Antigen antibody reactions

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Agglutination Tests

- In district laboratories, agglutination tests are frequently used because compared with other serological tests, they are :
- simpler to perform
- require no special equipment
- and are usually less expensive.

Agglutination test

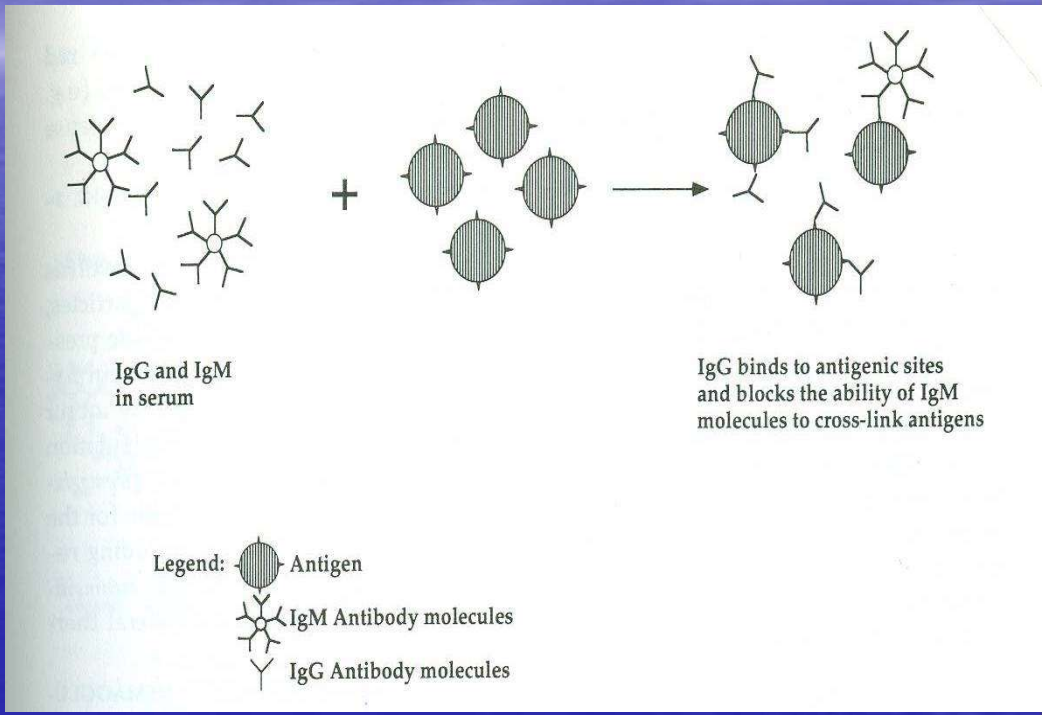
- It is one of important laboratory method to detect antigen antibody reaction.
- It provides flexible and useful method for semi quantitating of either antigen or antibody concentration.
- The reaction occurs between insoluble antigen and appropriate antibody.
- The reaction will results in forming aggregate or agglutinate.

- **Principle of agglutination tests**
- Agglutination is the visible clumping together of bacteria, cells, or particles, by an antigen combining with its specific antibody.
- The resulting clumps are referred to as agglutinates..
- In tests used to detect antibody **(**agglutinin) in a patient' serum, a known antigen(aggutinogen) suspension is used.
- The antigen particles are agglutinated if the serum contains the corresponding antibody. In general, to detect antibody in patients serum a known antigen suspension is added or to detect antigen in serum, a specific antibody is added.

Stages of agglutination reaction

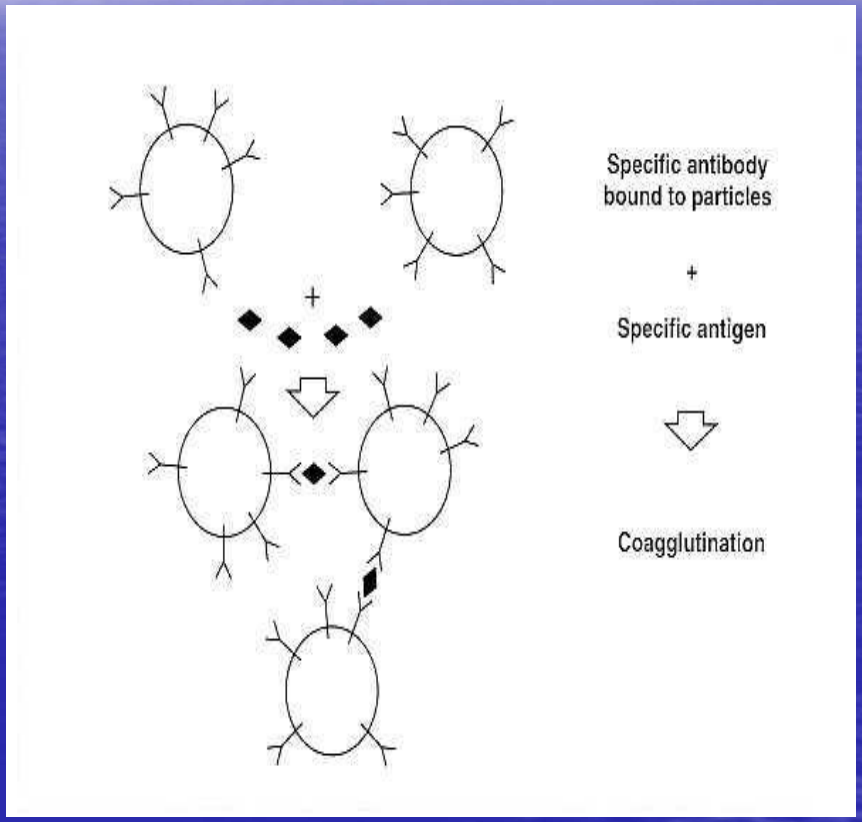
Phase one

- Antibody reacts with single antigenic determinants on or close to particle surface.
- It is a rapid reaction.



Secondary phase

- A single antibody molecule binds to antigenic determinants on adjacent particles.
- The visible reaction occur under appropriate conditions and over time, particles remain connected and interconnected by antibody bridge.



- **Agglutination tests can be performed:**

- A. On slides
- B. In tubes
- C. In microtitration plates

- **A. Slide agglutination tests**

1. These are rapid, easily performed techniques that give a reaction in minutes or even seconds.
2. They are, however, not usually as sensitive as tube or microtitration techniques.
3. Their specificity depends on the reagent used.
4. The type of agglutination can be either active or passive.

- **I. Active agglutination slide tests**
- These are tests in which there is a direct agglutination of bacterial antigen with its corresponding antibody.
- Example, the slide agglutination of *salmonellae, shigellae*, or *Vibrio cholera* by using specific antibody.
- Slide agglutination tests used to identify bacteria from cultures are difficult to standardize and control.
- False agglutination (auto- agglutination) may occur.
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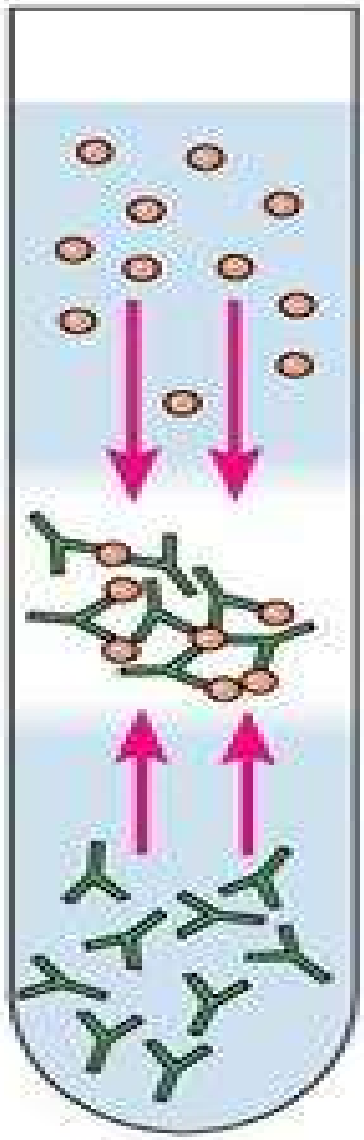
- **II. Passive agglutination slide tests**
- These are tests in which the specific antibody or known antigen is attached to inert particles or cells.
- When the known antigen or antibody combines with its corresponding antibody or antigen in the specimen,
- the particles or cells are used only to show that an antigen antibody reaction has occurred.
- Their role in the reaction is, therefore, passive.

- **The substances and cells used as carriers in passive slide agglutination tests include:**
- · **Latex particles**
- · **Carbon particles**
- · **Stabilized staphylococcal cells**

- **Latex particles:** these are polystyrene particles that can be coated with either known antigen or specific antibody.
- A example of a test in which antigen coated particles are used is the antistreptolysin O (ASO) slide test. This detects significant rises of ASO antibody in the serum of patients with poststreptococcal complications. Antibody coated latex particles are used in several tests including the detection of extracellular bacterial antigens in cerebrospinal fluid.
- **Carbon particles:** these are coated with cardiolipin antigen and used in the rapid plasma reagin (RPR) card test to screen for cardiolipin antibodies in the sera of patient with syphilis.

- **Precipitin Tests**
- The precipitin reaction provided the first quantitative assay for antibody.
- The precipitin reaction is based upon the interaction of antigen with antibody leading to the production of antigen-antibody complexes

- **Principle**
- To produce a precipitin reaction, varying amounts of soluble antigen are added to a fixed amount of serum containing antibody. As the amount of antigen added:
- In **the zone of antibody excess**, each molecule of antigen is bound extensively by antibody and cross linked to other molecules of antigen. The average size of antibody-antigen complex is small; cross-linking between antigen molecules by antibody is rare.
- In the zone of equivalence, the formation of precipitin complexes is optimal. Extensive lattices of antigen and antibody are formed by cross-linking.
- At high concentrations of antigen, the average size of antibody-antigen complexes is once again small because few antibody molecules are available to cross-link antigen molecules together



(a)

(b)

prozone

- Absence of agglutination at higher antibody concentration.
- It is due to many factors including
- Presence of blocking antibodies at low titers
- Inaccessible antigenic determinants
- Weak avidity
- Poor lattice formation.
- The problem can be avoided by use of standard serial dilution.

advantages of agglutination methods

- ease of performance.
- speed of performance, usually requiring few minutes.
- high degree of sensitivity.

Disadvantages of agglutination methods

- the reaction are only semiquantitative.
- the occurrence of the prozone phenomenon, in which agglutination is inhibited by extreme antibody excess as a result of poor lattic formation.

Application of agglutination test

- several antibodies can be detected by this method such as Rheumatoid factor.

- **Rheumatoid Factors (RF)** are autoantibodies that react with individuals own immunoglobulin.
- These antibodies are usually directed against the Fc fragment of the human IgG.
- RF have been associated with three major immunoglobulin classes: IgM, IgG, and IgA. Of these IgM and IgG are the most common.
- The formation of immune complex in the joint space leads to the activation of complement and destructive inflammation, causing **rheumatoid arthritis (RA)**

- As indicated by its name, RF has particular application to diagnosis and monitoring of rheumatoid arthritis.
- Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting primarily the joints and periarticular tissues.
- Rheumatoid factor is detected in 60-80% of cases of diagnosed rheumatoid arthritis.
- However, it is also detectable sometimes in the serum of patients with Systemic Lupus Erythematosus (SLE) and in certain non-rheumatic conditions.
- Elevated values may also be observed in normal elderly population.
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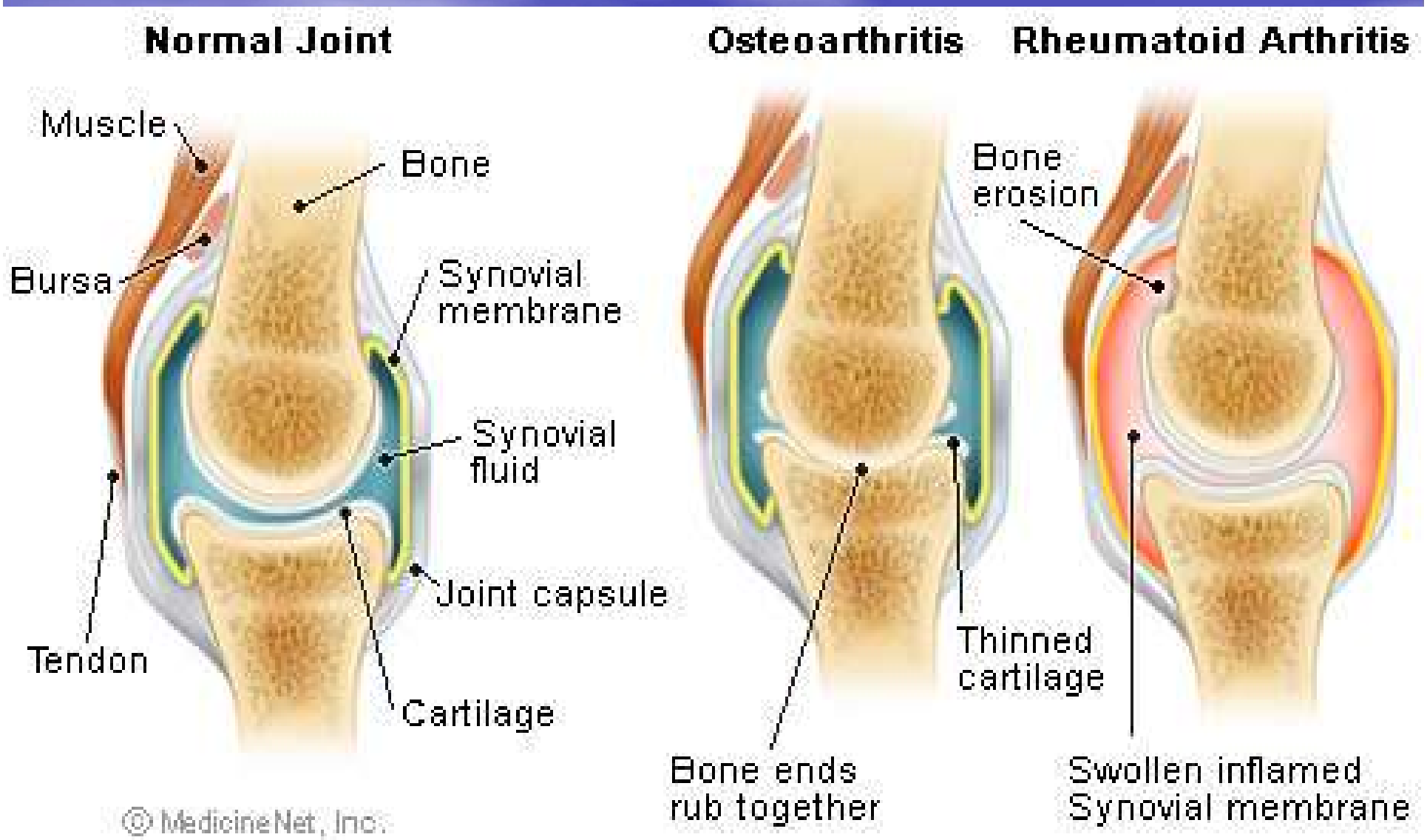
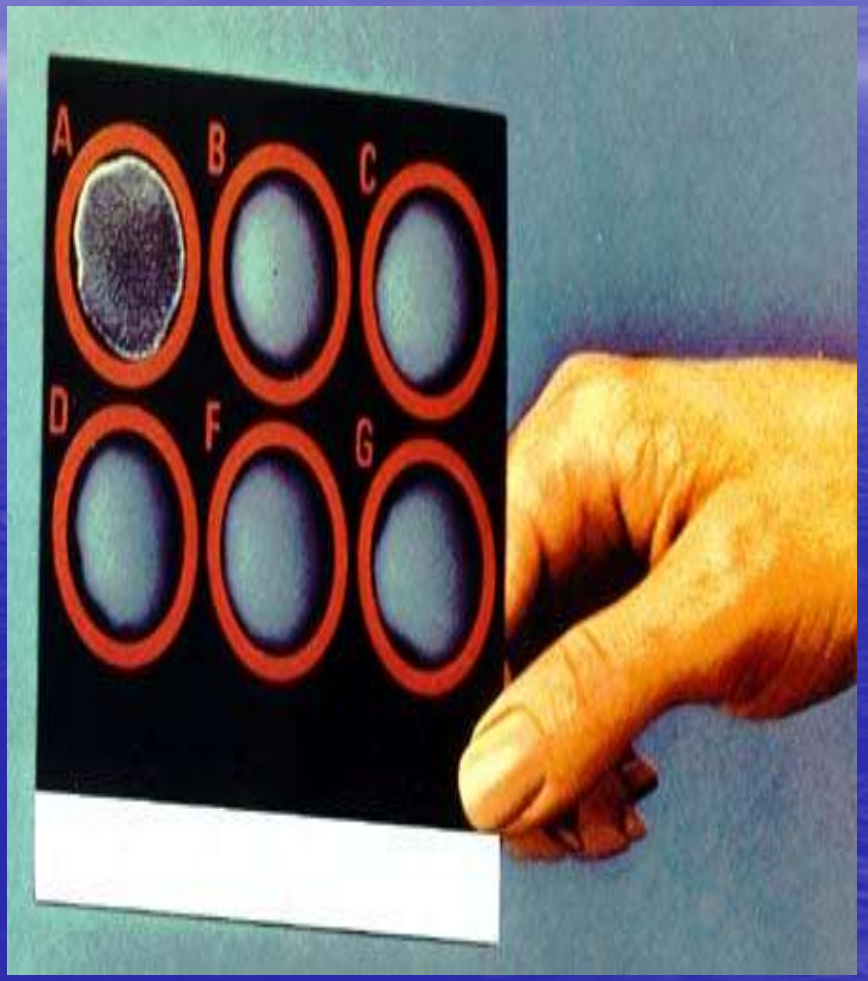


Normal and Arthritic Joints

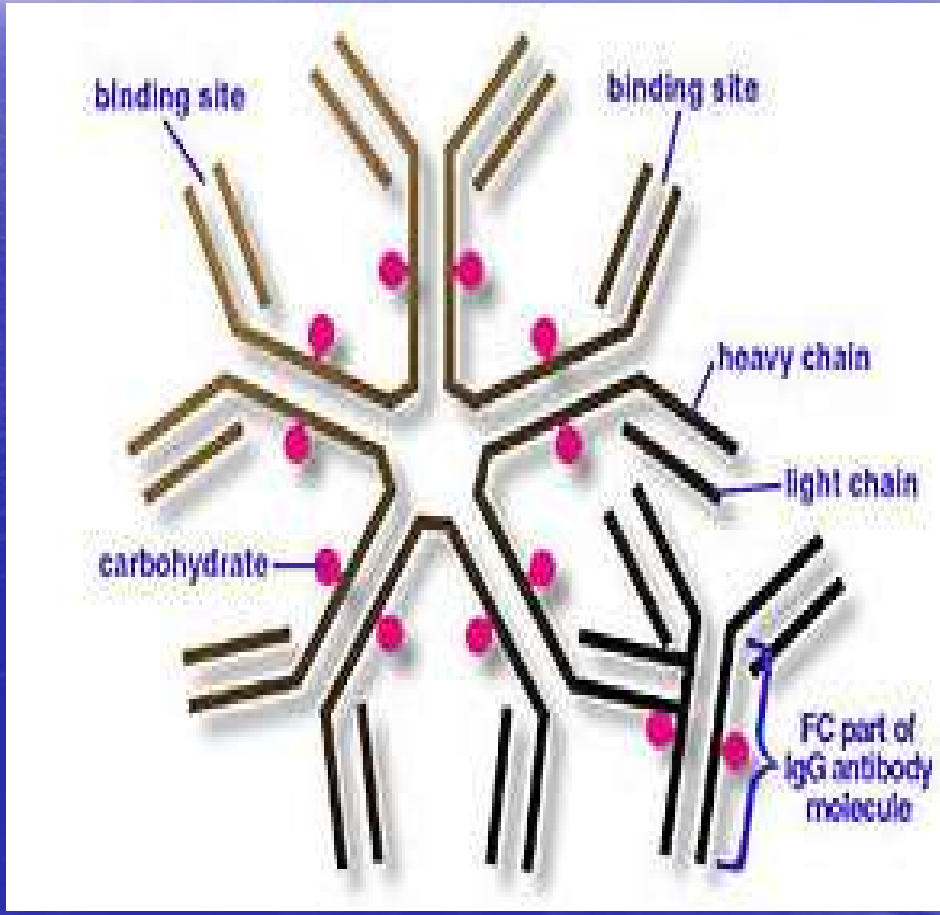
Latex agglutination test

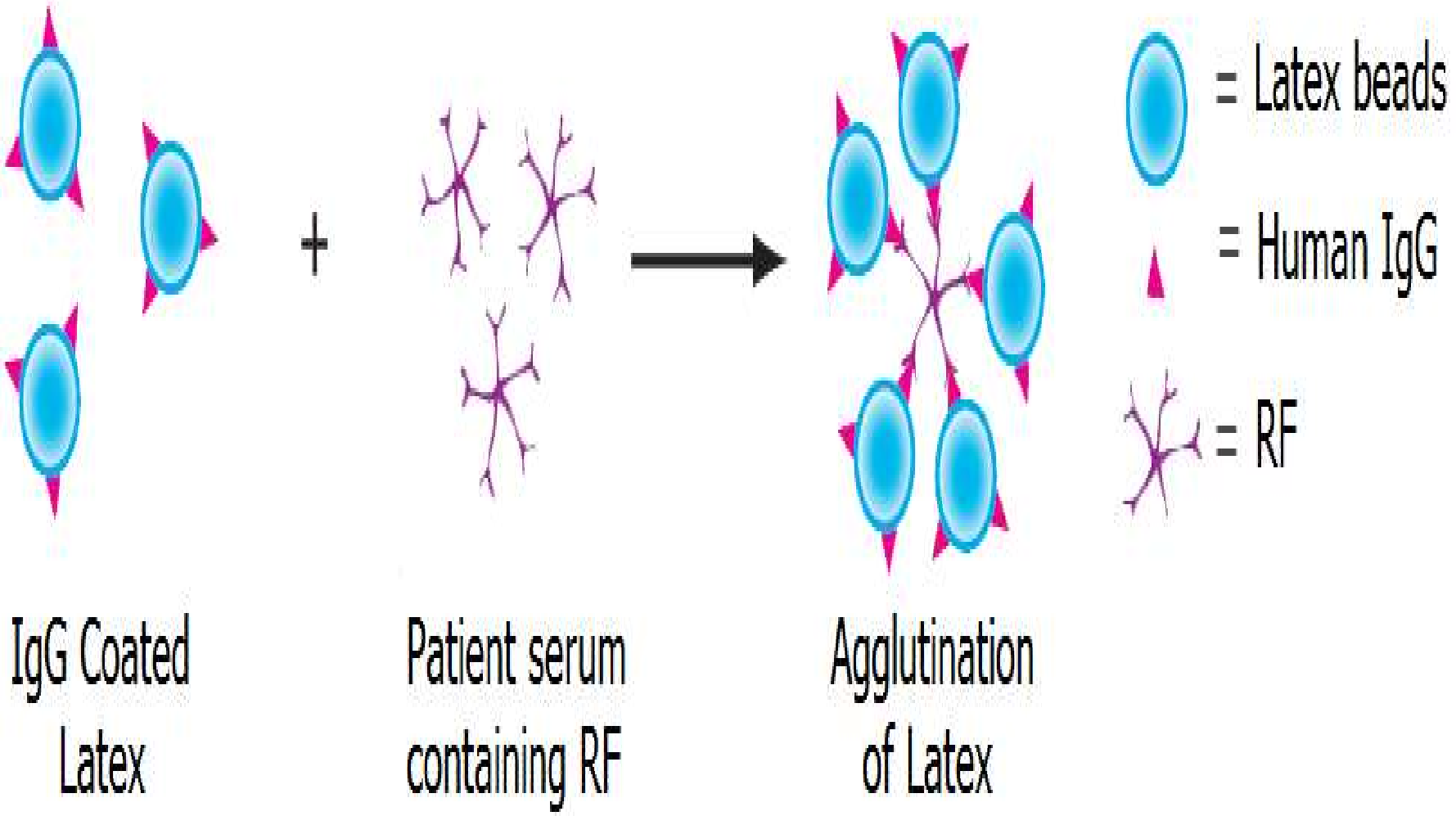


- **procedure**
- Bring all reagents and specimens to room temperature.
- Place one drop of the positive control and 40ul of the patient serum into separate circles on the slide.
- Gently and add one drop of RF latex reagent on each circle of sample to be tested and control.
- Use separate Applicator sticks/stir sticks to spread reaction mixture over entire area of the particular field.
- Tilt the slide back and forth for 2 minutes in a rotary shaker so that the mixture rotates slowly.
- Observe for agglutination after 2 minutes under bright artificial light.

Rheumatoid Factor (RF)

- his test is done to diagnosed Rheumatoid arthritis, which is one of important autoimmune disease.
- RF is an antibody (IgM or IgG classes) bind to the Fc portion of other IgG molecules, and form IgG-anti-IgG complexes in the circulation or **joint fluid.**




= Latex beads
= Human IgG
= RF
IgG Coated Latex
Patient serum containing RF
Agglutination of Latex

- **LIMITATIONS OF RF TEST**
- RF is not detected in all patients diagnosed with RA.
- RF may be detected in increased amounts in patients with infectious mononucleosis, sarcoidosis, systemic lupus erythematosus, TB or leprosy, and other conditions of acute or chronic immune response.
- The significance of a positive result should be interpreted with caution. Quantitative testing should be done to confirm diagnosis of RA.
- Highly haemolyzed and lipemic serum as well as plasma interfere with the test.
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Rheumatoid factor (RF)

- **RFs are detected in serum in up to 80% of adult patients with RA.**
- **RFs are not specific for RA and occur in other autoimmune disease, in chronic infectious diseases, such as infective endocarditis, tuberculosis, and hepatitis B.**
- **usually at low titer, in up to 20% of overtly normal elderly individuals**

Interpretation of the test

- Agglutination test is positive. Do titration and determine the end of titration.
- Normal range differ from lab to another, but in most lab titration of >1:20 consider positive.
- Positive test in 80% of Rheumatoid Arthritis.
- It also positive in other autoimmune disease.
- Positive in viral hepatitis.
- Positive in TB.

Methods used to detect RF

- Latex **agglutination method.**

mixes the blood being tested with (latex) beads that are covered with human antibodies. If rheumatoid factor (RF) is present, the latex beads clump (agglutinate).

- **Haemagglutination test.**

mixes the blood being tested with a sheep's red blood cells that have been covered with rabbit antibodies. If RF is present, the red blood cells agglutinate.

- **Nephelometry test**

Using an automated machine based on laser light scattered.

Factors interfere with positive result

- **Hyperlipedemia. Blood that is very high in fats.**
- **Age. About 5% to 10% of people over age 65 have an elevated RF level.**