# BHARATHIDASAN UNIVERSITY

Tiruchirappalli- 620024,

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## Programme: M.Sc., Biomedical Science

**Course Code: 18BMS59C17**

**Course Title: Immune & Molecular Diagnostics**

**Unit-III**

**Measurements of specific proteins in serum, CSF & Urine**

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**Unit III:**

Measurements of specific proteins in serum, CSF & Urine- Immunoglobulins (Igs), paraproteins & cryoglobulins, investigation of complement disorders- assays for individual components (EIA, RIA), Functional assay for complement pathways- AH50, C50- detection of complement breakdown products- C3nephritic factor, Functional assays for immune complexes. Clinical significance of C- Reactive proteins- cryoglobulins in patient specimens- test methods for detection. Tests for allergy- Total serum IgE, allergen specific IgE- serology based methods-in vivo-skin prick test, in- vitro- RAST ($1^{st}$, $2^{nd}$ & $3^{rd}$ generation methods)- cell based methods- Allergan induced mediator release assay- histamine release, LTC4 release (Cellular antigen Stimulation Test- CAST), Flow cytometric basophil activation assay (Flow Assay Stimulation Test- FAST)- CD63 CD203c, test for hypersensitivity III- precipitating antibodies.

# PRESENTATION: 2

# C- REACTIVE PROTEIN

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- Synthesize primarily in hepatocytes
- CRP Gene- Chromosome 1, Short arm
- Few cytokines especially, IL-6 and IL-1 regulate CRP at transcritional level
- Normal range: Less than 10mg/L
- It has highest affinity for,

    Phosphocholine on bacteria

    Mixture of sphingomyeline and phosphatidylcholine in eukaryotic membranes

# C- REACTIVE PROTEIN

- CRP can recognize self ligands,
  - Plasma lipoproteins
  - Damaged cell membrane
  - Several phospholipids
  - Small nuclear ribonucleoprotein components
  - Apoptotic Cells
- CRP Also bind to some extrinsic ligands,
  - Phospholipid
  - Capsular/cell body components of bacteria, fungi and parasite and
  - Plant particles

# Methods for the detection of CRP

- ELISA
- Immunoturbidimetry
- Rapid immunodiffusion and
- Visual Agglutination

# CLINICAL IMPORTANCE CRP

- **Elevated CRP levels:**
  Osteoarthritis
  Predictive of coronary events (esp. in stable angina)
  Proinflammatory or prothrombotic effects

- **Mild elevation of CRP levels:**
  SLE
  Scleroderma
  Sjogren syndrome
  Dermatomyositis/Polymyositis

# CLINICAL IMPORTANCE CRP

**Normalization of CRP levels:**

- Helpful tool in determining the response to Antibiotic therapy and duration of treatment

**Transplant cases,**

- Elevated levels were seen in majority of kidney or heart transplant
- Highly elevated in GVHD
- Changes in levels are not organ specific, like other inflammatory conditions

# CLINICAL IMPORTANCE CRP

- **Cerebral vein or sinus thrombosis:**

  An increase CRP is associated with a poorer short- term prognosis

- **Giant cell arteritis:**

  Thrombocytosis with platelet counts >400,000/μL and CRP levels >2.45 mg/dL have been found to be to the strongest laboratory predictors of a positive temporal artery biopsy.

- **Pancreatitis:**

  Level peaks 3 days after onset of pain

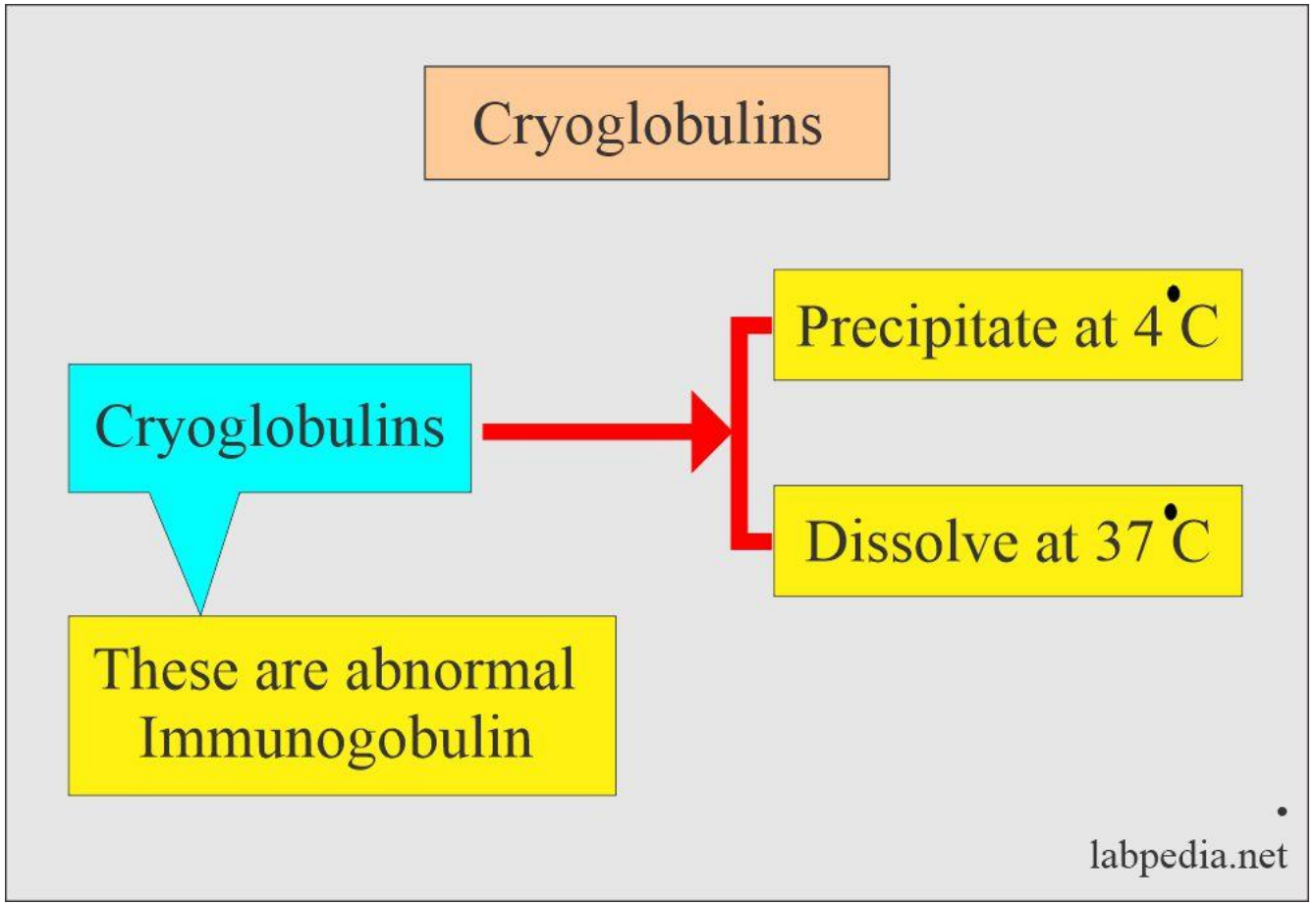
  At 48 hours, sensitivity = 65–100%, PPV = 37–77%.

  Level of 150 mg/L distinguishes mild from severe disease.

# CLINICAL SIGNIFICANCE OF CRYOGLOBULINS

# INTRODUCTION

- The term cryoglobulin’ was coined by Lerner and Watson in 1947.
- Precipitation of cryoglobulins is dependent on temperature, pH, cryoglobulin concentration, and weak noncovalent factors.
- Cryoglobulins - immunoglobulins that precipitate in vitro at temperatures < 37°C and redissolve after rewarming.
- Cryoglobulinemia - refers to the presence of cryoglobulins in serum.
- Cryoglobulinemic disease or cryoglobulinemic vasculitis - used to describe patients with symptoms related to the presence of cryoglobulins.

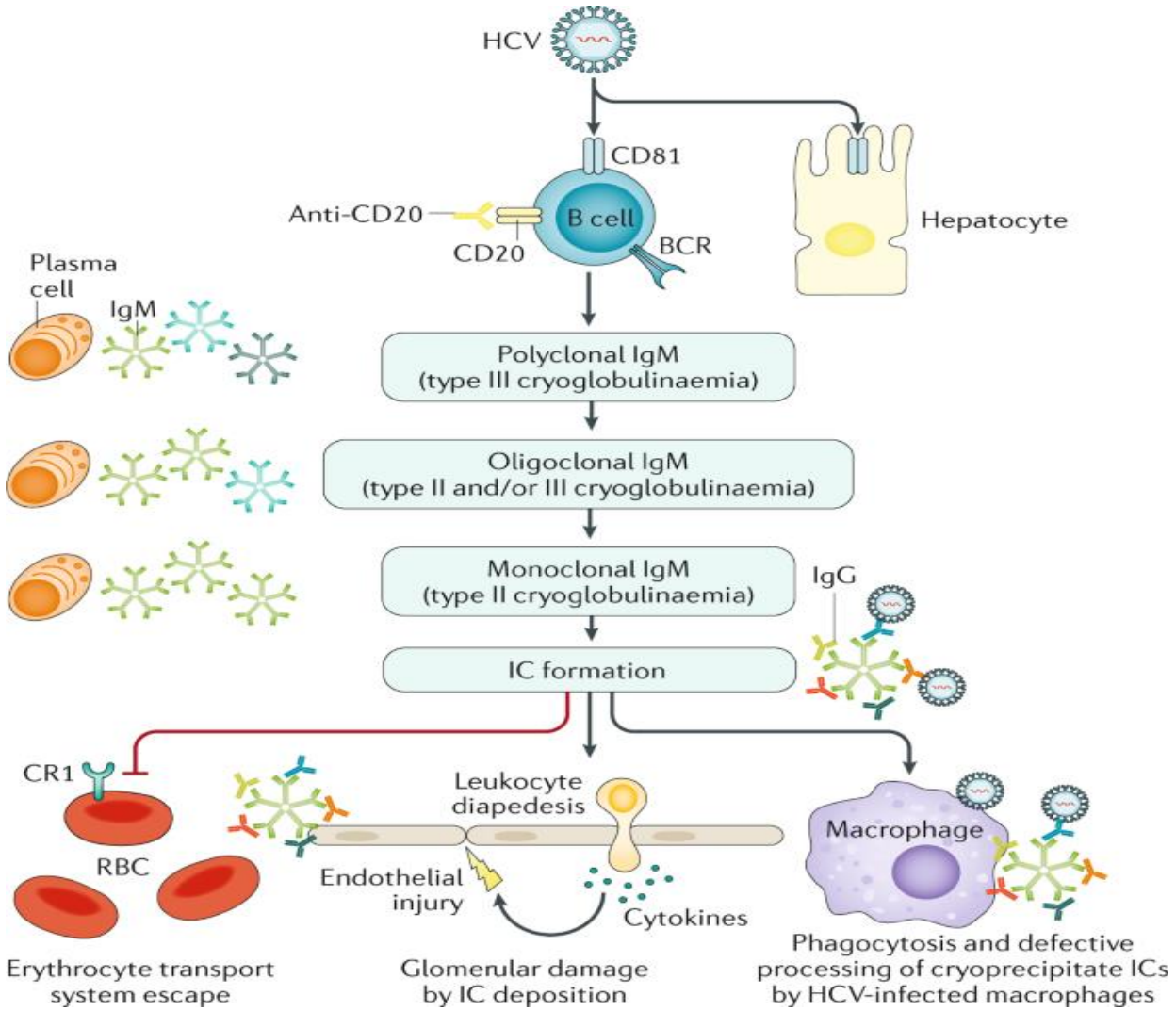

Cryoglobulins
Cryoglobulins
Precipitate at 4°C
Dissolve at 37°C
These are abnormal Immunogobulin
labpedia.net

# TYPE I CRYOGLOBULINEMIA

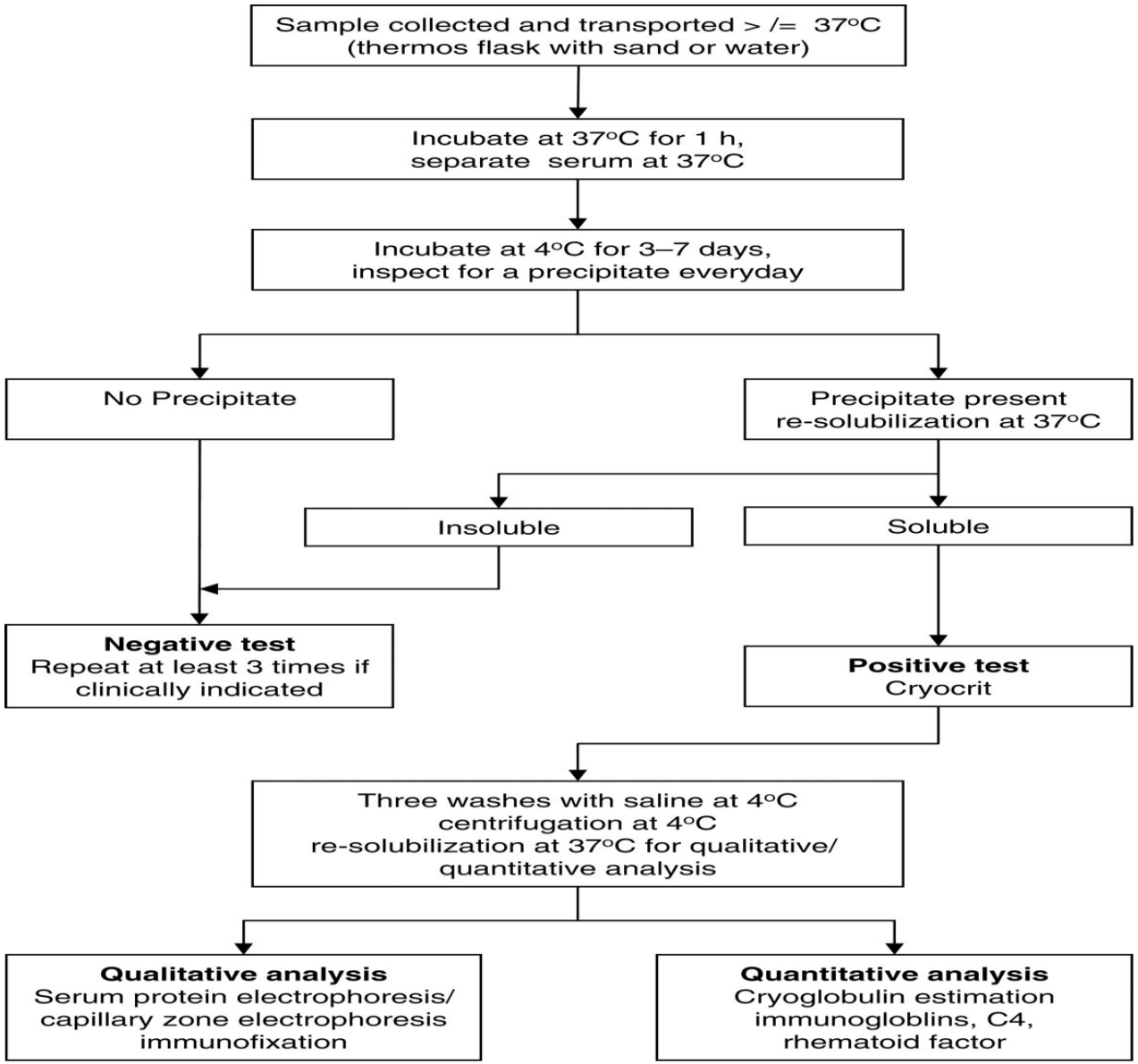
- With increase in  amount of B  lymphocytes the production of antibodies increases ( monoclonal antibodies)– IgM and IgG
- Ig’s are proteins, with increase in Ig’s total protein count level increase.
- With increase in serum protein concentration –Erythrocyte sedimentation rate increase
- With increase in protein level – blood viscosity increases
- The higher is the viscosity – the higher is the risk of thrombosis

  - Thrombosis in digits – Raynaud phenomenon
  - Thrombosis in cerebral circulation – blurry vision
  - Thrombosis in digits – distal ulcers
  - Thrombosis in peripheral arteries- peripheral neuropathy

# TYPE II CRYOGLOBULINEMIA



# DIAGNOSIS OF CRYOGLOBULINEMIA



# BLOOD TEST

- Any haemolysis or lipaemia should be noted since this may interfere with visual interpretation of cryoprecipitation.
- In patients who are receiving heparin, fibronectin–heparin complexes may lead to false-positive cryoprecipitation.
- Recommend collecting an EDTA sample at the same time as the serum routinely to exclude cryofibrinogenaemia as cryoglobulins and cryofibrinogens can lead to similar clinical symptoms and signs.
- Two aliquots of separated serum should be incubated for at least three days, one at 4°C and the other at 37°C and preferably for seven days and visually inspected every day.
- Type I cryoglobulins tend to precipitate within hours and become apparent by the next day.
- Mixed cryoglobulins at low concentrations may take several days to precipitate.
- A cryoprecipitate may be seen as gelatinous, flocculent, crystalline material or sometimes as a dusty hue.

# RE DISSOLUTION

- Re-dissolution of the cryoglobulin precipitate by re-warming to 37°C is very important.
- If the precipitate is not resoluble within a few minutes then the result is negative– no further analysis is required.

# SERUM PROTEIN ELECTROPHERESIS

**1. Sample Collection**

**2. Centrifugation**

**3.Sample Preparation**

**4. Gel Preparation**

**5. Loading Samples**

1. Load the prepared serum samples into wells on the gel.

**6. Electrophoresis**

1. Submerge the gel in an electrophoresis buffer.

2. Apply an electric current to separate proteins based on their charge and size.

**7. Staining**

1. After electrophoresis, stain the gel with a protein-specific stain (e.g., Coomassie Blue) to visualize protein bands.

**8. Destaining**

1. Rinse excess stain from the gel with a destaining solution.

**9. Drying**

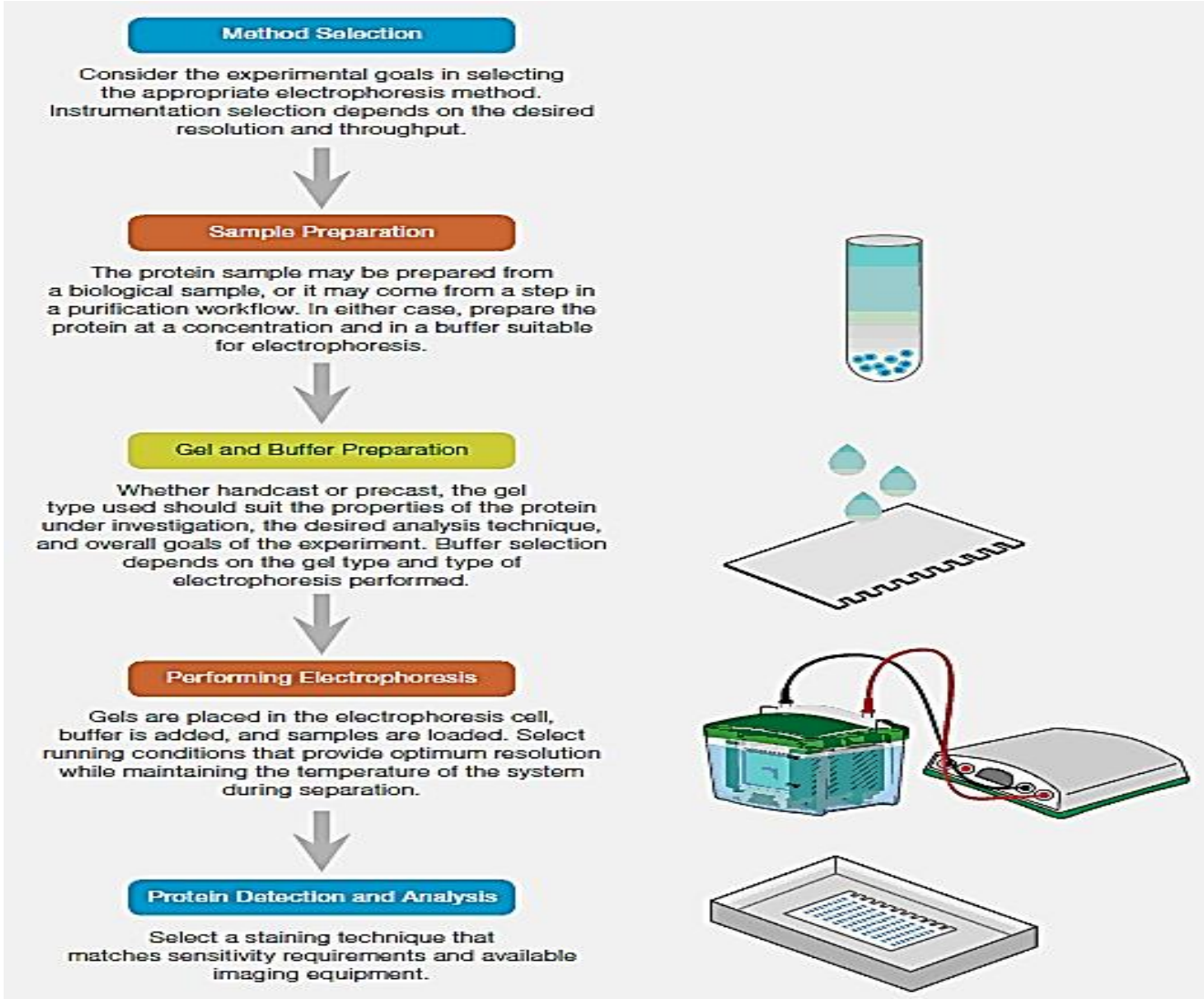
**10. Interpretation**

1. Examine the gel to identify protein bands.
2. Measure the distance traveled by each protein band from the origin.

**Reporting**

1. Generate a report summarizing the protein fractions (e.g., albumin, alpha-1, alpha-2, beta, and gamma globulins) and any abnormalities detected.

# SERUM PROTEIN ELECTROPHERESIS



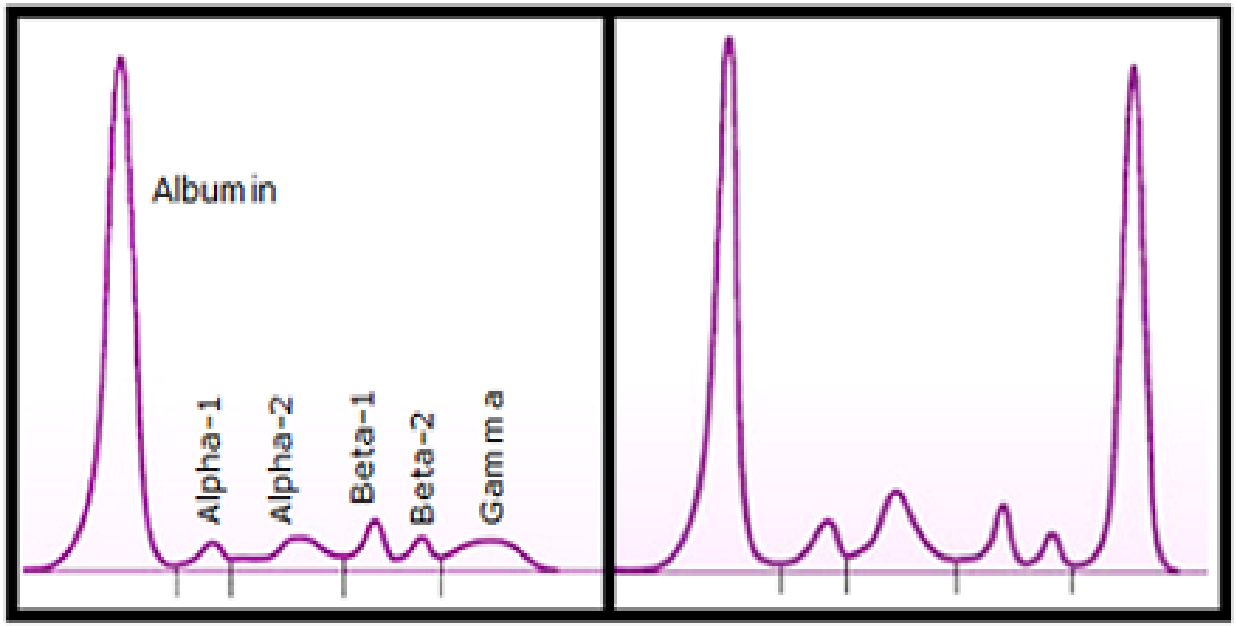



*Figure 3: Protein categories in a normal serum electrophoresis trace (left) and abnormal monoclonal protein (right) seen in a Myeloma patient*

# IMMUNO FIXATION

- **Immuno Fixation:**
  1. After electrophoresis, cut the gel into strips or segments for further analysis.
  2. Incubate each gel strip in separate trays with specific antisera:
     1. One tray with anti-IgG antiserum.
     2. One tray with anti-IgM antiserum.
     3. One tray with anti-IgA antiserum.
  3. Incubate the gel strips at 4°C or room temperature for an appropriate duration (typically 1-2 hours to overnight) to allow antigen-antibody reactions to occur.

- **Washing and Staining:**
  1. Wash the gel strips to remove excess antisera.
  2. Stain the gel strips with an appropriate staining solution (e.g., Coomassie Brilliant Blue or Ponceau S stain) to visualize the protein bands.

- **Interpretation:**
  1. Examine the stained gel strips for the presence of bands or precipitation lines indicating cryoglobulins and their respective immunoglobulin classes (IgG, IgM, IgA)

# Test for allergy- total serum Ig E, Allergen specific IgE

- Allergy testing is crucial for identifying specific allergens responsible for hypersensitivity reactions. Various tests are available to detect the presence of immunoglobulin E (IgE) antibodies, which are central to allergic responses.
- These tests include **Total Serum IgE**, **Allergen-Specific IgE** (via serology and cell-based methods), and **Skin Prick Test**. In-vitro methods like **RAST (Radioallergosorbent Test)** and its subsequent generations have also evolved to improve accuracy and specificity.

# 1. Total Serum IgE

## a. Overview

- Total serum IgE measures the overall level of IgE in the blood. Elevated IgE levels are associated with allergic diseases, parasitic infections, and some immunodeficiencies.

## b. Clinical Indications

- Helps in the diagnosis of atopic diseases like asthma, allergic rhinitis, and eczema.
- Used in conjunction with other tests to confirm allergies or monitor allergic conditions.

## c. Limitations

- Total IgE levels can be elevated in non-allergic conditions.
- It does not specify the allergen responsible for the allergic response.

# 2. Allergen-Specific IgE Testing

## a. Overview

- Allergen-specific IgE tests identify the presence of IgE antibodies against specific allergens. These tests can be performed in vivo (e.g., skin prick test) or in vitro (e.g., serology-based methods).

## b. In Vivo: Skin Prick Test (SPT)

### i. Principle

- A small amount of allergen is introduced into the skin, usually on the forearm or back.
- If the patient is sensitized to the allergen, a localized allergic reaction (wheal and flare) occurs within 15-20 minutes.

### ii. Procedure

1. **Preparation**: The skin is cleaned, and allergens are applied using a small lancet.
2. **Application**: Positive (histamine) and negative (saline) controls are included.
3. **Observation**: The size of the wheal is measured to determine the presence of an allergic response.

## iii. Advantages

- Quick and relatively inexpensive.
- Provides immediate results.

## iv. Limitations

- Risk of systemic reactions, although rare.
- Antihistamines and certain medications can interfere with results.

# c. In Vitro: Serology-Based Methods

## i. Radioallergosorbent Test (RAST)

- RAST was one of the first methods used to detect allergen-specific IgE in the blood.
- **1. First-Generation RAST**
- Used radioactively labeled anti-IgE to detect IgE bound to allergens.
- Limited by low sensitivity and safety concerns due to the use of radioactive materials.

- **2. Second-Generation RAST**
- Improved sensitivity by using enzyme-linked detection systems instead of radioactive labels.
- Utilized enzyme-linked immunosorbent assays (ELISA) for detection.
- **3. Third-Generation RAST (ImmunoCAP)**
- Further improvements with the use of fluorescent markers (e.g., ImmunoCAP).
- Enhanced sensitivity and specificity.
- Uses a solid-phase immunoassay with allergen-coated cellulose sponges to capture IgE.

## ii. Procedure for In Vitro Tests

1. **Sample Collection**: Blood is drawn from the patient.
2. **Incubation**: Serum is incubated with allergens bound to a solid phase.
3. **Detection**: IgE bound to allergens is detected using labeled anti-IgE antibodies.
4. **Quantification**: The amount of allergen-specific IgE is quantified and reported in kU/L.

**iii. Advantages**

- Safe for patients with severe allergies, as there is no risk of an immediate allergic reaction.
- Not influenced by antihistamines or other medications.

**iv. Limitations**

- More expensive and time-consuming compared to in vivo tests.
- Results can be influenced by technical factors, requiring well-standardized procedures.

# 3. Cell-Based Methods

## a. Basophil Activation Test (BAT)

- This test measures the activation of basophils (a type of white blood cell) in response to allergens.

### i. Principle

- Basophils express surface markers (e.g., CD63) upon activation by allergens.
- Flow cytometry is used to detect the expression of these markers, indicating an allergic response.

### ii. Procedure

1. **Sample Collection**: Blood is collected from the patient.
2. **Stimulation**: Basophils are exposed to allergens in vitro.
3. **Detection**: Activated basophils are identified using flow cytometry, based on the expression of activation markers.

**iii. Advantages**

- Provides functional information about basophil reactivity.
- Useful for diagnosing allergies when conventional tests are inconclusive.

**iv. Limitations**

- Requires specialized equipment and technical expertise.
- Not widely available and more costly.

# 4. Comparison of Allergy Testing Methods

| Test | Type | Advantages | Limitations |
|---|---|---|---|
| **Total Serum IgE** | In vitro (serology) | Simple, quick, indicates atopy | Non-specific, not allergen-specific |
| **Skin Prick Test (SPT)** | In vivo | Quick, cost-effective | Risk of local/systemic reactions |
| **RAST (1st Gen)** | In vitro (serology) | Safe, detects allergen-specific IgE | Low sensitivity, uses radioactivity |
| **RAST (2nd Gen)** | In vitro (serology) | Improved sensitivity | More expensive than SPT |
| **ImmunoCAP (3rd Gen)** | In vitro (serology) | High sensitivity, automated | Costly, requires specialized lab |
| **Basophil Activation Test** | In vitro (cell-based) | Functional test, useful in complex cases | Expensive, requires flow cytometry |

# ACKNOWLEDGEMENT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