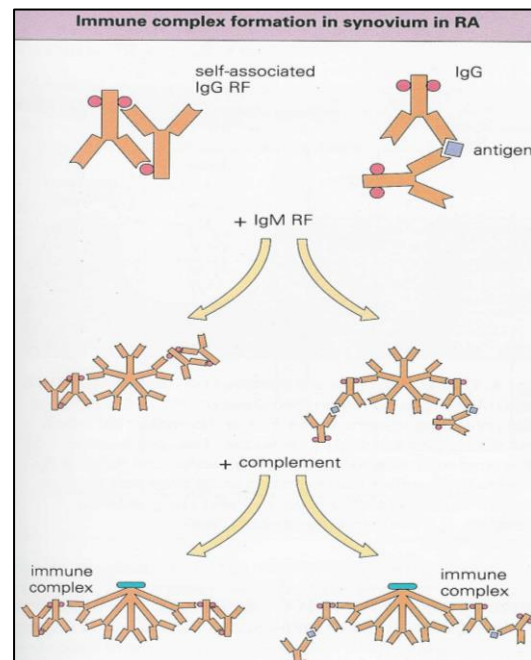
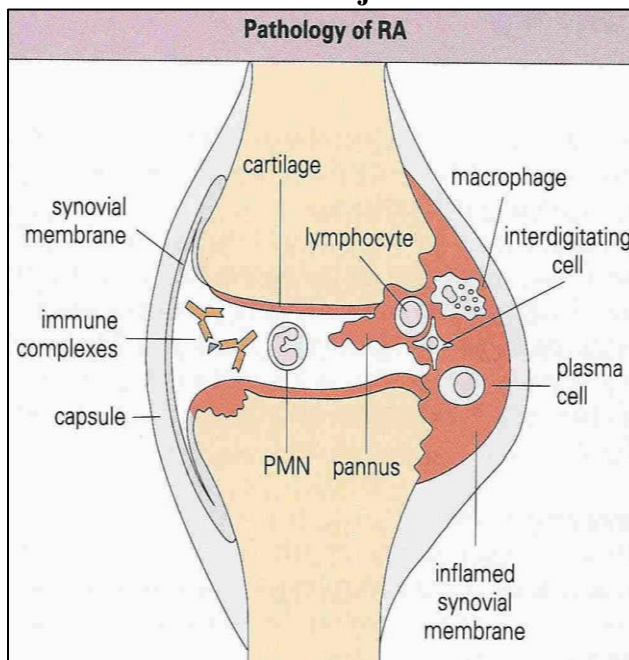




Unit VII – Problem 3 – Immunology Lab

- Pathogenesis of rheumatoid arthritis:

- Both genetic as well as environmental factors are implicated in the pathophysiology of the disease. **The genetic association is with HLA-DR4.**
- The negative feedback mechanisms that normally maintain tolerance of self are overtaken by aberrant positive feedback mechanisms for certain antigens such as the **Fc portion of IgG (bound by Rheumatoid Factor).**
- Once the abnormal immune response has become established (which may take several years before any symptoms occur), **plasma cells derived from B-lymphocytes produce Rheumatoid Factors (RFs)** -which are IgM directed against the Fc portion of IgG- in large quantities.
- These immune complexes will **activate macrophages** through Fc receptor and complement binding.
- This contributes to **inflammation in the synovium**, in terms of edema, vasodilation and **infiltration by activated T-cells** (mainly CD4 in nodular aggregates and CD8 in diffuse infiltrates).
- Synovial macrophages and dendritic cells further function as antigen presenting cells by expressing MHC class-II molecules leading to an established local immune reaction in the tissue.
- The disease progresses with **formation of granulation tissue at the edges of the synovial lining (pannus)** with extensive angiogenesis and production of enzymes that cause tissue damage.
- The synovium thickens, the cartilage and the underlying bone begin to disintegrate and evidence of **joint destruction occurs.**



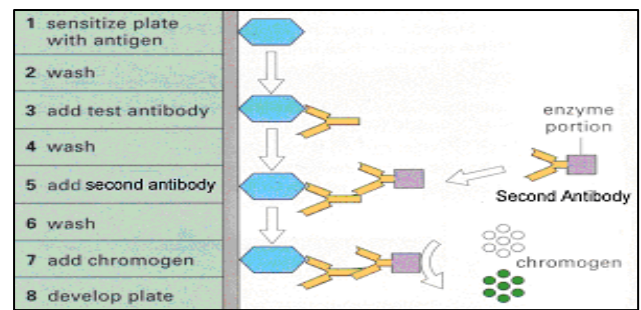
LABORATORY TESTS FOR RHEUMATIC/ AUTOIMMUNE DISEASES	
Initial tests	Further tests
ANA (Anti-Nuclear Antibodies)	Anti-ds DNA
C3-C4 (complement components)	Anti-ENA
RF (Rheumatoid Factor)	Anti-CCP

- ANA (Anti-Nuclear Antibodies):

- **The methods for this test are: *ELISA-screening*** which will be confirmed by *indirect immunofluorescence* (looking for antibodies).
- **ELISA (Enzyme-Linked Immuno-Sorbent Assay) steps:**
 1. Plate wells are sensitized with an antigen.
 2. Antibody is added to bind to the antigen.

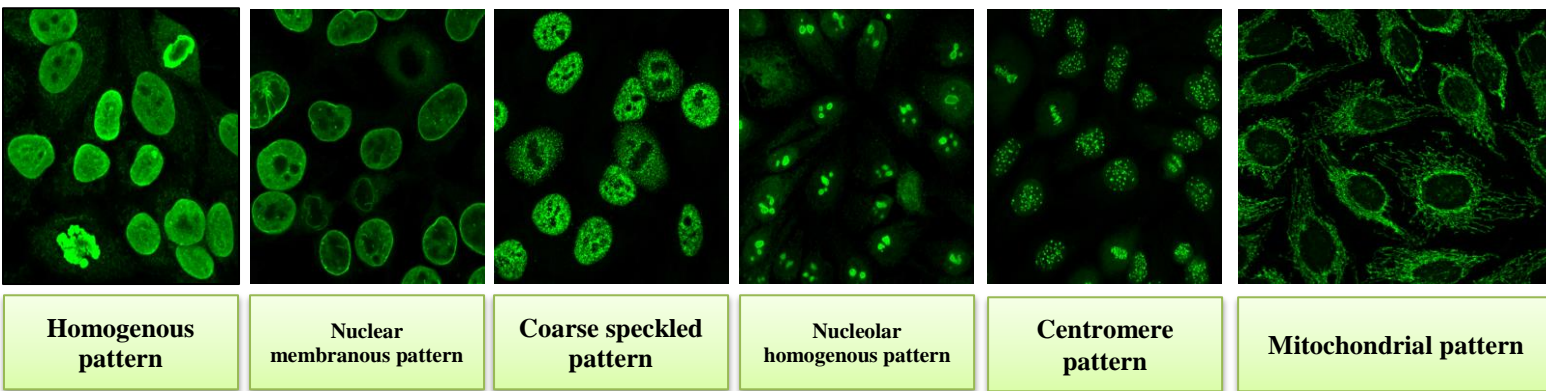
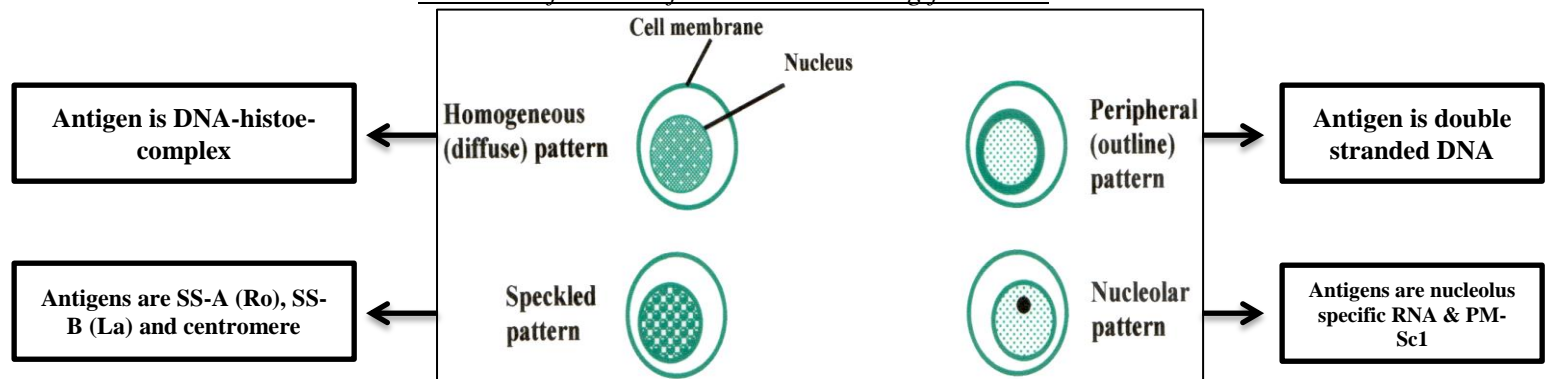


3. A second antibody (linked with an enzyme) will be added to bind to the antibody.
4. Substrate is added to give colour (if it reacts with the enzyme).
5. Stop reagent is added.
6. End product: antibody amount is measured by optical density screening.



- **Immunofluorescence: used extensively to detect auto-antibodies and antibodies to tissue and cellular antigens.**

- ✓ Direct Immunofluorescence: a fluoresceinated antibody is added to a section containing antigens → if antibodies bind to antigens, they will be revealed under the microscope (with UV light) and appear green in colour.
- ✓ Indirect Immunofluorescence: a fluoresceinated anti-immunoglobulin is added to a section containing antibodies → if anti-immunoglobulins bind to antibodies; they will be revealed under the microscope (with UV light) and appears green in colour.
- ✓ Patterns of immunofluorescent staining for ANA:



- If ANA test is positive and the patterns of immunofluorescent staining are known, further tests are required to be done because these patterns are not specific and may reflect more than one condition.

Pattern	Further test
Homogenous	Anti-ds DNA
Peripheral	Anti-ds DNA
Speckled	Anti-ENA (Extractable Nuclear Antigen)
Cytoplasmic	Anti-mitochondrial

- **RF (Rheumatoid Factor):**

- It is an antibody IgM present in the sera of patients with rheumatoid arthritis and directed against the Fc portion of IgG (acting as an autoantibody).
- **Latex agglutination slide test:** is used for the qualitative and semi-quantitative determination of rheumatoid factors in non-diluted serum. IgG coated in latex particles will react with RF (if they are present in the patient's serum) and this will result in agglutination.
- **Interpretation of the result of RF test:**



- ✓ A negative rheumatoid factor does not exclude the diagnosis of rheumatoid arthritis.
- ✓ Rheumatoid factors are not unique to rheumatoid arthritis:
 - ❖ It is present in 30% of SLE patients.
 - ❖ 90% of Sjogren's syndrome.
 - ❖ Some patients with scleroderma or polymyositis.
- ✓ False RF occurs in:
 - ❖ Chronic inflammatory conditions: hepatitis, sarcoidosis and neoplasia.
 - ❖ Chronic infectious diseases: tuberculosis, kala-azar and syphilis.
 - ❖ Viral infections: infectious mononucleosis.
 - ❖ Subacute bacterial endocarditis.
 - ❖ Following vaccination (transient).
 - ❖ Large proportion of elderly (20%).
- **Rheumatoid Arthritis (RA) is divided into 2 major clinical subtypes depending on the presence or absence of anti-CCP (anti-Cyclic Citrullinated Peptide) antibodies:**
 - ✓ Positive anti-CCP Rheumatoid Arthritis (2/3 of RA patients).
 - ✓ Negative anti-CCP Rheumatoid Arthritis.
- **C3-C4 (complement components) are detected by nephelometry (turbidimetry):**
 - Light rays from a laser are collected in the focusing lens and pass through the sample tube containing antigen and antibody.
 - Light passing through the tube and emerging at 70-degree angle is collected by another lens and focused into an electronic detector.
 - This signal is converted to a digital recording of the amount of turbidity in the sample tube and can be mathematically related to either antigen or antibody concentration in the sample.

