Clinical immunology

is the study of diseases caused by disorders of the immune system.

The diseases caused by disorders of the immune system fall into two broad categories:

- Immunodeficiency in which parts of the immune system fail to provide an adequate response (examples include chronic granulomatous disease and primary immune diseases)
- Autoimmunity, in which the immune system attacks its own host's body (examples include systemic lupus erythematosus, rheumatoid arthritis, Hashimoto's disease and others).

Rheumatoid arthritis (RA)

is a systemic autoimmune disease characterized by prominent joint involvement. Arthritis is typically associated with erosion of cartilage and subchondral bone, formation of an inflammatory tissue, consisting of activated macrophages, T cells, fibroblasts, and other immune cells (pannus). This can ultimately result in joint destruction and significant joint deformities. In addition to the joints, RA can cause vasculitis, splenomegaly, and leukopenia (Felty's syndrome), interstitial lung disease, and other abnormalities.

Cause of RA

The cause of RA is appearing to be multi-factorial. It is considered as autoimmune disease in which the body loses its ability to distinguish between synovial and foreign tissue.

Other factors involved in RA are as follow:

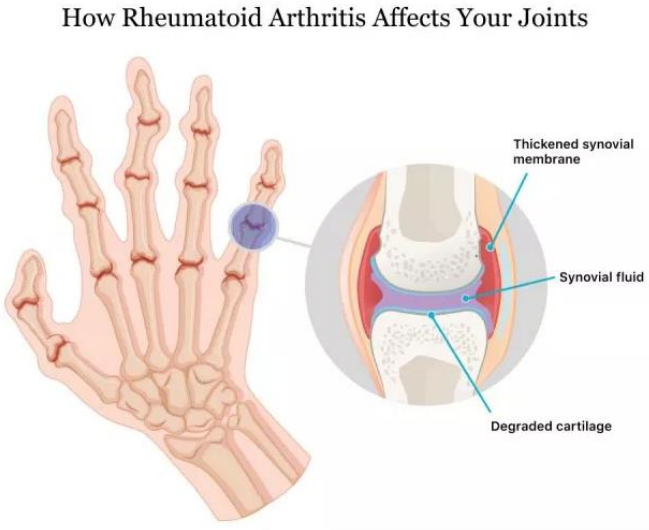
- Environmental influences:

- Infections
- Trauma thought to trigger the development of RA.
- Vitamin D deficiency
- Smoking and alcohol consumption.

- Genetic markers

- Clinical Features (Symptoms):

- Painful, warm, swollen joints of the hands and wrists most commonly
- Joint stiffness
- Loss of joint range of motion
- Joint deformity
- Pain sometimes affecting neck, shoulders, hips, knees, and/or feet
- Fatigue
- Fever



Laboratory diagnosis:

Diagnosis of RA is made on the basis of a combination of clinical manifestations, radiographic findings, and laboratory testing.

There are several types of blood tests that help diagnosis RA. These tests include:

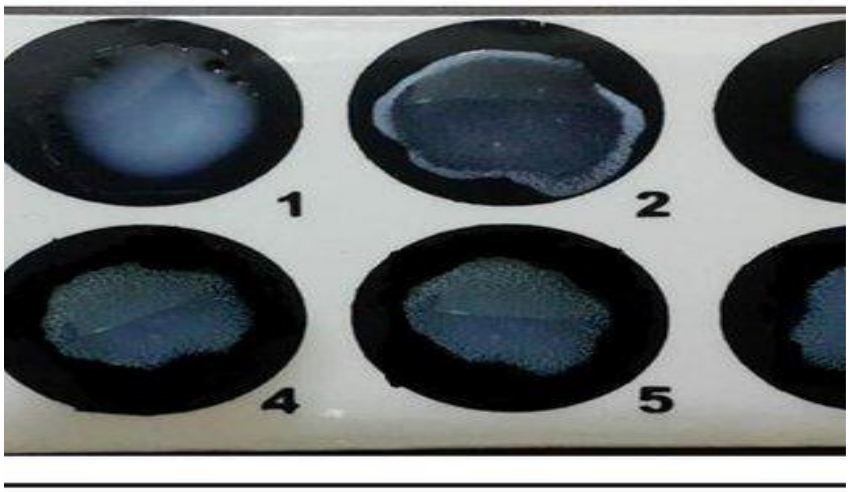
- Rheumatoid factor test (RF).
- Anti-cyclic citrullinated peptide antibody test (anti-CCP).
- Antinuclear antibody test (ANA).
- Erythrocyte sedimentation rate (ESR).
- C-reactive protein test (CRP).

Rheumatoid Factor Test: RF is the antibody that is most often tested for to aid in making the initial diagnosis. Thus, a negative result does not rule out the presence of RA. Conversely, a positive test result is not specific for RA, because RF can be found in other diseases such as syphilis, SLE, chronic active hepatitis, tuberculosis, leprosy, infectious mononucleosis, malaria, and Sjorgren's syndrome.

Test principle: The RF latex is slide agglutination method depends onto mixing of specimen which containing of RF with anti-RF coating with latex reagent and allowed them to react; if RF is present, the visible agglutination is observed but when RF is absent there is no agglutination is observed.

Procedure Rheumatoid factors (Qualitative method):

- place 50µl of the sample and one drop of each positive and negative control into separated circles on the slide test.
- Add one drop (50µl) of RF reagent to each circle.
- Mix the drop with stirrer, spreading them over the entire surface of the circle. Use different stirrer for each sample.
- Rotate the slide within two minutes



Systemic lupus erythematosus

Systemic lupus erythematosus (SLE), is the most common type of lupus. SLE is an autoimmune disease in which the immune system attacks its own tissues, causing widespread inflammation and tissue damage in the affected organs. It can affect the joints, skin, brain, lungs, kidneys, and blood vessels. There is no cure for lupus, but medical interventions and lifestyle changes can help control it.

Causes:

Multiple factors are associated with the development of SLE, including:

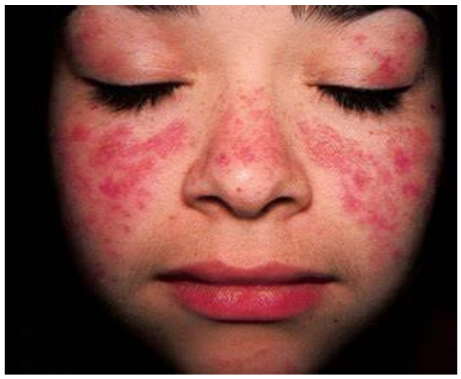
genetic, hormonal, and environmental factors.

Clinical features:

Systemic lupus erythematosus is a chronic autoimmune disease that can affect

almost any organ system. symptoms include:

- 1. Fatigue, fever, and arthralgia.
- 2. Arthritis of the small joints of the hands, wrists, and knees may occur
- 3. Multiple cutaneous manifestations of SLE include redness of the face, takes the form of the butterfly, the rash is most often seen over the cheeks and bridge of the nose, but can be widespread. It gets worse in sunlight
- 4. Excessive sensitivity to light
- 5. The kidney is the most commonly involved visceral organ in SLE.
- 6. Acute nephritic disease may manifest as hypertension and hematuria. Nephrotic syndrome may cause edema, weight gain, or hyperlipidemia.



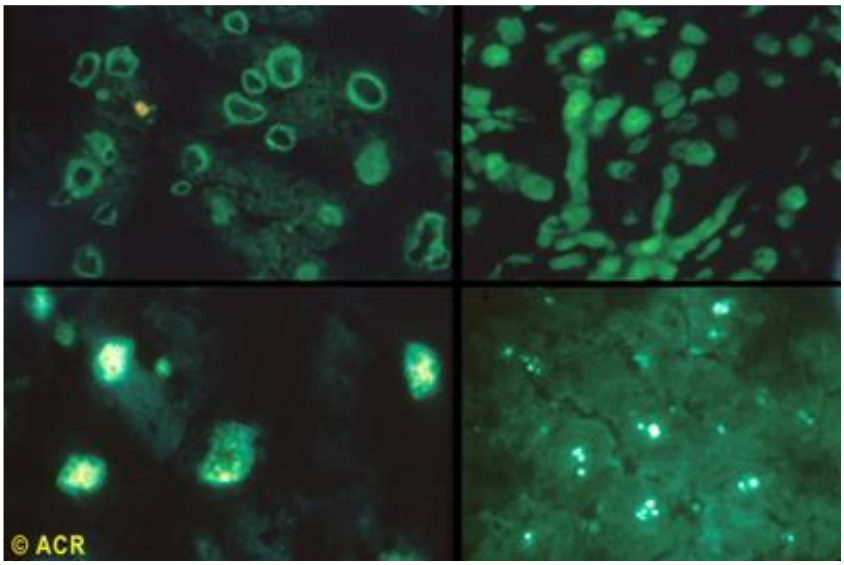
Laboratory Finding:

- 1. ANA-Screening test; sensitivity 95%; not diagnostic without clinical features.
- 2. Anti-dsDNA-High specificity; sensitivity only 70%; level is variable based on disease activity
- 3. Anti-Sm-Most specific antibody for SLE; only 30-40% sensitivity
- 4. Anti-SSA (Ro) or Anti-SSB (La)-Present in 15% of patients with SLE
- 5. Anti-ribosomal P-Uncommon antibodies that may correlate with lupus cerebritis
- 6. Lupus anticoagulant-Multiple tests (eg, direct Russell viper venom test) to screen for inhibitors in the clotting cascade in antiphospholipid antibody syndrome
- 7. Direct Coombs test-Coombs test-positive anemia to denote antibodies on RBCs
- 8. Anti-histone- Drug-induced lupus ANA antibodies.

What is antinuclear antibody test?

The antinuclear antibody (ANA) test is used as a primary test to help evaluate a person for autoimmune disorders that affect many tissues and organs throughout the body (systemic) and is most often used as one of the tests to help diagnose systemic lupus erythematosus (SLE).

There are several methods used to test for ANAs. One method is a blood test called the Fluorescent Antinuclear Antibody Test or FANA. This test involves viewing fluorescent-labeled antibodies on a glass side under the microscope and determining the pattern and intensity of the fluorescence.



(The figure explain Immunofluorescence of ANA test)

What does the result reading mean?

A negative ANA reading means no autoantibodies are present in the body. However, a positive ANA reading alone does not indicate an autoimmune disease.

Why?

- The prevalence of ANAs in healthy individuals is about 3-15%. The production of these autoantibodies is strongly age-dependent, and increases to 10-37% in healthy persons over the age of 65. Even healthy people with viral infections can have a positive ANA.
- Some medications can cause a positive ANA.
- Other conditions, such as cancer, can cause a positive ANA.

Ankylosing spondylitis (AS)

Ankylosing spondylitis (AS) is a type of arthritis (chronic inflammatory arthritis) that mainly affects the back, by causing inflammation in the spine. This can make back, rib cage and neck stiff and painful.

In response to the inflammation, the body produces extra calcium around the bones of the spine. This can make extra bits of bone grow and cause back and neck to be more stiff.

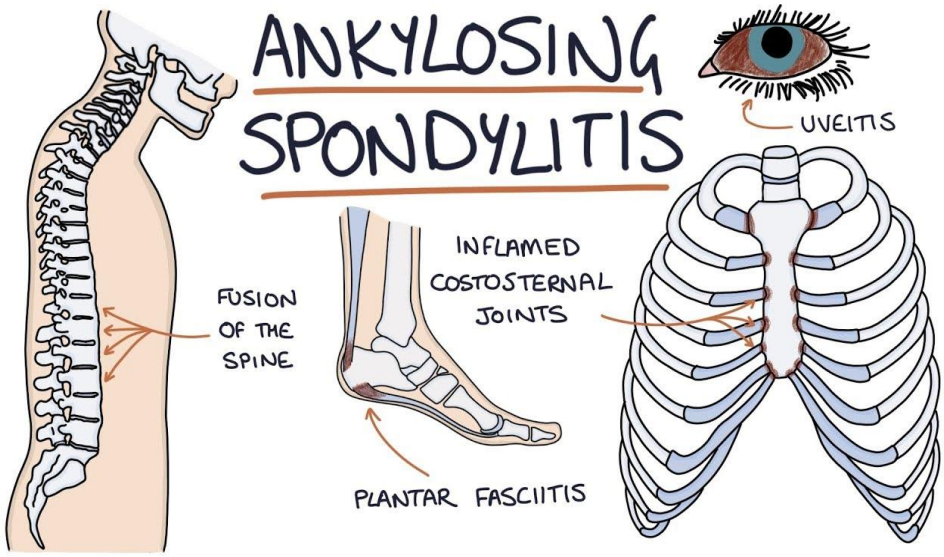
Ankylosing spondylitis is a type of spondyloarthritis. This is the name for a group of conditions with similar symptoms – mainly pain and stiffness around the spine.

While it mainly affects the neck and back, it can also cause pain and stiffness elsewhere in the body, including in the hips, shoulders and feet.

Clinical feature (symptoms)

Symptoms of ankylosing spondylitis can be similar to more common back problems, especially in the early stages.

1. stiffness and pain in lower back in the early morning that lasts at least 30 minutes and then eases through the day or with activity.
2. pain that wakes in the night.
3. pain in one or both buttocks and sometimes the backs of the thighs.
4. soreness at the heel or in the arch of foot.
5. pain and swelling in a finger or toe.
6. tenderness at the base of your pelvis, which can make sitting on a hard chair uncomfortable.
7. chest pain or a tightness around the chest that comes on gradually. This can make it difficult to take deep breaths.
8. Ankylosing spondylitis affects the eyes in more than 30 percent of cases, leading to episodes of eye inflammation called acute iritis.



Causes

1. Genetic.

Ankylosing spondylitis has unknown specific cause, though genetic factors seem to be involved. In particular, people who have a gene called HLA-B27 are at a greatly increased risk of developing ankylosing spondylitis.

Men are more likely to develop ankylosing spondylitis than are women.

Onset generally occurs in late adolescence or early adulthood.

Other genes associated with Ankylosing spondylitis:

- CARD9 (Caspase Recruitment Domain Family Member 9)
- ERAP1 (Endoplasmic Reticulum Aminopeptidase 1)
- HLA-B
- IL1A
- IL23R
- STAT3 (Signal Transducer and Activator of Transcription 3)

2. environmental factors.

Diagnosis

Symptoms of ankylosing spondylitis can be similar to more common back problems, especially in the early stages, the condition develops slowly and there's no definitive test. So it can be difficult to diagnosis.

A diagnosis will be made based on several things, including:

1. Blood tests. to check for signs of inflammation in the body. Inflammation in spine and joints is a main symptom of the condition.
2. an X-ray.
3. a MRI scan.
4. an ultrasound scan.
5. Genetic testing. A genetic blood test may sometimes be carried out to see if the patient carries the HLA-B27 gene, which is found in most people with AS.

Sjögren's Syndrome

Sjögren's syndrome (SS) is a systemic autoimmune disease that targets the exocrine glands. It is characterized by xerostomia (dry mouth), xerophthalmia (dry eyes), and is usually accompanied by production of auto-antibodies specific for the Ro RNA-binding protein.

one-half of all cases of SS are primary and the remainder occurs as secondary SS.

Secondary SS is defined as the former definition of primary Sjögren's in the presence of another autoimmune disorder such as RA or SLE. Similar to most autoimmune disorders, most cases (approximately 90%) occur in women. The majority of cases occur in midlife; however, the disorder is also seen in children and the elderly.

Sjogren's syndrome typically occurs in people with one or more known risk factors, including:

1. **Age.** Sjogren's syndrome is usually diagnosed in people older than 40.
2. **Sex.** Women are much more likely to have Sjogren's syndrome.
3. **Rheumatic disease.** It's common for people who have Sjogren's syndrome to also have a rheumatic disease — such as rheumatoid arthritis or lupus.

Clinical Features

1. The most common clinical manifestations of SS are ocular and oral. Patients most often perceive xerophthalmia as a foreign body-type sensation manifested by scratchiness, grittiness, or irritation.
2. Patients with xerostomia will complain of a parched sensation in the mouth, which often extends to the throat. Eating can be difficult without supplemental fluids.
3. Other xeroses such as dry skin and dry vaginal mucosa leading to irritation and dyspareunia may also occur.
4. Extra glandular manifestations of SS include musculoskeletal symptoms, with arthralgias and transient synovitis.
5. Pulmonary involvement, which most commonly is cough due to xerotrachea.
6. Neurologic disease is perhaps the most common significant extra glandular manifestation of SS and can involve the cranial nerves, peripheral nerves, and rarely the central nervous system.

Causes

1. Genetics. especially (HLA-DR and HLA-DQ) gene
2. Sex hormones. the condition affects more women than men.(sex hormones, especially estrogen).
3. Viral infections.
4. Environmental factors.

Laboratory diagnosis:

1- Immunological tests:

- ANA (Anti-Nuclear Antibody): About 70% of Sjögren's patients have a positive ANA test result.
- RF: About 60-70% of Sjögren's patients have a positive RF.
- SS-A (or Ro) and SS-B (or La): these are the marker antibodies for Sjögren's. 70% of Sjögren's patients are positive for SS-A and 40% are positive for SS-B (these may also found in lupus patients).

2- Blood tests

- ESR: This test measures inflammation. An elevated ESR indicates the presence of an inflammatory disorder, including Sjögren's.
- IGs: These are normal blood proteins that participate in immune reactions and are usually elevated in Sjögren's patients.

3- The ophthalmologic (eye) tests include:

- Schirmer Test: Measures tear production.
- Rose Bengal and Lisamine Green: Eye drops containing dyes that an eye care specialist uses to examine the surface of the eye for dry spots.

4- The dental tests include:

- Salivary Flow: Measures the amount of saliva produced over a certain period of time.
- Salivary scintigraphy: A nuclear medicine test that measures salivary gland function.
- Salivary gland biopsy: (usually in the lower lip) Confirms inflammatory cell (lymphocytic) infiltration of the minor salivary glands.

5- X-ray Finding (imaging studies)

Behçet's Disease

Behcet's disease is a rare, chronic, lifelong disorder that involves inflammation of blood vessels throughout the body.

Behçet's syndrome is an inflammatory condition that may affect many different parts of the body.

Often the skin and the lining of the mouth and genital areas (mucosa) can be inflamed. Joint pains, headaches, tiredness and stomach pains are also common. In some people, the eyes, blood vessels or (rarely) the brain or nerves may become involved.

It is a form of vasculitis that can lead to ulceration and other lesions. It can be interpreted as a chronic disturbance in the body's immune system.

HLA-B5, and more specifically its predominant sub allele HLA-B51, are associated with BD.

Clinical features (signs and symptoms):

1. Ulcer

- Mouth ulcer: Recurrent mouth ulcers are the hallmark of Behçet's syndrome. Ulcers can be small, large or multiple. They are often on the inner lips but can also occur on the tongue, the roof of the mouth and occasionally the throat. The ulcers are usually painful and occasionally leave a scar. Mouth ulcers affect up to 10% of the population so the presence of mouth ulcers alone is not enough for a diagnosis of Behçet’s Syndrome.

- Genital ulcer: Genital ulcers occur less frequently than oral ulcers. They resemble oral ulcers in appearance, are usually painful and, when deep, may heal with scarring.

2. Skin.

Skin problems are very common in Behçet's syndrome, occurring in about 80% of patients. Some people may develop acne-like sores on their bodies. Others may develop nodules on the lower legs.

3. Eyes.

Behcet's disease may cause inflammation in the eye – condition called uveitis. In people with Behcet's disease, uveitis causes redness, pain and blurred vision in one or both eyes. It is thought that 50-70% of people with Behçet's develop inflammation of the eye. If this is going to happen, it usually starts within 2-3 years of the diagnosis of Behçet's syndrome.

4.Headache.

Headaches are very commonly reported in people with Behçet's syndrome. Over 80% of people will report this symptom.

5. Muscle and joint pains.

Pain in the muscles and joints are common in Behçet's syndrome. Occasionally joint swelling (arthritis) affect the knee.

6. Digestive system.

Behçet's disease may cause abdominal pains, diarrhea or bleeding.

7. Tiredness.

Most patients with Behçet's syndrome report feeling fatigued and this may well impact on quality of life

8. Thromboses.

There is an association between a subset of patients with BD and the development of thrombosis, or blood clots within the veins.

9. Brain.

Inflammation in the brain and nervous system that lead to headache, fever, disorientation, poor balance or stroke.

Laboratory diagnosis:

Laboratory Data:

1. There is no diagnostic test for Behcet's disease.
2. The erythrocyte sedimentation rate **(ESR),**
3. C-reactive protein **(CRP),** C9 and/or C3, C4 complements may be elevated during the active phases of disease.
4. Immunoglobulins **(IgM and IgG)** may be elevated and immune complex are also found in serum of some patients.
5. Antineutrophil cytoplasmic antibody: Occasionally, patients are found with positive test results for Perinuclear Antineutrophil Cytoplasmic **(p-ANCA)** antibody, although positive or negative results on this test do note change prognosis or therapy.
6. Synovial fluid: Synovial fluid usually is cloudy with variable viscosity, and the WBC counts are 300-36,000/µL (either noninflammatory or inflammatory). Polymorphonuclear leukocytes and protein elevations are the predominant findings, and glucose levels are near normal.

7. Cerebrospinal fluid: These findings may show local inflammation with increased WBC counts, lymphocyte predominance, and elevated protein levels, as well as Ig levels and Ig index that reflect local production of Ig. Opening pressures are very high in some patients.

Imaging Studies

1. Radiography, MRI, and CT scanning.
2. Brain CT scanning: Acute areas of ischemia can be identified.
3. Brain MRI/magnetic resonance angiography (MRI/MRA.
4. Single-photon emission computed tomography (SPECT).
5. Angiography

Behçet's disease

is a systemic vasculitis of unknown aetiology characteristically affecting venules

Onset is typically in young adults with recurrent: oral and genital ulceration, uveitis, skin manifestations, arthritis , neurological involvement, and a tendency thrombosis.

Clinical Symptoms

- Oral ulcers are a defining feature (97%-100%) of cases and it can be minor, major, or herpetiform.

- Genital ulcers are less common than oral ulcers occurring in 60%-80% of cases.

- Skin lesions occur in about 80% of patients.

- Erythema nodosum.

- The papulopustular/acneiform lesions.

- A widespread vasculitis is the primary lesion in Behçet's disease and vessels of all sizes, both arteries and veins, may be involved in 9%-25% of patients.

- Neuro-Behçet's occurs in approximately 5% of cases.

Causes of Behcet's disease

* Autoimmune disorder.

* Genetic Factor.

* Environmental Factor.

* Chemical Exposure.

Risk Factors

Age: Behcet's disease commonly affects men and women in their 20 and 30

Place : Middle East

Gender : is usually more severe in me

Genes : Having certain genes is associated with a higher risk of developing Behcet's .

Diagnosis of Behcet's disease

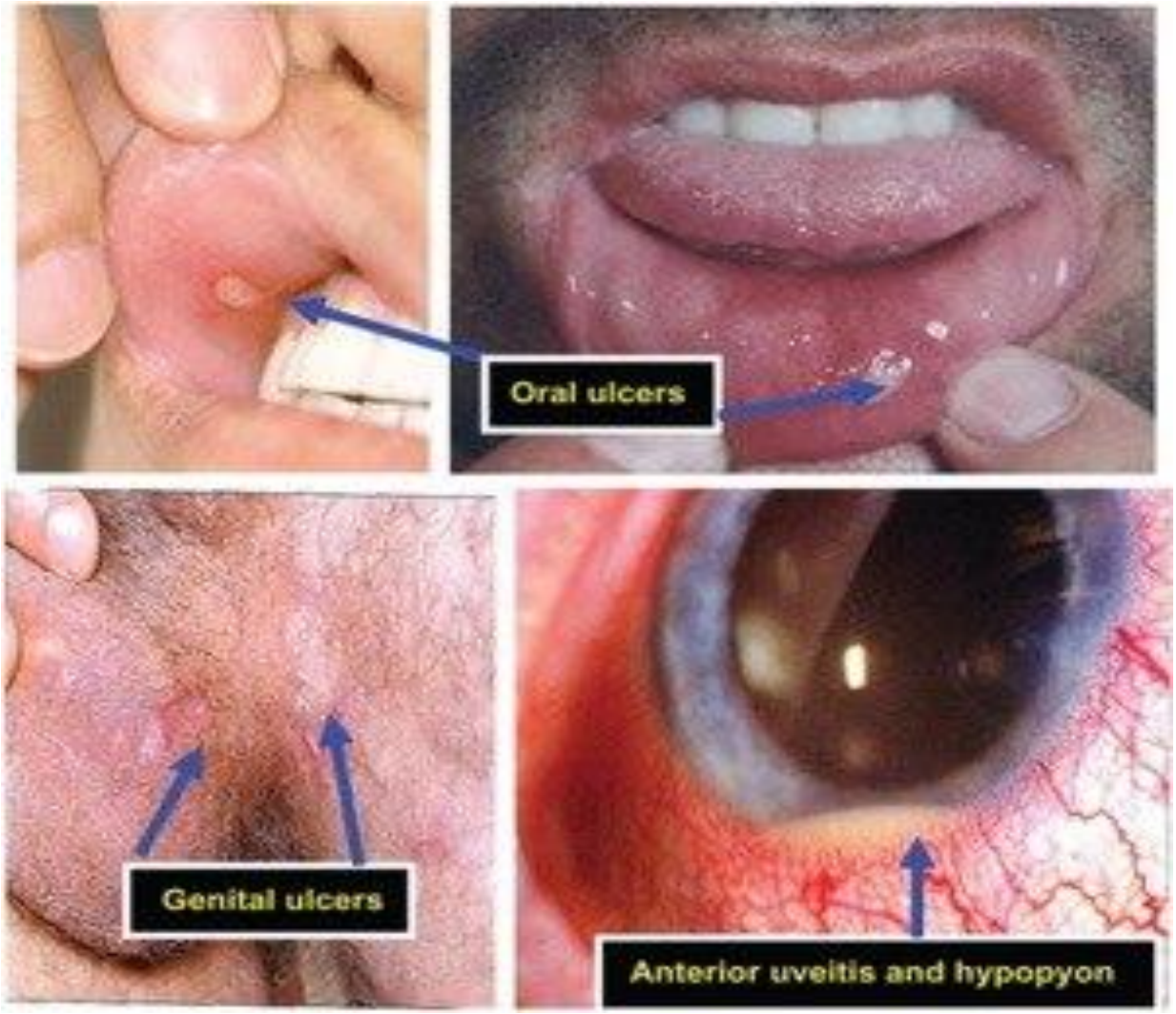
• Mouth ulcers : Recurred at least three times in 12 months two additional signs, such as:

-Genital ulcers

-Eye problems

-Skin sores: Rashes or acne-like sores

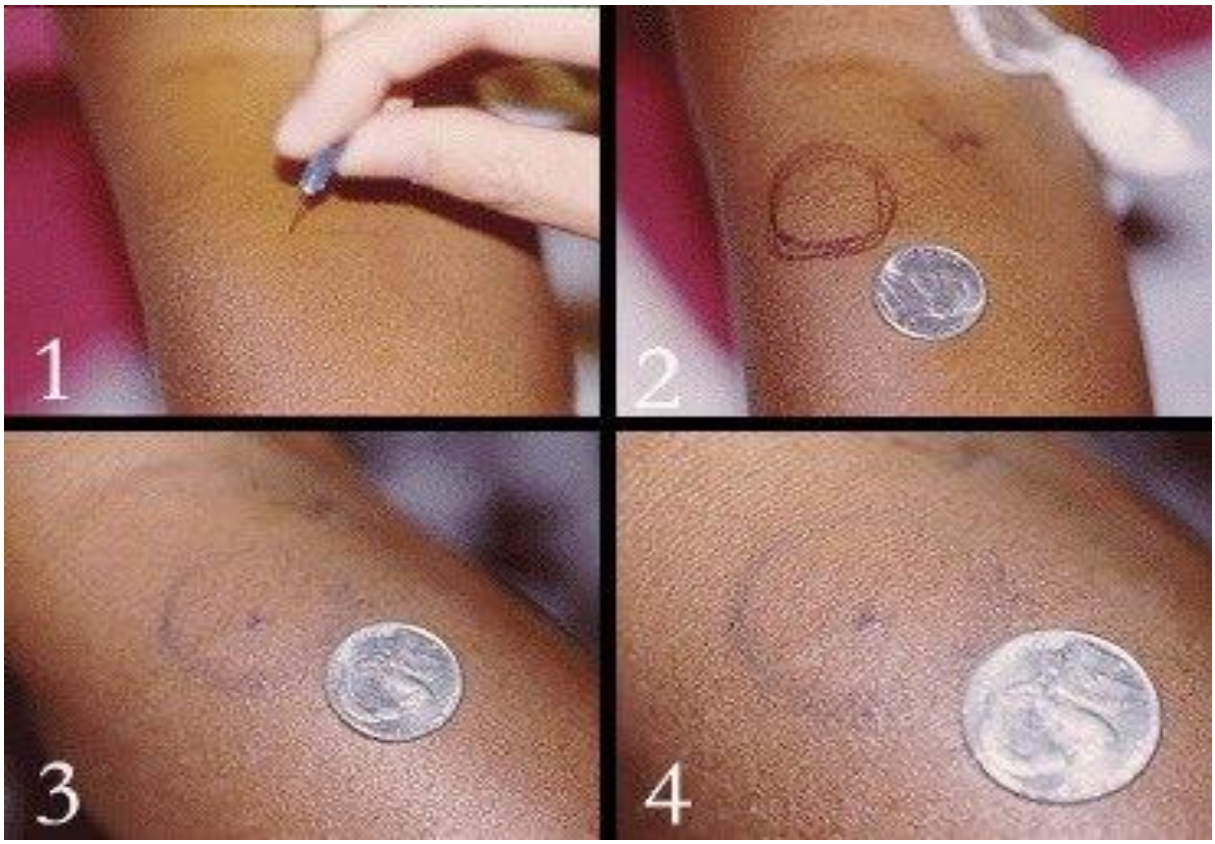
-Positive ′ In a pathergy test .



Pathergy test :

Procedure **:** inserts a sterile needle into the skin and then examines the area(1 to 2) days later.

If the pathergy test is positive, a small red bump forms under skin where the needle was inserted. This indicates your immune system is overreacting to a minor injury . The pathergy test has a specificity of 95% to 100% .



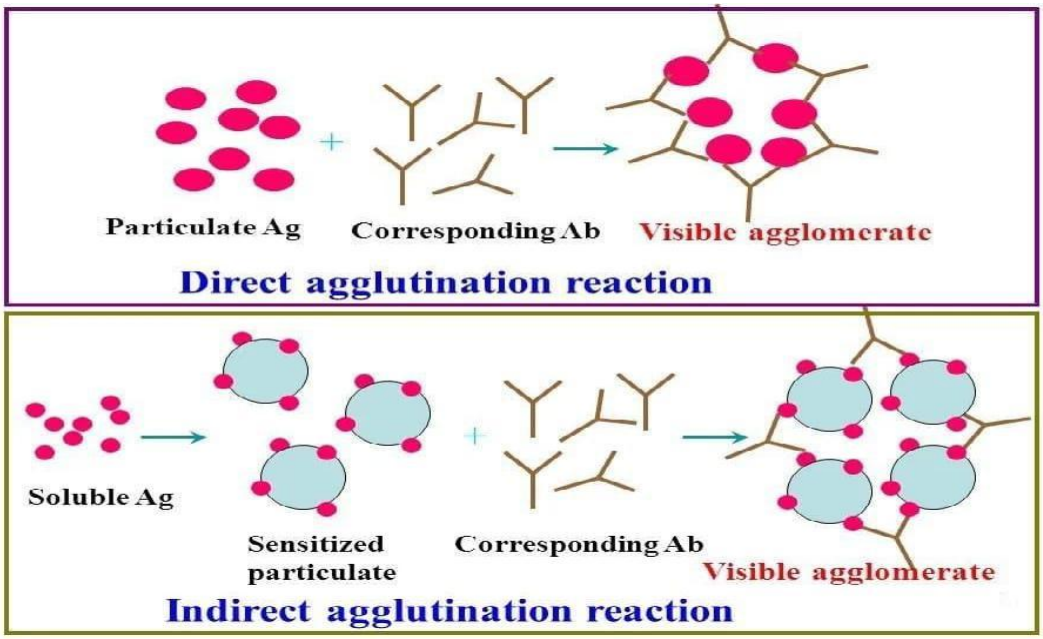
Pathergy test

Types of agglutination reactions

Agglutination reactions: Are based on the interaction of antibodies (agglutinins) and particulate antigens (e.g. bacteria) in presence of electrolytes (NaCI) at a suitable temperature and pH; the binding of multivalent antibodies with particulate antigens form large clumps or aggregates, easily visible without magnification, when exposed to specific antibodies.

Types of agglutination reactions: Precipitation reaction can be broadly of the following types:

1. Direct (active) agglutination reactions, where the antigens are found naturally on a particle are known as direct agglutination, example between a cellular antigen and its antibody (Blood group typing, etc.).
2. Indirect (passive) agglutination reactions employing carrier particles that are coated with soluble antigens, using biological carrier (e.g., erythrocytes); using artificial carrier (e.g., latex or charcoal particles).
3. Co-agglutination - using protein (A).

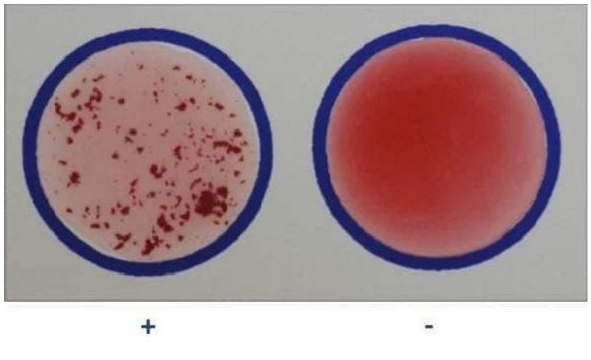


** Direct bacterial agglutination uses whole pathogens as a source of antigen, the binding of antibodies to surface antigens on the bacteria results in visible clumps.

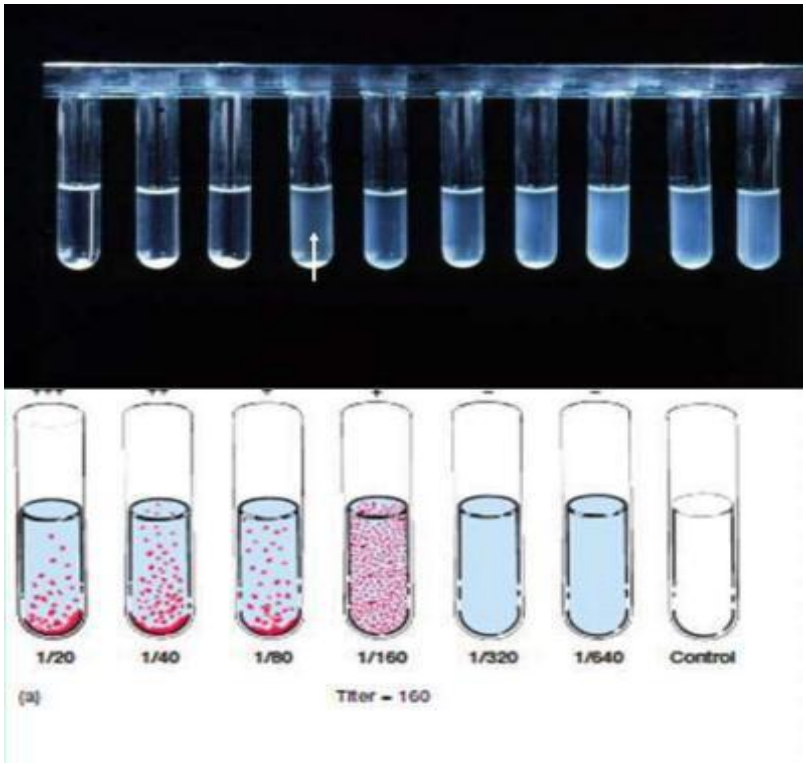
** Direct agglutination reactions can be of the following types:

1. Slide agglutination.
2. Tube agglutination.
3. Heterophile agglutination: demonstration of heterophilic antibodies in serum present in certain bacterial infections.
4. Anti-globulin (Coombs') test.

* **Slide agglutination:** Basic type of agglutination reaction that is performed on a slide. Identification of Blood typing and Slide tests are commonly used for rapid identification of bacteria such as *Salmonella* pullorum plate test, Lancefield method for serotyping of *streptococci*, etc. The method consists of mixing a drop of unknown antigen and known antibody on a glass slide or a porcelain plate, a positive reaction is indicated by formation of visible clumps within a minutes. An antigen control (negative = no formation of visible clumps) by mixing a drop of antigen with normal saline should be included to rule out false positives due to auto-agglutination of the antigen and also to validate the results.



** **Tube agglutination:** It is agglutination test performed in test tubes, tube tests are commonly used as a quantitative test to determine the titer of antibodies in a given serum sample, examples Brucellosis, Widal test for typhoid, etc.; in this method serum is diluted in a series of tubes and standard antigen suspensions (consists of adding a fixed volume of antigen into test tubes containing serial dilutions of antiserum in normal saline), after incubation, a positive reaction is indicated by formation of visible clumps and the reciprocal of highest dilution of anti-serum giving positive reaction is the titer of antibodies. Appropriate antigen and serum controls should be included to validate the results. Prozone phenomenon may be observed.



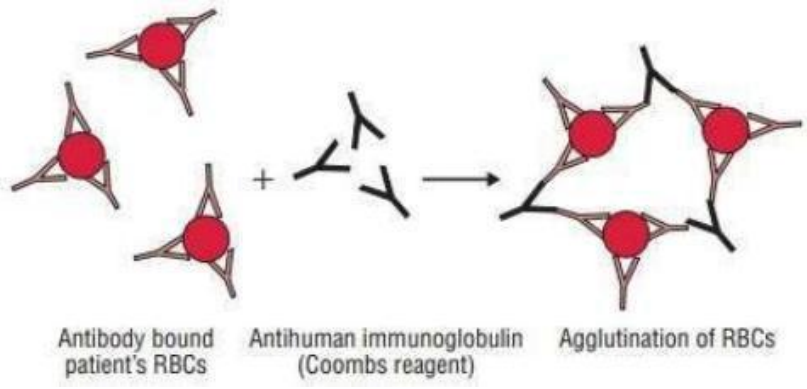
*** **Heterophile agglutination test:** Heterophile antibodies are antibodies induced by external antigens (heterophile antigens), some cross-react with self-antigens for example, in rheumatic fever, antibodies against group (A) streptococcal cell walls can also react with (and thus damage) human heart tissues; this test depends on demonstration of heterophilic antibodies in serum present in certain bacterial infections.

**** **Anti-globulin (Coombs') test:** Coombs' test was devised originally by Coombs', Mourant, and Raceb for detection of incomplete anti-Rh antibodies, these incomplete anti-Rh antibodies coats the surface of erythrocytes, but does not cause any agglutination when serum is mixed with Rh1 erythrocytes in saline, but on adding antiglobulin or Coombs' serum (rabbit anti-human IgG) to such anti-Rh antibodies coated erythrocytes, however, the latter are agglutinated. Coombs' tests are used for detection of anti-Rh antibodies, autoimmune hemolytic anemia, and incomplete antibodies in brucellosis and other diseases. Coombs' test is of two types:

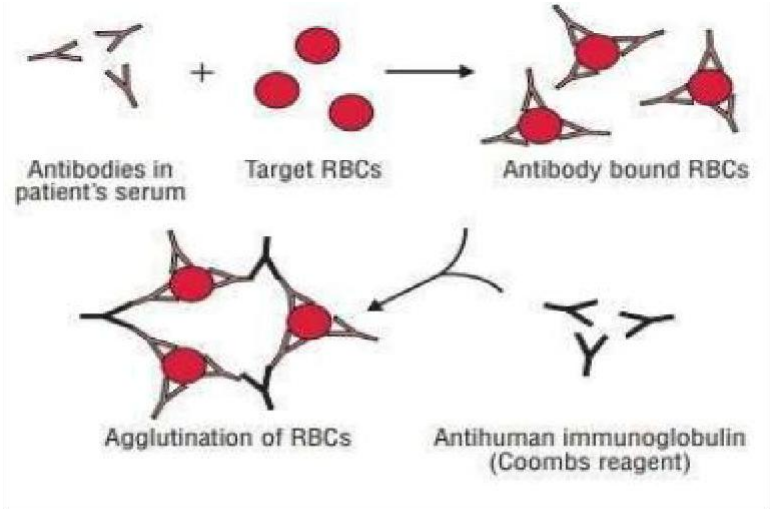
(a) Direct Coombs' test: Detection of incomplete antibodies on patients RBCs.

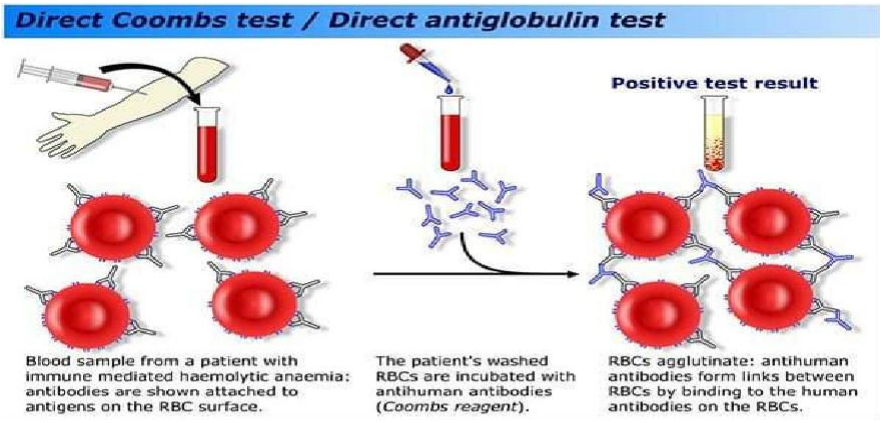
(b) Indirect Coombs' test: Detection of incomplete antibodies in patient's sera.

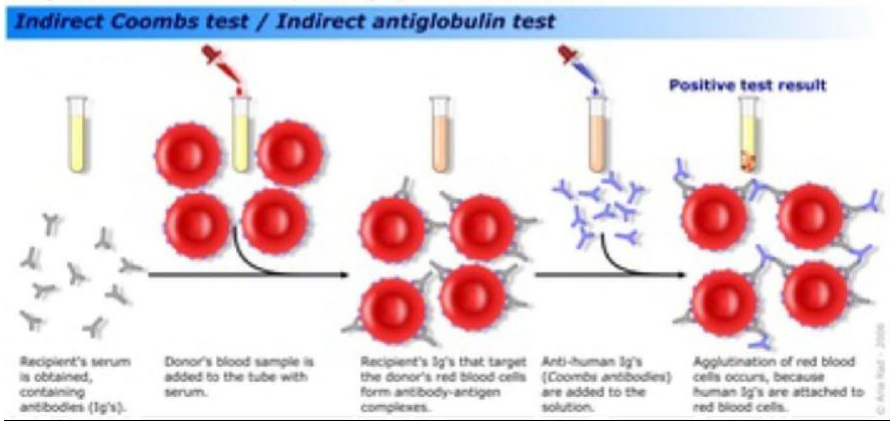
* **Direct Coombs' test**: In this test, the sensitization of red blood cells (RBCs) with incomplete antibodies takes place in vivo, the test procedure involves the addition of Coombs serum (AHG anti-human IgG) directly to a patient's washed RBCs, the occurrence of agglutination means that the patient's RBCs have cell bound antibodies, direct Coombs test is used for diagnosis of: hemolytic transfusion reaction, hemolytic disease of the fetus and newborn (HDFN) and autoimmune hemolytic anemia (AIHA) etc.



Indirect Coomb's Test: The indirect Coombs test, detects antibodies against human RBCs in the patient's serum, in this method a patient's serum is incubated with RBCs of a known type followed by the addition of adding Coombs serum (AHG), if test serum contains incomplete circulating (Ab) they will sensitize RBCs in vitro and agglutination will result, the indirect Coombs test is used in cross-matching before blood transfusions and in prenatal testing of pregnant women.

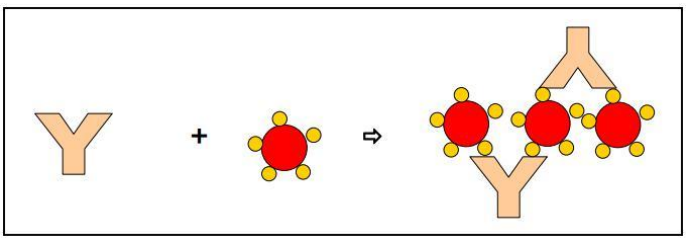






2. Indirect (Passive) Agglutination: Agglutination reactions where the soluble antigens are coated or adsorbed on a cell or particle, called as carrier, are known as direct agglutination, these antigens are not normally found on the surfaces of such carriers, coating is usually done to convert precipitation reactions into agglutination reactions, since the latter are easier to perform and interpret and are more sensitive than precipitation reactions for detection of anti-bodies, the binding of antibodies to homologous antigens adsorbed on the surface of carriers results in the agglutination of carrier particles forming visible clumps as in direct agglutination (e.g. Latex particles, Carbon particles, Bantonite are used as inert carriers; antigens coated in latex particles used in ASO test), if the antibody instead of antigens is adsorbed on the carrier particle for detection of antigens, it is called as reverse passive agglutination. Types of particles that participate in such reactions: Erythrocytes, Bacterial cells and inert carriers such as latex particles.

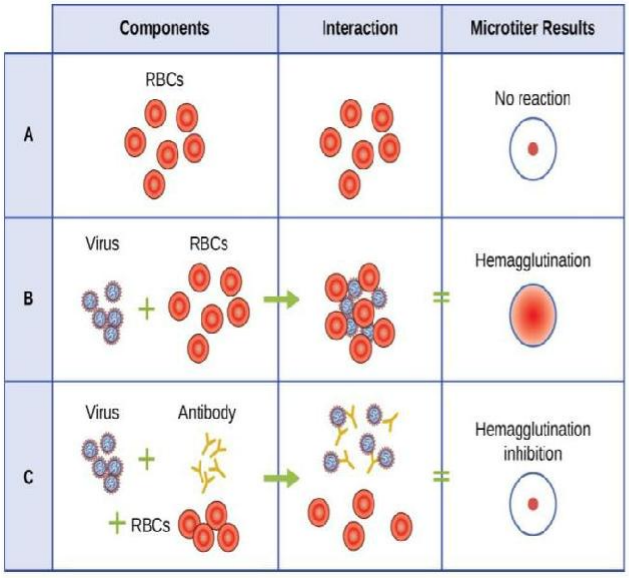
** **Latex Agglutination:** A group of passive agglutination tests carried out by coating either antigen or antibody on an artificial carrier particle, called latex bead, are known as latex agglutination test, if antigen and antibodies are homologous, then clumping of beads occur, latex agglutination test can be used either to detect antibody or antigen (known as Reverse passive latex agglutination test). Latex beads are polystyrene latex particles; 0.8- 1 μm in diameter, the number of antibody or antigen molecules bound to each latex particle is large, resulting in a high number of exposed potential binding sites, antigen or antibody present in serum specimen binds to the combining sites of the corresponding antibody/antigen exposed on the surfaces of the latex beads, forming cross-linked aggregates of latex beads and antigen/antibody, which are visible as clumps.

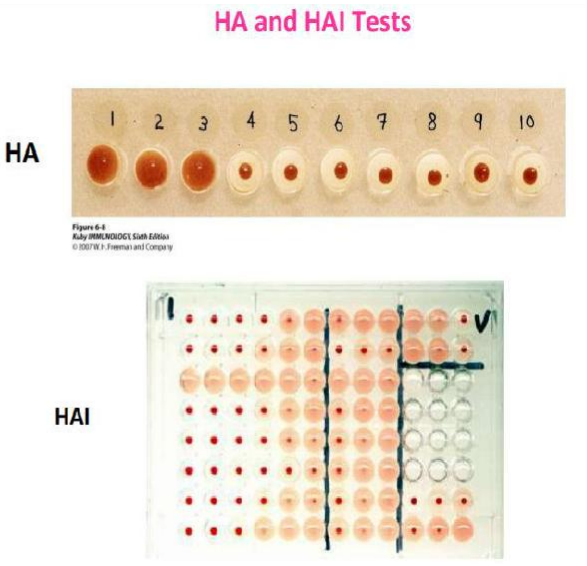


Hemagglutination test: RBCs are used as carrier particles in hemagglutination tests (RBCs of sheep, human and chick) are commonly used in the test. When RBCs are coated with antigen to detect antibodies in the serum, the test is called indirect hemagglutination (IHA) test. Hemagglutination uses erythrocytes as the biological carriers of bacterial antigens, and purified polysaccharides or proteins for determining the presence of corresponding antibodies in a specimen. When antibodies are attached to the RBCs to detect microbial antigen, it is known as reverse passive hemagglutination (RPHA).

Viral hemagglutination: Many viruses including influenza, mumps, and measles have the ability to agglutinate RBCs without antigen antibody reactions, this process is called viral hemagglutination, this hemagglutination can be inhibited by antibody specifically directed against the virus, and this phenomenon is called hemagglutination inhibition.

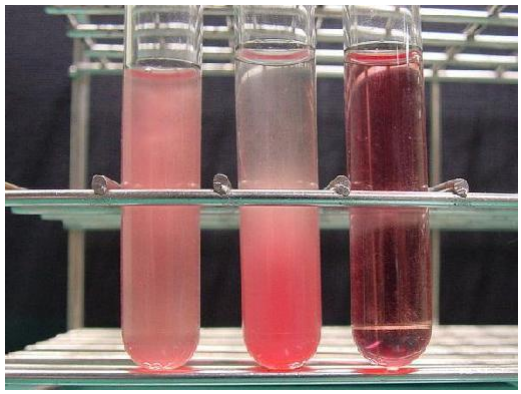
Hemagglutination Inhibition Assay: Is used in titrate the antibody response to a viral infection. The HI assay takes advantage of some viruses' ability to hemagglutinate (bind) red blood cells, therefore forming a "lattice" and preventing the red blood cells from clumping.





Tube testing ABO (preparation 3-5% RBC suspensions)

Is review basic hemagglutination and detect the antigen-antibodies reaction, take red cells that we know are group ABO and Rh as group A for example, in first step is prepare 3-5% cell suspension, it's important to label the test tubes for you'll no forget what you're doing, then get some red cells from the bottom of tube (test tube or EDTA tube) and put 1-2 drops of the red cells into each of labeled test tubes and fill them with saline, which will wash the red cells because it provides for a cleaner cell and remove the extra proteins or antibodies surrounding the cells, then centrifuge for a minute, red cells will be at the bottom of the tube and the saline supernatant will be clear, dump the saline to make the tube cleaner, the dump its quick flick of the wrist because if you do it too slowly the air bubbles from and the saline won't come out, then would fill the tube with saline and centrifuge it again for a minute, and again the wash to make 3 time. In the end the important tool that used in hemagglutination testing to have a 3-5% cell suspension is commercially prepared cells (red reagent known what concentration) as a point of comparison as you learn to make cell suspensions by add saline to the tube until think it looks right.



** A 3-5% red cell suspension is used for the following tube examination procedures:

- ABO and Rh typing.
- Direct antiglobulin test and auto control.
- Donor unit compatibility (crossmatch).
- Red cell phenotyping.

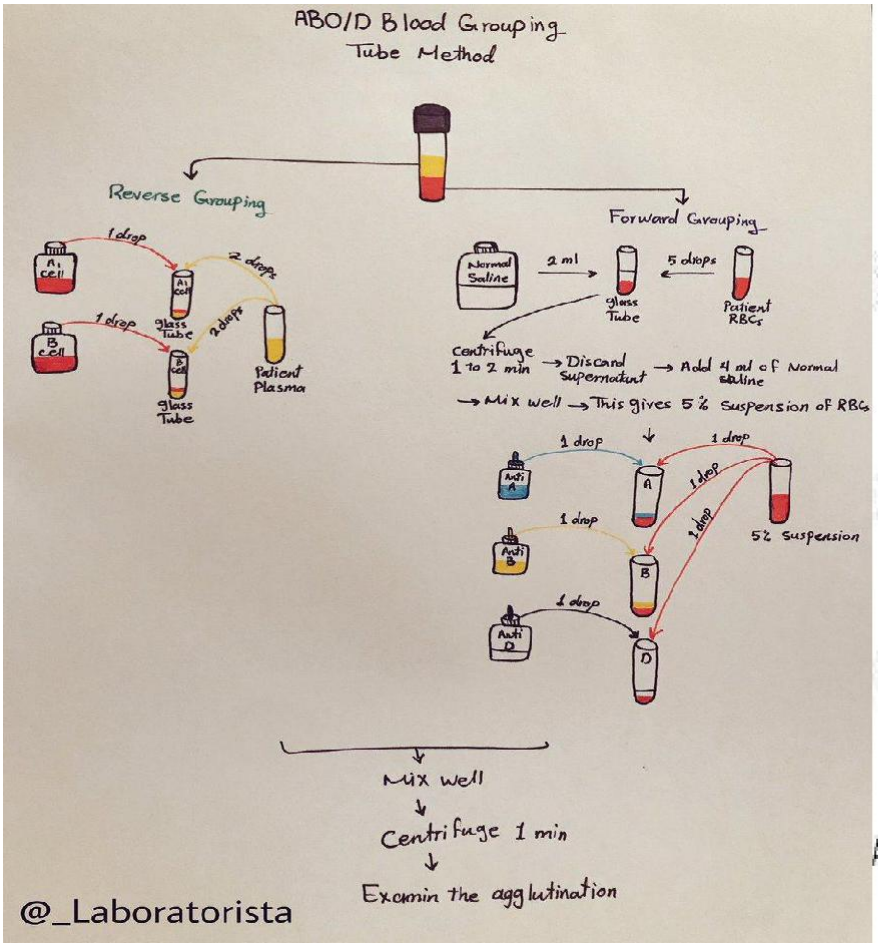
Specimens: EDTA anticoagulated whole blood and Cord blood collected in a plain tube

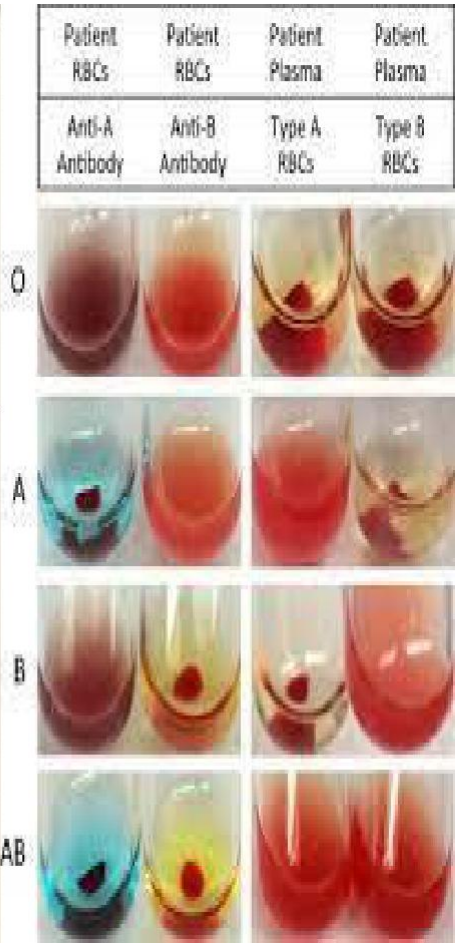
Procedure of Preparing a 3-5% Red Cell Suspension

1. Label a tube with the patient identifier or donor unit number and add 2 drops of whole blood or 1 drop of packed cells into the appropriate labelled tube.
2. Fill test tube ¾ full with saline to re-suspend the cells. Avoid contamination of tubes when dispensing saline into several tubes.
3. Compare the red cell suspension to a commercial 3% suspension (e.g. reverse grouping cells) if necessary, to ensure the appropriate strength has been achieved. Adjust to a 3-5% suspension by adding saline or centrifuging to remove saline.
4. Centrifuge the tube to obtain clear supernatant and a defined red cell button. (Speed and time as recommended by manufacturer's directions). Decant the supernatant.
5. Following the removal of supernatant, gently re-suspend the red cell button.If more than one wash is required repeat steps of washing. After the last wash, gently re-suspend the red cells with saline to a 3-5% red cell suspension.
6. Compare the red cell suspension to a commercial 3% suspension if necessary, to ensure the appropriate strength has been achieved. Adjust to a 3-5% suspension by adding saline or centrifuging to removing saline. Mix the red cell suspension immediately before performing the serological procedure.

ABO tube test: In comparison to the slide test, the tube test is more sensitive and reliable; therefore, it can be used conveniently for blood transfusion. In this method, both forward (cell), as well as reverse (serum) grouping is carried out. The forward grouping suggests the presence or absence of A and B antigens in RBCs, whereas reverse grouping indicates the presence or absences of anti-A and anti-B in serum. In forward grouping, blood cells are placed in two test tubes along with saline as a diluent media, and then one drop of each anti-A and anti-B is added separately in these samples. These tubes are subjected to centrifugation for few minutes, and then, the resultant matrix is gently shaken for observing agglutination.

For precise blood grouping, the two tubes can be categorized according to the extent of blood clumping. The purpose of centrifugation is to ensure enhanced chemical interactions, particularly for weaker antibodies to react, thus leading to agglutination. Some potentiators could also be added to promote the agglutination; moreover, the long incubation of tubes also favors these reactions without drying of the test samples. In a similar fashion, reverse grouping can be performed, as here, the blood serum is treated against RBC reagent groups of A1 and B, and the subsequent agglutination pattern is monitored. The grading of agglutinates in both forward and reverse grouping is useful in comparing the difference in the strength of hemolysis reactions. In general, the tube method is much more sensitive than the slide test and requires a low volume of reagents, and some unexpected antigens can also be detected; therefore, it is a better option for safer transfusions. However, in infants, reverse grouping is somewhat difficult to perform, since they produce insufficient amounts of antibodies to be determined.

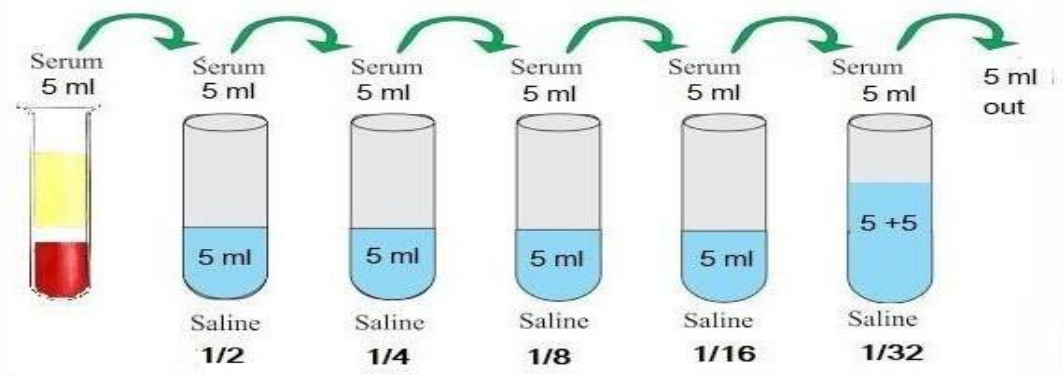




Dilutions

- **A dilution** is a process that reduces the concentration of a substance in a solution and is a common laboratory technique used to obtain the desired concentration.
- The serology tests (usually refers to the diagnostic concentration of antibodies in the serum) can be classified into:
  1) Qualitative tests (positive and negative).
  2) Quantitative tests (such as 300 µl).

  To make the quantitative tests we have to make several dilutions. Usually, in immunology lab the specimen is the serum and the diluent is the normal saline.
- **Serum:** is that part of blood which is similar in composition with plasma but exclude clotting factors of blood, which can be obtained from put the blood in the plane tube and left stand at room temperature for 15- 30 minutes to coagulate and a centrifuge for 10 minutes at 3000 rpm.
- It may be found that the concentration of the substance being measured is too high for measurement with a certain instrument . In such cases, a dilution is necessary.
- **Antibody Titer**: the relative amount of antibody present in an antiserum that can bind to a constant amount of antigen. The relative amount is usually determined by making dilutions of an antiserum and testing the dilutions for their ability to react with an antigen then greatest reacting dilution is taken as the Titer.
- The diluent is **Normal Saline** (N.S) prepared by weighing the 85 gm NaCl to 1L distilled water.



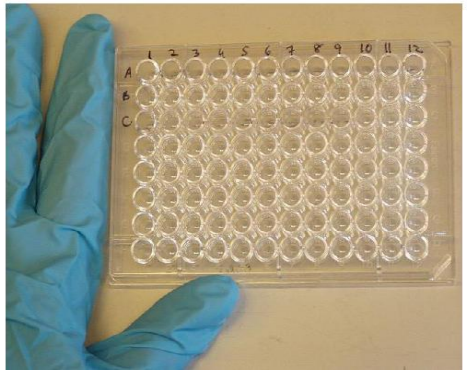
Lab 8

ELISA

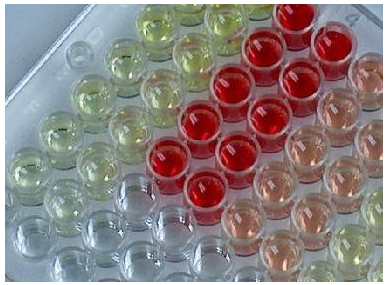
Clinical Immunity

Msc. Anmar Saeed Almusawi

ENZYME LINKED IMMUNO SORBENT ASSAY (ELISA)



INTRODUCTION TO ELISA

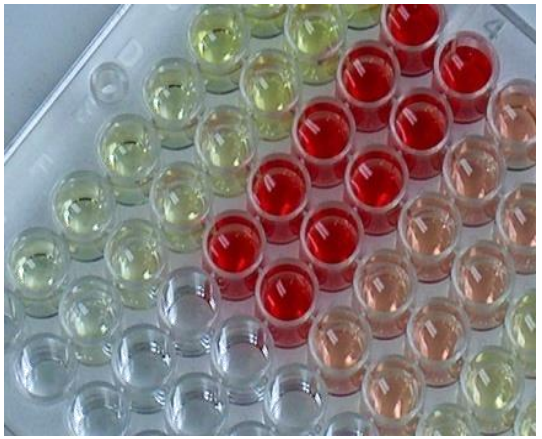


- ELISA, or enzyme-linked immunosorbent assay, are quantitative immunological procedures in which the Ag- Ab reaction is monitored by enzyme measurements.

The ELISA test, or the enzyme immunoassay (EIA), was the first screening test commonly employed for HIV. It has a high sensitivity.

Why known as .....?

Enzyme Linked Immunosorbent Assay



1. Antigen of interest is absorbed on to plastic surface (‘*sorbent*’).
2. Antigen is recognised by specific antibody (‘*immuno*’).
3. This antibody is recognised by second antibody (‘*immuno*’) which has enzyme attached (‘*enzyme-linked*’).
4. Substrate reacts with enzyme to produce product, usually coloured.

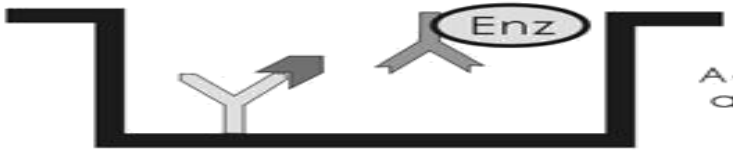
BASIC PRINCIPLE OF ELISA

- Use an enzyme to detect the binding of antigen (Ag) antibody (Ab).
- The enzyme converts a colorless substrate (chromogen) to a colored product, indicating the presence of Ag : Ab binding.
- An ELISA can be used to detect either the presence of Antigens or antibodies in a sample depending how the test is designed.
- ELISA was dveloped in 1970 and became rapidly accepted

Basic procedure of ELISA

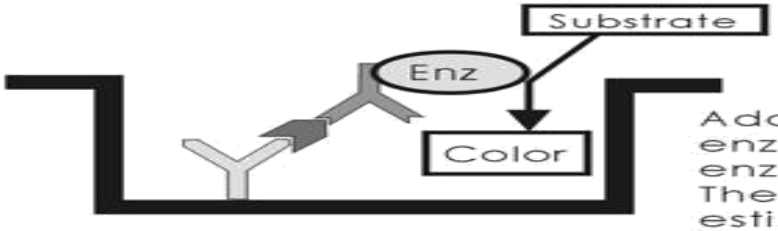
Add sample solution containing antigen to be measured to the well where antibody is solidified.

Antigen is captured by antibody.
Wash out excessive substances.



Add enzyme-labeled second antibodyto the well.

The enzyme-labeled second antibody binds to the captured antigen.
Wash out excessive substances.



Add a chromogenic substrate of the enzyme which shows coloration by enzyme.
The amount of the antigen is estimated from the absorbance.

Materials Needed

- Testing sample
- Antibody (1^{st}, 2^{nd}) / Antigen
- Polystyrene microtiter plate
- Blocking buffer
- Washing buffer
- Substrate
- Enzyme
- Micro-plate reader

Specimen Sample For ELISA

SERUM
CSF
SPUTUM
URINE
SEMEN
SUPERNATANT OF CULUTRE
STOOL

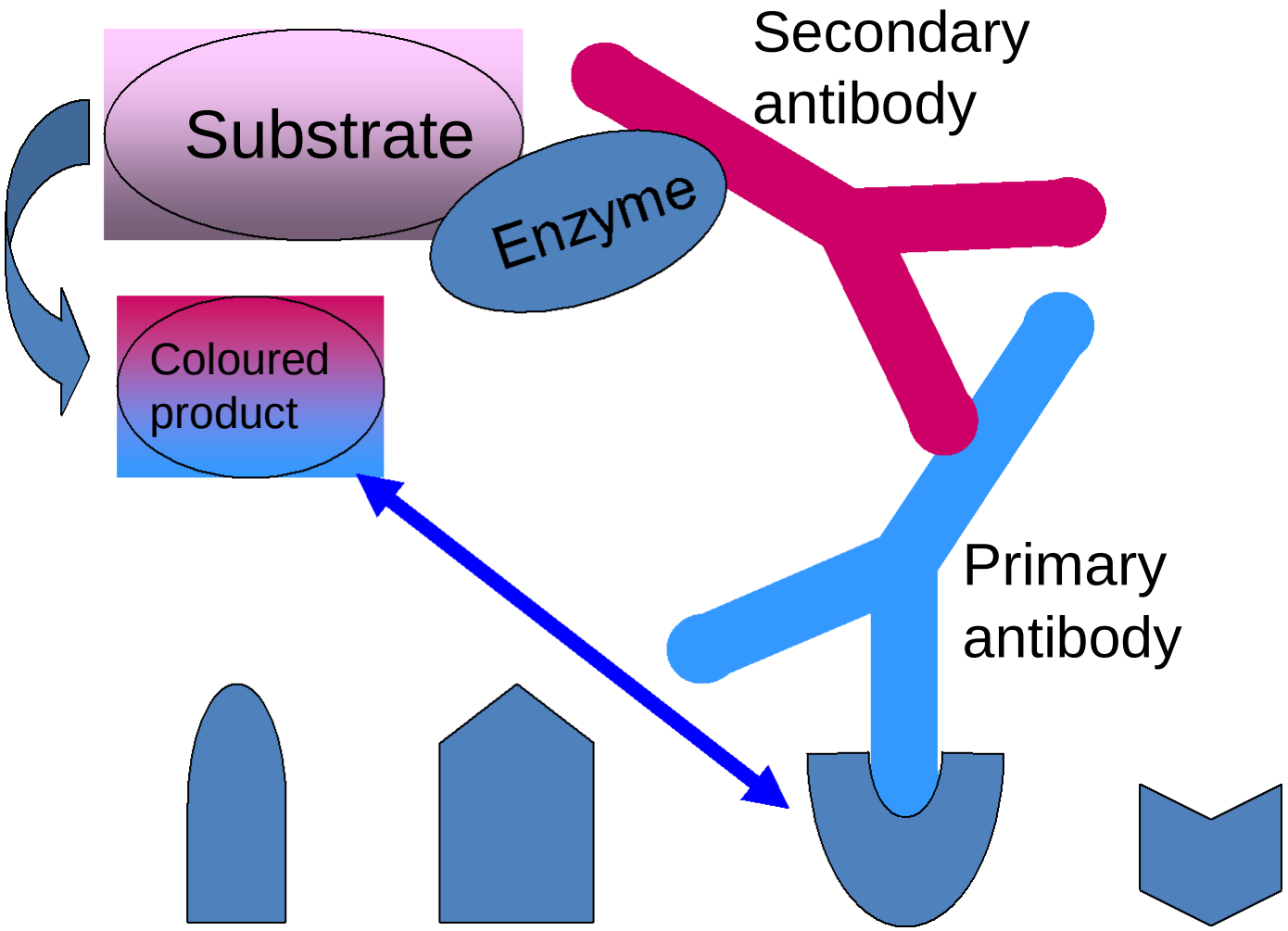
Enzymes Used in Elisa

- Horseradish peroxidase (most commonly used)
- Alkaline Phosphatase

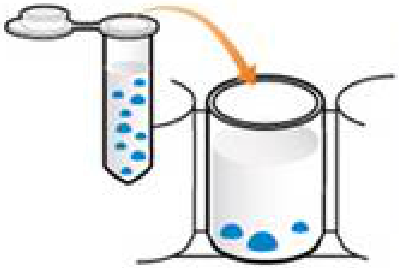
ENZYME SUBSTRATE

- Initially the substrate should be colorless
- After degradation by the enzyme it should be strongly colored or fluorescent.

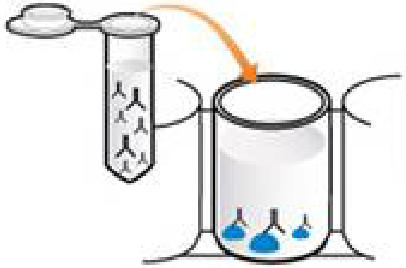
ENZYME	SUBSTRATE	CHROMOGEN	STOPPING
Alkaline Phosphatase	p-NPP	p-NPP+ diethandamine+Mg Cl2	1 M NaOH
Horse radish Peroxidase	H2O2	Tetramethylbenzidine + Phosphate – Citrate buffer	1 M H2SO4
Horse radish Peroxidase	H2O2	O – Phenylenediamine + HCl	1 M HCl


Secondary antibody
Substrate
Enzyme
Coloured product
Primary antibody
Different antigens in sample

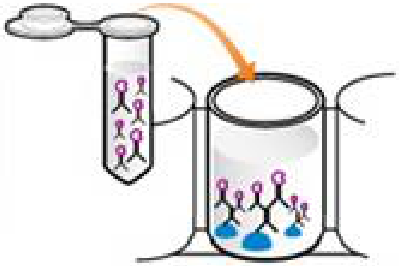
Basic Steps Of Enzyme-Linked Immunosorbant Assay



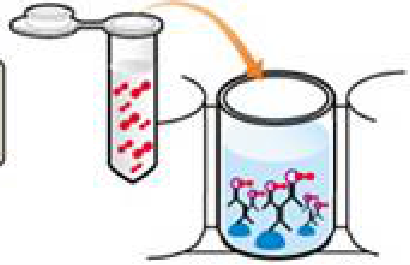
1. Load student samples (unknowns) in triplicate into wells of the microplate strips. Incubate for 5 minutes. Rinse.



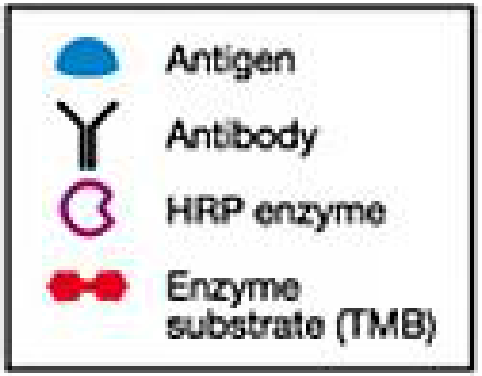
2. Add primary antibody to all wells. Incubate for 5 minutes. Rinse.



3. Add enzyme-linked secondary antibody to all wells. Incubate for 5 minutes. Rinse.



4. Add enzyme substrate to all wells. Incubate for 5 minutes.



Advantages of ELISA

- Reagents are relatively cheap & have a long shelf life
- ELISA is highly specific and sensitive
- No radiation hazards occur during labelling or disposal of waste.
- Easy to perform and quick procedures
- Equipment can be inexpensive and widely available.
- ELISA can be used to a variety of infections.

Disadvantages of ELISA

- Measurement of enzyme activity can be more complex than measurement of activity of some type of radioisotopes.
- Enzyme activity may be affected by plasma constituents.
- Kits are commercially available, but not cheap
- Very specific to a particular antigen. Won't recognize any other antigen
- False positives/negatives possible, especially with mutated/altered antigen

Limitations

- Results may not be absolute
- Antibody must be available
- Concentration may be unclear
- False positive possible
- False negative possible

APPLICATIONS OF ELISA

1- Hormones	**7- Vaccine Quality Control**
2- Proteins	8- FOR GMO (Genetically modified organism)
3- Infectious Agent (Viral, Bacterial, Parasitic, Fungal)	9- For Rapid Test
4- Drug Markers	10- IgG, IgM, IgA
5- Tumor Markers	11- In New Born Screening
6- Serum Proteins	12- In Clinical Research

Equipments for performing the ELISA test

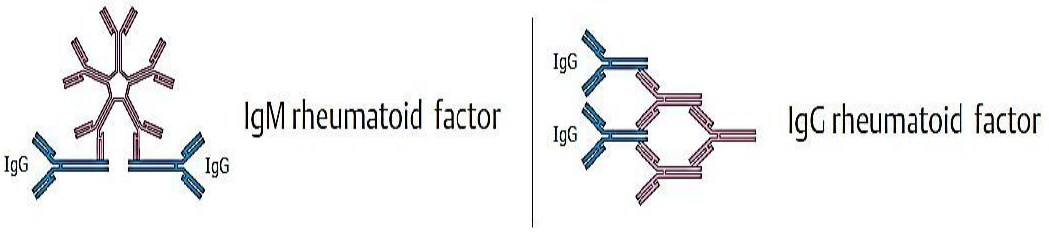
(CRP)

Is protein produced by liver in response to inflammation, elevated values can be found among people with certain chronic inflammatory diseases, i.e. rheumatoid arthritis, juvenile chronic arthritis, ankylosing spondylitis and Crohn's disease.

Normal range of CRP is less than **10 mg/l.**

(RF)

Are autoantibodies against antibodies (IgG OR IgM), this immune complex may precipitate in joint and causing **rheumatoid arthritis (RA)**.



Presence:

1- 60-80% of rheumatoid arthritis cases

2- May be in Systemic Lupus Erythematosus (SLE)

3- May be in SS

4- May be normally elevated normal elderly population.

Normal range= 0-18 IU/ml.

(Anti-ccp)

ACPAs target a type of protein that is called a citrullinated protein that can be found in some people's joints. The anti-CCP antibody test can be helpful in diagnosing Rheumatoid Arthritis (RA).

Arginine is a standard amino acid. Arginine can be converted to the non-standard amino acid *citrulline* by an enzyme called *Peptidyl-Arginine-Deiminase (PAD)*.

ACPAs are present in **60-70% of people who get RA**.

This test is **97% specific for RA if it is present**.

The anti-CCP antibody test can help **distinguish RA from other possible types of arthritis.** The ACPAs it looks for are almost always associated with RA, they are not associated with many other types of arthritis, and they are only rarely found in certain other autoimmune conditions.

RA patients with anti-ccp positive called **seropositive RA**, BUT About **20% of RA patients are seronegative** meaning that anti-ccp is negative and doctor diagnose the RA in this case by imaging and physical examination.

Normal range= **less than 20 u/ml**

Psoriatic arthritis(PsA)

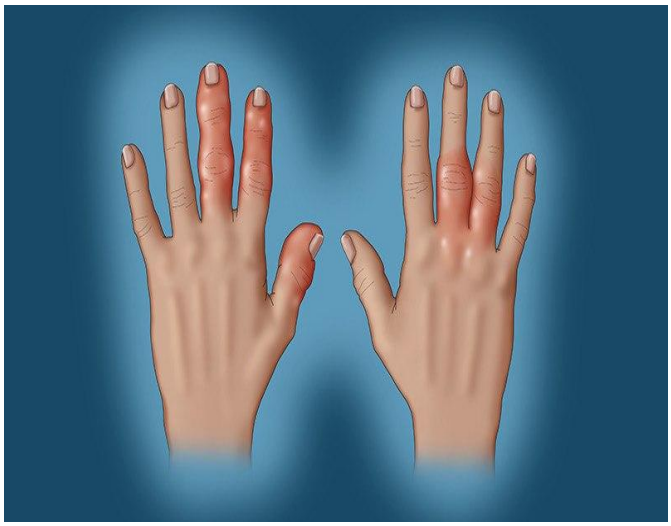
Psoriatic arthritis is a type of arthritis that affects some people with the skin condition psoriasis. It typically causes affected joints to become swollen, stiff and painful.

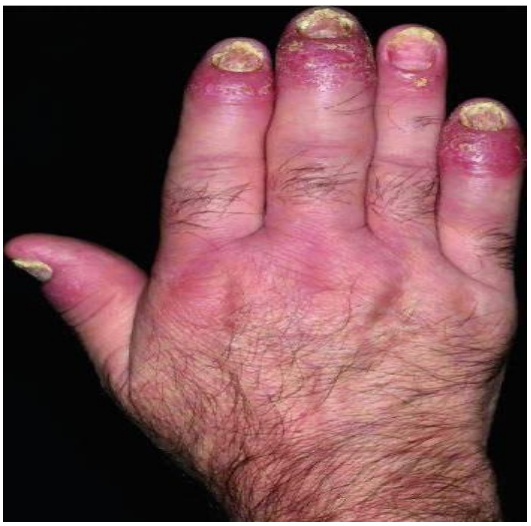
Like psoriasis, psoriatic arthritis is a long-term condition that can get progressively worse. If it's severe, there's a risk of the joints becoming permanently damaged or deformed, and surgery may be needed.

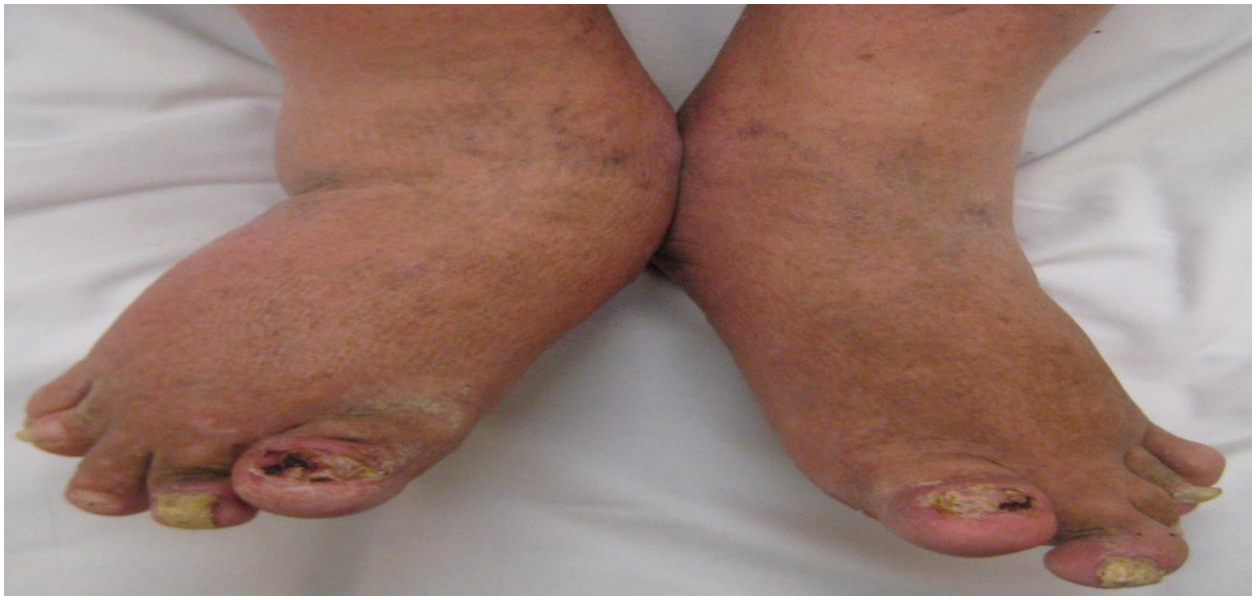
But if psoriatic arthritis is diagnosed and treated early, its progression can be slowed down and permanent joint damage can be prevented or minimised.

PsA is classified as one of the subtypes of spondyloarthropathies. Males and females are equally affected. PsA can range from mild nondestructive disease to a severely rapid and destructive arthropathy.

*Clinical manifestations include skin and nail psoriasis, dactylitis, enthesitis, osteoperiostitis, large joint oligoarthritis, arthritis mutilans, sacroiliitis, spondylitis and distal interphalangeal arthritis.







*pathophysiology and Etiology

1- Genetic Factor: HLA-B27.

2-Immunological factors:

A- Autoantibodies against nuclear antigens, cytokeratins, epidermal keratins, and heat-shock proteins have been reported in persons with psoriatic arthritis, indicating that the disease has a humoral immune component.

B- The cytokine profile for psoriatic arthritis reflects a complex interplay between T cells and monocyte (macrophages). Th1-cell cytokines (eg, TNF-α, IL-1β, IL-10) are more prevalent in psoriatic arthritis than in RA, suggesting that these 2 disorders may result from a different underlying mechanism.

C- Dendritic cells have been found in the synovial fluid of patients with psoriatic arthritis and are reactive in the mixed leukocyte reaction; the conclusion is that the dendritic cells present an unknown antigen to CD4+ cells within the joints and skin of patients with psoriatic arthritis, leading to T-cell activation.

D- Fibroblasts from the skin and synovial of patients with psoriatic arthritis have an increased proliferative activity and the capability to secrete increased amounts of IL-1, IL-6, and plateletderived growth factors. Several studies suggest that cytokines secreted from activated T cells and other mononuclear proinflammatory cells induce proliferation and activation of synovial and epidermal fibroblast.

3- Infections: psoriatic arthritis has been reported to be associated with bacterial infection as in Streptococcus species infection and viral infection such as HIV.

4- Enviromental factors

*Diagnosis of (PsA)

1-By Clinical symptoms

2- Blood tests They include: A- C-reactive protein. B- Rheumatoid factor (RF): People with PsA are almost always RF-negative C- Erythrocyte sedimentation rate (ESR) is often done. The higher ESR the greater the level of inflammation in the body.

3-X-rays

4-MRIs

Rheumatoid factor (RF)

RF is an immunoglobulin (autoantibody) which can bind to other antibodies normally, antibodies produce by the immune system to help destroy and eliminate invading foreign bacteria and viruses that can causes disease but the RF antibody can attach to normal body tissue and cause damage in resulting. The incidence of RF increases with age and about (20%) present of people over (65 years old) have an elevated RF. The RF increased in many diseases such as: Rheumatoid arthritis, chronic viral infection, Tuberculosis, Chronic hepatitis, Leukemia, Syphilis and Renal diseases.

RF test: Specimen; this test should be performed on fresh serum and the samples may be stored refrigerated (2^{o}- 8^{o}C) for a maximum of (7 days), lf longer storage is required store at (-20^{o} C); the bacterial contamination may cause positive agglutination.

Principle of the method:

The principle of the test is immunologic reaction (agglutination) between the Rheumatoid Factor (RF), a macromolecular molecule globulin found in serum and the corresponding lgG coated onto finely dispersed **polystyrene latex particles**.

Reagents:

* RF Latex direct reagent: Polystyrene latex particles coated with human lgG and suspended in a glycine buffer.

* Positive control serum: A stabilized human serum containing rheumatoid factors RF reactive with the latex reagent.

* Negative control serum: a stabilized human serum nonreactive with the latex reagent.

Materials provided with test set (kit):

1. RF Latex, Direct Reagent.

2. Positive Control Serum.

3. Negative Control Serum.

4. 6- Well Test Slide.

5. Disposable Pipettes.

6. Product Instructions (leaflet).

Materials NOT provided:

1. Test Tubes (for quantitative method).

2. Serological Pipettes.

3. Laboratory Timer.

4. Laboratory Rotator (optional).

5. Isotonic Saline (0.85% sodium chloride for quantitative method).

Test procedure (method I, qualitative)

1. Bring all reagents and specimens to room temperature (25^{o} C).

2. Shake the latex reagent gently, expel the contents of the dropper and refill.

3. Deliver on drop (50µ of patient sample to a circle on the test. Use a new pipet for each sample.

4. Place a drop of the latex reagent next to each specimen on the test slide.

5. Mix each specimen and latex with a disposable stirrer and spread over surface of each circle.

6. Rotate the slide (80 - 100 r.p.m.) for 2 minutes.

7. Examine under light source for the presence of agglutination.

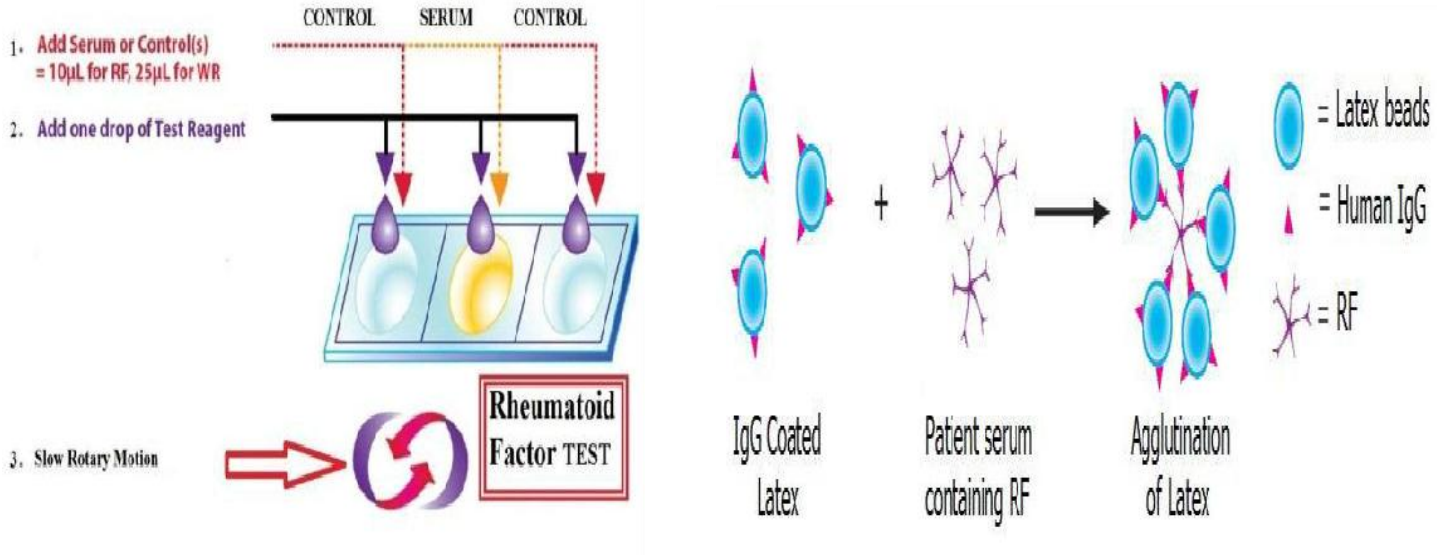
RESULTS

Positive: Agglutination (clumping of the latex particles) indicates a positive result.

A weakly reactive serum produces Avery fine granulation or partial clumping.

Negative: The absence of agglutination indicates a negative result.

** Positive and negative controls should be tested with each series of test sera.



RF test

Serum

Kit compound

Test positive (method II, semi-quantitative)

1* The semi-quantitative test can be performed in the same way as the qualitative test using isotonic saline is normal saline (0.85% sodium chloride), prepare a serial dilution of the serum starting all (1:2 thru l: 64 or more) as follows:

Dilution	Serum	Saline
1/2	50 µl undiluted serum	50 µl
1/4	50 µl 1/2 diluted serum	50 µl
1/8	50 µl 1/4 diluted serum	50 µl
1/16	50 µl 1/8 diluted serum	50 µl
1/32	50 µl 1/32 diluted serum	50 µl
1/64	50 µl 1/64 diluted serum	50 µl

2* Test each dilution in the same way as the qualitative technique above.

3* Agglutination of the sera indicates:

Dilution	RF Levels (IU/mL)
1/2	16 (8x2)
1/4	32 (8x4)
1/8	64 (8x8)
1/16	128 (8x16)
1/32	256 (8x32)
1/64	512 (8x64)

Results:

The titer is reported as the reciprocal of the highest dilution which gives a visible agglutination: e.g. if this occurs in dilution 1/8, the titer is

T= (DxF IU/mL)(8x8 IU/mL) = 64 IU/mL

*Normal levels of RF in adults is (0 – 18) IU/mL

Rheumatoid factor (RF)

RF is an immunoglobulin (autoantibody) which can bind to other antibodies normally, antibodies produce by the immune system to help destroy and eliminate invading foreign bacteria and viruses that can causes disease but the RF antibody can attach to normal body tissue and cause damage in resulting. The incidence of RF increases with age and about (20%) present of people over (65 years old) have an elevated RF. The RF increased in many diseases such as: Rheumatoid arthritis, chronic viral infection, Tuberculosis, Chronic hepatitis, Leukemia, Syphilis and Renal diseases.

RF test: Specimen; this test should be performed on fresh serum and the samples may be stored refrigerated (2^{o}- 8^{o}C) for a maximum of (7 days), lf longer storage is required store at (-20^{o} C); the bacterial contamination may cause positive agglutination.

Principle of the method:

The principle of the test is immunologic reaction (agglutination) between the Rheumatoid Factor (RF), a macromolecular molecule globulin found in serum and the corresponding lgG coated onto finely dispersed **polystyrene latex particles**.

Reagents:

* RF Latex direct reagent: Polystyrene latex particles coated with human lgG and suspended in a glycine buffer.

* Positive control serum: A stabilized human serum containing rheumatoid factors RF reactive with the latex reagent.

* Negative control serum: a stabilized human serum nonreactive with the latex reagent.

Materials provided with test set (kit):

1. RF Latex, Direct Reagent.

2. Positive Control Serum.

3. Negative Control Serum.

4. 6- Well Test Slide.

5. Disposable Pipettes.

6. Product Instructions (leaflet).

Materials NOT provided:

1. Test Tubes (for quantitative method).

2. Serological Pipettes.

3. Laboratory Timer.

4. Laboratory Rotator (optional).

5. Isotonic Saline (0.85% sodium chloride for quantitative method).

Test procedure (method I, qualitative)

1. Bring all reagents and specimens to room temperature (25^{o} C).

2. Shake the latex reagent gently, expel the contents of the dropper and refill.

3. Deliver on drop (50µ of patient sample to a circle on the test. Use a new pipet for each sample.

4. Place a drop of the latex reagent next to each specimen on the test slide.

5. Mix each specimen and latex with a disposable stirrer and spread over surface of each circle.

6. Rotate the slide (80 - 100 r.p.m.) for 2 minutes.

7. Examine under light source for the presence of agglutination.

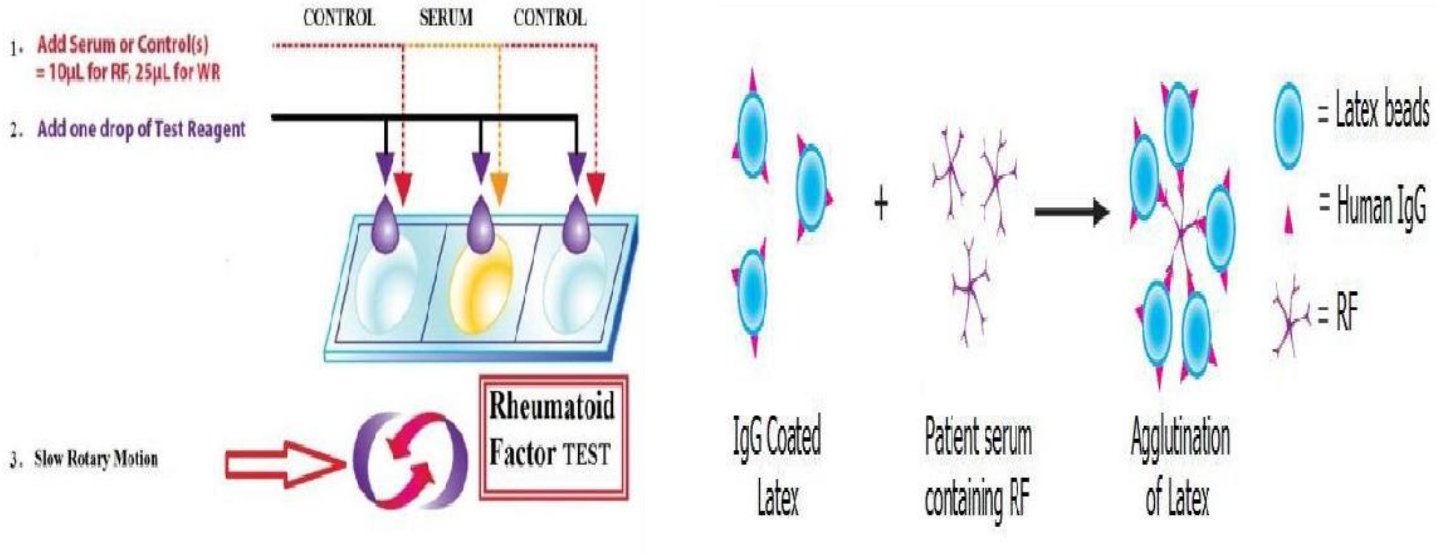
RESULTS

Positive: Agglutination (clumping of the latex particles) indicates a positive result.

A weakly reactive serum produces Avery fine granulation or partial clumping.

Negative: The absence of agglutination indicates a negative result.

** Positive and negative controls should be tested with each series of test sera.



RF test

Serum

Kit compound

Test positive (method II, semi-quantitative)

1* The semi-quantitative test can be performed in the same way as the qualitative test using isotonic saline is normal saline (0.85% sodium chloride), prepare a serial dilution of the serum starting all (1:2 thru l: 64 or more) as follows:

Dilution	Serum	Saline
1/2	50 µl undiluted serum	50 µl
1/4	50 µl 1/2 diluted serum	50 µl
1/8	50 µl 1/4 diluted serum	50 µl
1/16	50 µl 1/8 diluted serum	50 µl
1/32	50 µl 1/32 diluted serum	50 µl
1/64	50 µl 1/64 diluted serum	50 µl

2* Test each dilution in the same way as the qualitative technique above.

3* Agglutination of the sera indicates:

Dilution	RF Levels (IU/mL)
1/2	16 (8x2)
1/4	32 (8x4)
1/8	64 (8x8)
1/16	128 (8x16)
1/32	256 (8x32)
1/64	512 (8x64)

Results:

The titer is reported as the reciprocal of the highest dilution which gives a visible agglutination: e.g. if this occurs in dilution 1/8, the titer is

T= (DxF IU/mL)(8x8 IU/mL) = 64 IU/mL

*Normal levels of RF in adults is (0 – 18) IU/mL

SLE Latex test

Specimen; this test should be performed on fresh serum and the samples may be stored refrigerated (2^{o}- 8^{o}C).

Principle of the method:

The SLE latex agglutination test provides a means of detection anti-DNP in human serum. SLE latex reagent is a stabilized buffered suspension of polystyrene latex particles that have been coated with DNP, when the latex reagent is mixed with the serum containing antibodies to DNP, agglutination occurs:

Reagents:

SLE Latex reagent: Polystyrene latex particles coated with desoxyribonucleoprotein DNP (calf thymus) Sodium azide.

Positive control: Liquid containing desoxyribonucleoprotein antibodies (Human) to give agglutination Sodium azide.

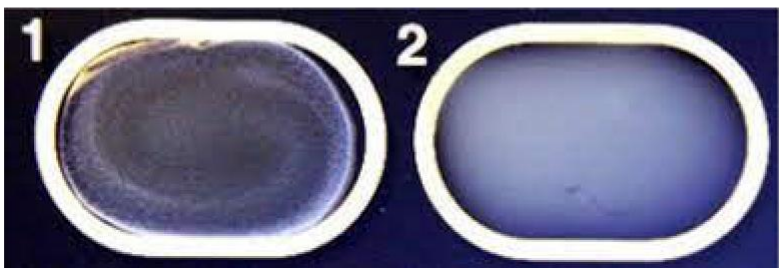
Negative control: Liquid control; non-reactive with Sodium azide.

I. Qualitative method

1. Bring all reagents and specimens to room temperature (25^{o} C).
2. Shake the latex reagent gently.
3. Deliver on drop (30µl) of patient sample to a circle on the test.
4. Place a drop (40µl) of the latex reagent next to each specimen on the test slide.
5. Mix each specimen and latex with a disposable stirrer.
6. Rotate the slide (80 - 100 r.p.m.) for (1) minute.
7. Examine under light source for the presence of agglutination.

Results:

Positive: Agglutination (clumping of the latex particles). A weakly reactive serum produces Avery fine granulation or partial clumping. Negative: Absence of agglutination indicates a negative result.



Positive (+ve) **Negative (- ve)**

Il. Semi quantitative method

The titer: This part of the ANA test gives an estimate of how many anti-nuclear antibodies are present.

1. Semi-quantitative test can be performed in the same way as the qualitative test using isotonic saline is normal saline (9 g/l NaCl), prepare a serial dilution of the serum starting all (1:2 thru l: 64) as follows:

Dilution. No.	Serum	NaCl	Titer and Result
1	250 µl undiluted serum	250 µl	1/2 1:20
2	250 µl 1/2 diluted serum	250 µl	1/4 1:40
3	250 µl 1/4 diluted serum	250 µl	1/8 1:80
4	250 µl 1/8 diluted serum	250 µl	1/16 1:160
5	250 µl 1/16 diluted serum	250 µl	1/32 1:320
6	250 µl 1/32 diluted serum	250 µl	1/64 1:640

2. Test each dilution in the same way as the qualitative technique above.
3. Agglutination of the sera indicates:

Results

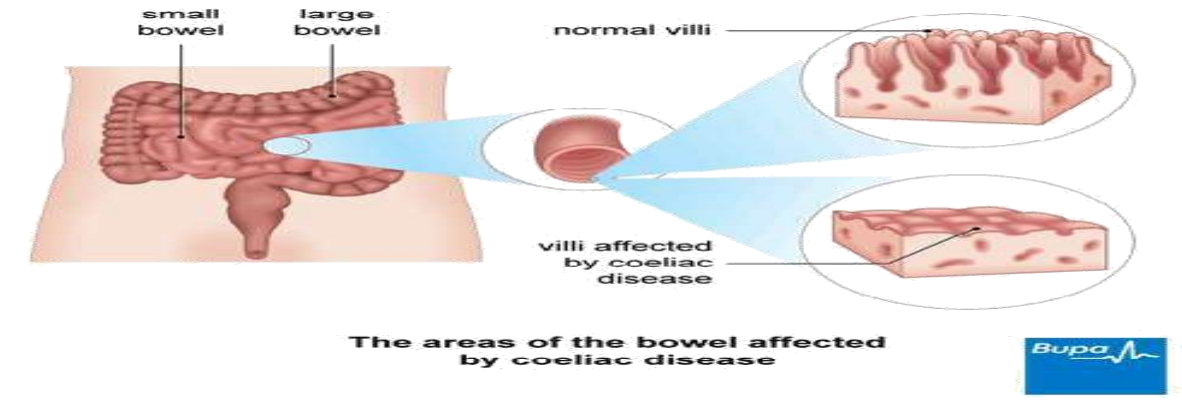
The one part of the serum is diluted into 20 parts of diluent (called a 1:20) and **the titer** (DxF) of antinuclear antibodies (anti DNP) is the reciprocal of the highest dilution which gives a visible agglutination a positive reaction. The lowest dilution is 1:640 when antibodies are present at the lowest dilution; this indicates that there are a very high number of antibodies in the blood and that the body has mounted a substantial immune response against nuclear protein.

* Normal levels of SLE (antinuclear antibodies ANA) in adults is < 1:10 mg/mL

Gastrointestinal Autoimmune Diseases

Coeliac disease: (Celiac disease or gluten-sensitive enteropathy)

is an autoimmune disease affecting the small intestine **,** that it is associated with a known environmental trigger dietary gluten. Gluten is a protein complex found in wheat, barley, and rye that is poorly digested by the upper gastrointestinal system. It contains an alcohol-soluble component called gliadin . Gliadin is resistant to digestive enzymes in the small intestine and therefore remains intact in the lumen, or space within the intestines, after ingestion. undigested gliadin is able to pass through the epithelial barrier of the intestine and trigger immune response. The immunogenicity of gliadin is enhanced when it is acted on by **tissue transglutaminase (tTG),** an intestinal enzyme that converts the gliadin to glutamic acid. that lead to cause inflammatory reaction , villous atrophy and malabsorption.



Etiology

- ✓ Genetic factors: in person carrier HLA-DQ-8 and HLA-DQ-2.
- ✓ Microbial infection like some viruses as *rotavirus* .
- ✓ Early exposure to gluten before completing the gut barrier development (1-3months after birth) is high risk for CD when second exposed (4-6months after birth).

Signs and symptoms of CD

- Common

- Diarrhea
- Fatigue
- Borborygmus
- Abdominal pain
- Weight loss
- Abdominal distention

- Uncommon

- Osteopenia/ osteoporosis
- Abnormal liver function
- Vomiting
- Iron-deficiency anemia
- Neurologic dysfunction
- Constipation

**Laboratory Diagnostic tests of CD:

- Serum Autoantibodies for diagnosis of celiac disease

- **Anti-tissue transglutaminase (tTG) antibody – IgA**

Detection of IgA antibodies to tTG is the serological method of choice for initial testing. This is because automated, ELISA-based assays , tTG antigen have a high sensitivity (91% to 95%) and specificity (95% to 97%) for celiac disease.

- **Antiendomysial (EMA) - IgA .**

Positive anti-tTG results can be followed by testing for endomysial antibodies (EMA). EMA tests are highly specific for celiac disease, but are more costly.

- **Antibodies against synthetic deamidated gliadin peptides (DGPs)**

In patients who are IgA deficient, testing for IgG anti-tTG or for IgG antibodies to DGPs can be performed. Automated ELISA tests for anti- DGPs are a more recent development and show an especially high sensitivity in children under the age of 2 to 3 years

((All of these antibodies are based on (IgA) or (IgG). But IgG-based tests Specifically, are useful for detecting celiac disease in selected IgA-deficient patients))

- **Endoscopy and biopsy:**

Upper endoscopy with at least 6 duodenal biopsies is considered the criterion standard to help establish a diagnosis of celiac disease.

- **Intestinal biopsy**

Biopsy of the small intestine should be performed to confirm the diagnosis.

Histological examination of biopsy tissue characteristically shows an increase in the number of intraepithelial lymphocytes, elongation of the intestinal crypts, and partial to total atrophy of the villi.

-Histologically, the classic histological changes of CD in the small bowel are categorized by the "**Marsh classification**":

MARSH – OBERHUBER CLASSIFICATION	
MARSH I	Increased Intraepithelial Lymphocytes
MARSH II	Increased Intraepithelial Lymphocytes and Crypt Hyperplasia
MARSH IIIA	Increased Intraepithelial Lymphocytes, Crypt Hyperplasia and Partial Villous Atrophy
MARSH IIIB	Increased Intraepithelial Lymphocytes, Crypt Hyperplasia and Subtotal Villous Atrophy
MARSH IIIC	Increased Intraepithelial Lymphocytes, Crypt Hyperplasia and Total Villous Atrophy

Celiac Quick Test

IgA Anti –tissue Transglutaminase (tTG) **ELISA Kit**