pathways of activation:**
  - Classical pathway
  - Alternative pathway
  - Both result in low plasma C3 concentrations.
Other commonly measured specific proteins...
\[ \beta_2 - \text{Microglobulin} \]

- Also known as \textbf{B2M} is a component of MHC class I molecules, which are present on all nucleated cells (excludes red blood cells).
- Cleared from plasma by glomerular filtration followed by tubular reabsorption and catabolism.
  - Plasma concentrations reflect cell turnover and renal function.
  - Concentrations increase in conditions where there is increased cell turnover e.g. malignancy (lymphoid), acquired immune deficiency syndromes, inflammation.
- \textbf{Important prognostic indicator in myeloma} (non-specific, therefore not useful for diagnosis).
- May also help to determine disease severity, progression/tumour burden and to evaluate effectiveness of treatment.
α₁-Antitrypsin

- A glycoprotein, part of a family of serine protease inhibitors (anti-protease). Synthesised in the liver.
- Constitutes 90% of serum α₁ globulin seen in protein electrophoresis.
- Controls the inflammatory response to minimise damage to host tissue.
  - Protects tissues from effects of neutrophils.
- **α₁-antitrypsin deficiency** associated with pulmonary and liver disease at any age.
  - Accumulation of abnormal protein causing inflammation and damage, often progressing to cirrhosis in liver (mechanism ill understood).
- Genetic variants of α₁-antitrypsin characterised by their different electrophoretic mobilities on Isoelectric Focusing:
  - Normal phenotype = protease inhibitor MM (PiMM)
  - Homozygote for Z variant = PiZZ (seen in most patients with clinical disease)
  - Heterozygote phenotype = PiMZ and PiSZ
  - S phenotype mainly associated with lung disease
  - Z phenotype strongly associated with liver and lung disease.
- **Initial screening test = plasma [α₁-antitrypsin] – low or zero.**
  - NB: raised concentrations during acute phase
Caeruloplasmin

- Principle Cu-containing protein in plasma.
- Ferroxidase activity essential for oxidation of Fe(II) to Fe (III).
- Important role in Wilson’s Disease:
  - Rare inborn error of Cu metabolism, resulting in Cu deposition in organs – liver (acute hepatitis, cirrhosis), basal ganglia of brain, cornea of eye (colouration).
  - Autosomal recessive mutation of Cu-transporting ATPase gene ATP7B, >100 mutations worldwide
  - Result = failure to incorporate Cu into caeruloplasmin and biliary excretion of Cu, reduced circulating [caeruloplasmin]
  - Presentation in children is mainly hepatic problems, in young adults more neurological problems.
  - Investigation/diagnosis: Low serum Cu and caeruloplasmin (NB: Caer can be increased in acute phase), increased urine Cu excretion (24 hr urine Cu, penacillamine chelating test), abn LFTs, gold standard = liver biopsy.
Cryoglobulins

- Proteins that precipitate out of serum at temperatures below body temperature (37°C)
- Most cryoglobulins are either:
  - **Monoclonal immunoglobulins** - typically IgM (but IgG, IgA or rheumatoid factors are also found), or
  - **Polyclonal** - usually immune complexes containing more than one class of immunoglobulins, e.g. IgM rheumatoid factor antibody bound to IgG.
- Can occur in any conditions in which there are high concentrations of immunoglobulins e.g. Systemic Lupus Erythematosus (SLE).
- The insoluble fibrillar protein complexes in Amyloid Disease may also cause Cryoglobulinaemia.
- **Cryoglobulinaemia can cause clinical symptoms (Raynaud’s Syndrome) when precipitation occurs above 21°C:**
  - Intolerance to cold, purpura, gangrene of the extremities and skin sores.
Cryoglobulin Determination

- Blood collected, transported and separated at above 37°C (flask filled with sand stored at 50°C).
- Serum divided into two aliquots; stored at 4°C to encourage cryoglobulin precipitation, and at 37°C to prevent cryoglobulin precipitating from the serum.
- Specimens visually inspected for up to 7 day for any signs of precipitation/gel formation.
- If sample positive and forms adequate amount of cryo-precipitate than that sample will be further investigated and typed:
  - Cryo-precipitate washed and reconstituted in saline.
  - Protein electrophoresis (SPE) performed to identify any monoclonal or polyclonal components.
  - Immunotyping (IT) to identify the presence of IgM, IgG, IgA or kappa/lambda light chains.
  - SPE and IT by capillary electrophoresis
  - Cryoglobulin classified according to the immunoglobulin present in the precipitate (Type 1, 2, 3).
Paraproteinaemia

- **Paraprotein** – an immunoglobulin (protein) produced by a single clone of myeloma cells (B cells), most frequently plasma cells, found in excess in the blood and/or urine. Since all molecules identical, seen as discrete band (usually in γ region) on serum electrophoresis.

- Paraproteins can be found in both malignant and non-malignant conditions including:
  - Multiple myeloma (associated with older adults).
  - Waldenström’s Macroglobulinaemia (non-hodgkin lymphoma) - macroglobulin = IgM, symptoms related to macroglobulin (increased plasma viscosity).
  - Monoclonal Gammopathy of Undetermined Significance (MGUS) - Pre-malignant (benign).

- **Hyperviscosity syndrome** - a group of symptoms triggered by increase in the viscosity of the blood.
  - Symptoms: spontaneous bleeding from mucous membranes, visual disturbances due to retinopathy, neurologic symptoms (headache, vertigo, seizures, coma).
  - Occurs from pathologic changes of cellular/protein fractions of blood found in polycythaemias, multiple myeloma, leukaemia, monoclonal gammopathies, (e.g. Waldrenströms, sickle cell anaemia, and sepsis).
Myeloma

- Malignancy of plasma cells, usually in marrow (of lumbar spine, skull, long bones, pelvis).

- Variants:
  - IgG, IgA, IgM, IgD, IgE
  - Light chain (kappa $\kappa$ or Lambda $\lambda$) myeloma
  - Heavy chain disease
  - Biclonal gammopathy
  - Non-secretory myeloma

- **Clinical Presentation**: generally bone pain (back, ribs), pathological fracture/osteolytic lesions (“pepper pot skull” on x-ray, hypercalcaemia, recurrent infections, fatigue/anaemia

- **Diagnosis 2/3 of**:
  - Serum and/or urine paraprotein
  - Osteolytic lesions on x-ray
  - >25% plasma cells in marrow (or clonal population)

- **Treatment**: (symptomatic, complications)
  - Chemotherapy
  - Consider BM transplant
Myeloma: Investigation

- **Biochemistry:**
  - Serum immunoglobulins (turbidimetry)
    - IgG >20g/L, IgA/M >10 g/L, Ig D/E paraprotein @ any conc.
  - Serum protein electrophoresis (gel or capillary elect) if identify abnormally high/low immunoglobulins
  - Quantification of paraprotein by densitometry (relate density of band to measures total protein)
  - Urine electrophoresis for Bence Jones Protein (BJP) or light chains
  - Confirmation/typing of paraprotein in serum or urine by immunofixation (if present)
  - ?serum Free Light Chains

- **Other Investigations:**
  - FBC/Hb (anaemia)
  - ESR (inc. due to high [protein])
  - Calcium (hypercalcaemia)
  - B2M (prognosis)
  - BM biopsy/aspirate (to study plasma cells)
  - Skeletal survey (bone/skull x-ray)
Myeloma: Complications & Prognosis

- **Complications:**
  - Increased risk of chronic renal failure - tubular damage due to accumulation of free light chains (BJP)
  - Increased risk of acute renal failure – hypercalcaemia due to localised action of cytokines stimulating osteoclastic bone resorption (therefore measure urea/cre to investigate poss. Renal impairment)
  - Fractures/lesions - Bone x-rays, skeletal survey, MRI or CT
  - Immunoparesis/ increased risk of infection - due to suppressed Immunoglobulin levels
  - Increased plasma viscosity

- **Poor prognosis indicated by:**
  - Presence of urine BJP
  - Low serum albumin (<30 g/L – inverse correlation with tumour burden)
  - Anaemia (normochromic, normocytic, BM failure)
  - Increased B2M (increases as tumour mass increases)
  - Also presence of hyperCa, renal dysfunction, progressive increase in [paraprotein]

- **Monitor by:**
  - Paraprotein quantification by densitometry (related to tumour burden)
    - Can’t use turbidimetry/nephelometry for Igs quantification
MGUS

- “Monoclonal Gammopathy of Undetermined Significance”.
- Non-malignant, benign (pre-malignant).
- Presence of paraprotein in absence of features of myeloma:
  - Paraprotein <30 g/L, no associated FLCs.
  - Plasma cells in BM <10%.
- Repeat investigations in 6 months to see if paraprotein size has increased.
Urine Proteins

- **Proteinuria** – presence of excess serum proteins in the urine.
  - Often causes urine to become ‘foamy’.

- **Causes of proteinuria**:
  - **Pre-renal** – heavy exercise, fever, hypertension, multiple myeloma, pre-eclampsia
  - **Renal** – acute and chronic glomerulonephritis, renal tubular dysfunction, polycystic kidney, nephrotic syndrome
  - **Post-renal** – acute and chronic cystitis, tuberculosis

- **Key proteinuria mechanisms**:
  - **Overflow** – high conc. of low MW protein filtered in quantities exceeding tubular reabsorptive capacity (e.g. Urine BJP)
  - **Glomerular** – increased glomerular permeability (e.g. Albumin)
  - **Tubular** – impaired/saturated reabsorption of protein filtered by normal glomeruli (e.g. B2M)

- Measurement of protein in urine important to diagnose, stage and monitor chronic kidney disease (together with GFR).
  - NICE: Albumin:Creatinine Ratio (ACR) should be used in preference to Protein:Creatinine Ratio (PCR) or 24 hr urine TP and dipstick.
Urine Bence Jones Protein (BJP)

- Bence Jones protein is a **monoclonal globulin or immunoglobulin light chain (kappa or lambda) found in the urine** (MW 22-24 kDa).
  - Free light chains (FLCs) have a low molecular weight and are freely filtered at the glomeruli and largely reabsorbed by proximal tubular cells. However when there is overproduction the reabsorptive capacity is exceeded and they pass into the urine.
- Detection of urine BJP may be suggestive of multiple myeloma or Waldenström’s macroglubulinaemia.
  - Monoclonal free light chains may be found in myeloma in addition to a serum paraprotein or may occur in isolation (i.e. in light chain only myeloma, seen in approximately 2% myeloma cases where undetectable in serum but detectable in urine).
- These FLCs may eventually cause renal impairment due to deposition of the protein in renal tubular cells and can cause formation of large casts and the characteristic myeloma kidney.
- Identified by urine electrophoresis and typed as either kappa (κ) or Lambda (λ) by urine immunofixation.
Protein Separation and Analysis

- Variety of laboratory methods to separate/measure proteins...
  - Electrophoresis (gel, capillary)
    - Immunofixation
  - Chromatography
  - Nephelometry
  - Turbidimetry
  - Immunoassay
Protein Separation

- Properties of proteins that aid separation:
  - Molecular weight (MW)
  - Charge
  - Solubility
  - Affinity
Electrophoresis

- **Definition**: Migration of all charged particles in a liquid medium under the influence of an electric field.
- Used to separate range of ionised analytes e.g. proteins, amino acids, nucleic acids, organic acids.
- Rate of migration dependent on:
  - Net electrical charge of particle
  - Particle size and shape
  - Electric field strength
  - Interaction with support medium (e.g. endo-osmosis, wick flow effects)
  - Temperature
- Migration of proteins based on charge-to-mass ratio
  - Proteins possess aa residues which can donate/accept protons and alter their overall charge, depending on pH of surrounding environment (buffers).
Electrophoresis - Components

- **Support media** (e.g. agarose, polyacrylamide gel) – in contact with buffer via wicks, where separation takes place
- **Buffers** (pH/Ionic strength)
- **Electrodes connected to power supply** (provides electric field)
  - As current passes through support medium, proteins will migrate towards anode (+) or cathode (-), depending on charge
- **Protein visualisation** method (stains, UV detection for CE)
- **Quantification** method (e.g. densitometry)
Capillary Electrophoresis

- **Definition:** A family of related techniques including capillary zone electrophoresis (CZE), Isoelectric Focusing (IFE) and capillary gel electrophoresis.
  - Exploit liquid phase of HPLC and the electric field of electrophoresis
- Solid support = **fine bore fused silica capillary** with an inherent negative charge on the surface of the capillary
- Mechanism responsible for protein separation = **electro-osmotic flow**
- Detection by UV, MS, chemiluminescence etc.
Electro-Osmotic Flow

- **Negatively charged proteins attracted to the cathode (-)** by a strong endo-osmotic flow of positive buffer ions (put buffer through capillary with positive charge) and electrical field.

- Separation of proteins results in differences in migration back to the anode (+)

- Therefore gamma globulins elute first (*good separation in gamma region*) – **ideal for Immunoglobulin separation**
  - I.e. cathodic movement of gamma globulins (despite neg. charge) from point of origin
Order of proteins detected is in the following manner, start with Gamma globulins and ending with albumin:

- **Gamma(γ)-globulins**: Immunoglobulins (some also found in the α₂ and β regions)
- **Beta(β)-globulins**: β₁ consists mainly of transferrin and some LDL, β₂ consists of C3 component of complement.
- **Alpha(α)₂-globulins**: mainly α₂-macroglobulin and haptoglobin.
- **Alpha(α)₁-globulins**: almost entirely α₁-antitrypsin and α₁-antichymotrypsin.
- **Albumin**: usually a single protein, makes up the obvious band.

Note: If plasma used rather than serum (i.e. not clotted): fibrinogen appears in β-γ region.
Serum Electrophoresis: Patterns in Disease

- **Acute Phase Reaction**: Synthesis of acute phase proteins – increased $\alpha_1$ and $\alpha_2$ globulins
  - Responsible for rise in ESR and increased plasma viscosity.

- **Chronic Inflammation**: Diffuse rise in $\gamma$-globulins.

- **Nephrotic syndrome**: In established cases, reduced albumin, $\alpha_1$ and $\gamma$-globulins, increased $\alpha_2$ globulin.

- **$\alpha_1$-antitrypsin deficiency**: Absence or obvious reduction in density of $\alpha_1$ band.

- **Hypogammaglobulinaemia**: Reduced $\gamma$-globulins.

- **Multiple Myeloma/Paraproteinaemia**: Discrete band(s) in the in $\gamma$-globulin region.

*Fig. 19.2* Serum protein electrophoretic patterns in disease.
CE Examples

Example 1: Normal Curve (P1)

Example 2: Polyclonal Increases in Gamma Globulins (P2) on Curves
Previously on Gel Electrophoresis it is difficult to determine when a band is over a beta 2 region.
## Capillary vs Gel Electrophoresis

<table>
<thead>
<tr>
<th>Advantages of CE</th>
<th>Disadvantages of CE</th>
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<tbody>
<tr>
<td><strong>High efficiency of separation</strong> and <strong>high resolution</strong> of small and large molecules (in β and γ regions)</td>
<td><strong>Different method of electrophoresis interpretation</strong> (&quot;electrophoretograms&quot;) – requires staff re-training</td>
</tr>
<tr>
<td><strong>Multiple sample simultaneously/rapid analysis</strong> - multiple capillaries, fully automated</td>
<td><strong>High capital cost of equipment</strong></td>
</tr>
<tr>
<td><strong>No gel staining procedures</strong> – measurement by direct UV at SWBH</td>
<td><strong>No gel for record keeping</strong> – all computer records must be securely backed-up</td>
</tr>
<tr>
<td><strong>Improved linearity and real-time quantification</strong> of paraproteins (during analysis)</td>
<td><strong>Cannot identify IgD/E paraproteins by Immunosubtraction</strong> – requires Immunofixation</td>
</tr>
<tr>
<td><strong>Small sample volumes</strong> (nL)</td>
<td><strong>Increased Immunofixation rate</strong> – Increased workload</td>
</tr>
<tr>
<td><strong>Analyser interfacing with LIMS</strong></td>
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</table>
Electrophoresis: Clinical Application

- E.g. **Serum Immunotyping** to identify the *monoclonal immunoglobulin* composition of suspected paraproteins detected in serum by protein electrophoresis.
- Essential for the complete **investigation of Myeloma**. Also used to monitor **Monoclonal Gammopathy of Uncertain Significance (MGUS)**.
- Immunotyping Capillary Electrophoresis currently used at SWBH:
  - Diluted serum mixed with individual specific antisera
  - Forms large insoluble complexes with heavy and light chains which thus move more slowly then other components of the serum when electrophoresed.
  - The immune complex migrates anodically.
  - After, treated and untreated curves are overlaid to see which peaks have been removed under which antisera.
Serum Immunofixation

Clinical Application:
- Used to identify the monoclonal immunoglobulin composition of suspected paraproteins detected in serum by protein electrophoresis.
- Essential for the complete investigation of myeloma.
- Also used to monitor monoclonal gammopathy of uncertain significance (MGUS).

Method:
- Following electrophoretic separation, individual heavy and light chain immunoglobulins are identified by their reaction with specific antisera raised against gamma (IgG), alpha (IgA) and mu (IgM) heavy chains and kappa (free and bound) and lambda (free and bound) light chains, respectively.
- The resulting insoluble complexes are trapped in the gel matrix (fixed).
- Non-reacted proteins are then removed by blotting and washing and the insoluble complexes visualised with acid violet stain.
Serum Immunofixation: Results Interpretation

- **ELP G A M K L**
  - No abnormal band detected in serum by immunofixation

- **ELP G A M K L**
  - IgA Kappa

- **ELP G A M K L**
  - IgA Lambda

- **ELP G A M K L**
  - IgG Lambda

- **ELP G A M K L**
  - IgM Lambda

- **ELP G A M K L**
  - Immunofixation shows Kappa band only
  - Later identified as IgD (Kappa monoclonal band)
**Urine Electrophoresis: BJP**

- The demonstration of paraproteinaemia and immunoparesis by protein capillary electrophoresis and urine BJP are diagnostic of multiple myeloma.

- Urine protein gel electrophoresis (Sebia Hydrasys) currently used at SWBH for identification of urine BJP:
  - Proteins separated and visualised by high resolution electrophoresis using highly sensitive protein specific stain Acid Violet.
  - Method provides a rapid initial screening method for detecting abnormal bands in urine, in particular Bence Jones Protein.
Urine Electrophoresis: Results

Appendix 1  Interpretation of urine BJP electrophoresis band patterns

This is an example of a made up mixed run.

- **Lane 1/N** – FLCs not detected (normal)
- **Lane 2, 21** – Generalised proteinuria (FLCs not detected)
- **Lane 11** - Abnormal band detected – hold results and investigate further by urine Immunofixation
- **Lanes 14 15, 25, 27** – Abnormal bands detected, hold (as above)
- **T** – Would require further invx if patient
- **Pos** – Band in βγ region
Urine Immunofixation

- BJP immunofixation electrophoresis is used to identify the monoclonal kappa (κ) or Lambda (λ) free light chains in urine following the detection of abnormal bands in serum by protein electrophoresis and urine by BJP electrophoresis.

- **Method:**
  - Electrophoretic separation of urine proteins.
  - Individual heavy and light chain immunoglobulins identified by their reaction with a gamma (IgG), alpha (IgA) and mu (IgM) combined trivalent antiserum, and antiserum raised against kappa and lambda light chains (both free and bound).
  - Resulting insoluble complexes trapped in the gel matrix (FIXED).
  - Non-reacted proteins removed by blotting and washing.
  - Insoluble complexes visualised with acid violet stain.
Urine Immunofixation: Results Interpretation

Free κ light chains detected

Free λ light chains detected

Free light chains not detected
Nephlometry & Turbidimetry

- **Methods used to measure scattered light** using ‘Light Scattering Theory’:
  - A physical phenomenon resulting from interaction of light with insoluble particles in solution
  - Transmitted light reduces as the reaction progresses
  - Factors affecting: particle size, wavelength dependence, measuring angle etc..

[Diagram of light scattering apparatus]
## Nephelometry vs Turbidimetry

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<th>Nephelometry</th>
<th>Turbidimetry</th>
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<tr>
<td><strong>Definition</strong></td>
<td>Detection of scattered light that is not in the direct path of the transmitted light</td>
<td>Measures a decrease in intensity of incident light transmission caused by scattering</td>
</tr>
<tr>
<td><strong>Type of light measured</strong></td>
<td>Scattered light</td>
<td>Transmitted light</td>
</tr>
<tr>
<td><strong>Measuring angle/arrangement of photometer</strong></td>
<td>Measurement of light at right angle (90°) to direction of incident light. Could be movable detectors which allow operator to vary the angle of detection.</td>
<td>180° from the incident light (same direction as the propagation of the light from the source)</td>
</tr>
<tr>
<td><strong>Instrument used</strong></td>
<td>Dedicated Nephelometry analyser (e.g. Siemens Dade Behring BN II Nephelometer)</td>
<td>Spectrophotometer</td>
</tr>
<tr>
<td><strong>Advantages</strong></td>
<td>Better sensitivity than turbidimetry (particularly for small immune complexes) – superior at &lt;20mg/L (no advantage over turbidimetry above this)</td>
<td>Performed on routine chem analysers, high throughput, accurate and precise</td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
<td>Requires dedicated analyser, cost and TAT implications, antigen (ag) excess/hook effect</td>
<td>Lower sensitivity compared with nephelometers (but improving with technological advances), ag excess</td>
</tr>
<tr>
<td><strong>Clinical use/examples</strong></td>
<td>Ag-AB rxn, immunocomplex rxn, lipoprotein (e.g. serum FLCs, IgG subclasses)</td>
<td>Ag-Ab rxn, immunocomplex rxn (e.g. CRP, urine and CSF protein, IgA, IgG, IgM, C3, C4, A1AT, B2M)</td>
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CSF Proteins

- CSF is a clear body fluid produced by the choroid plexuses that occupies the subarachnoid space and ventricular system around and inside the brain.
  - Acts as a “cushion” for the cerebral cortex of the brain
- CSF samples obtained by lumbar puncture.
- **CSF composition:**
  - Less protein/bilirubin and similar electrolyte composition when compared with plasma
  - **CSF total protein** (albumin, IgG, transthyretin) **derived from plasma proteins (80%) and manufacture within the brain (20%).** Increases as a result of a breakdown of the integrity of the blood-brain barrier
  - E.g. Froin’s syndrome (block to spinal circulation of CSF), Guillain Barre syndrome (deposition of immune complexes), multiple sclerosis.
Multiple Sclerosis (MS)

- Autoimmune condition in which immune system attacks the central nervous system (CNS) leading to **demyelination**.
- Neurological symptoms occur in discrete attacks or progressively.
- Clinical diagnosis difficult, although MRI can form part of the diagnosis.
- Biochemically, the hallmark of MS is the **presence of oligoclonal IgG bands in CSF** (absent from paired serum)
  - Found in at least 98% of patients with MS
  - BUT not specific for MS – can be found in other inflammatory and autoimmune conditions affecting the brain
- Presence of oligoclonal bands and a CSF protein >1g/L points to a diagnosis other than MS.
Oligoclonal Bands by IFE

- CSF oligoclonal band determination by **Isoelectric Focusing (IFE)** coupled to nitrocellulose immunoblotting (Western blotting) or immunofixation.

- IFE is a type of electrophoresis technique for separating different molecules by differences in their isoelectric point (dependent on pH of surroundings)
  - Significantly more sensitive at detecting CSF IgG than quantitative methods.

**Results interpretation:**
- No oligoclonal bands = normal
- Presence of oligoclonal bands in CSF and not serum = MS (local synthesis)
- Presence of bands in both CSF and paired serum = systemic response due to inflammation
Further Reading

- Kit Inserts – CRP, A1AT, B2M, Urine/CSF protein, Caer, Ig A/G/M etc...
- SOPs on iPassport:
  - Immunotyping of Serum Proteins by Sebia Capillrys (Serum protein electrophoresis)
  - Serum/urine immunofixation electrophoresis
  - Urinary Bence Jones Protein by Capillary Electrophoresis
  - Bence Jones Protein - Sebia Hydrasys LC
  - Cryoglobulin identification and typing