Science Serology

Which is a branch of immunology that deals with serum blood and study the interactions of antibodies and antigens in vitro and based science serums on the interaction of (antibody - antigen) in the diagnosis of some diseases, either through the presence of a specific antigen in the serum of the patient and the different interactions serological and according to the nature of the antigen common in the interaction and the way prepared these include:

1- Precipitation reactions: where the soluble antigen

Precipitation: When a specific antibody **precipitin** combines with a colloidal antigen **precipitinogen** in solution or in gel the antigen - antibody complex is thrown out of solution **precipitate**. The precipitate is heaviest in the **equivalence zone**, when antigen and antibody are fully combined. In some tests optimum relation between antigen and antibody must be kept up to carry out the reaction the so called **flocculation.** Flocculation test is used for the quantitative measurement of toxin, toxoid or antitoxin.

Precipitation reactions may be carried out in various ways:

1- **Capillary tube precipitation (Ring Test)**: layer Ag over Ab, Simplest test, qualitative, pecipitate occurs at the interface of the two reagents, forming a ring.
2- **Ouchterlony Double Diffusion (Immunodiffusion):** As the materials diffuse toward one another, ppt. lines form resulting from the Ag-Ab interactions.
3- **Radialimmunodiffusion (RID):** Antibody mixed with agar poured into plate, holes punched, add standards, controls and patients to wells, antigen will diffuse out and form precipitin ring, the diameter of the ring directly proportional to concentration, create standard curve and read results.
4- **Immunoelectrophoresis (IEP)**: serum sample is electrophoresed through an agar medium, trough is cut in the agar and filled with Ab, precipitin arc is then formed, because Ag diffuses radially and Ab from a trough diffuses, the reactants meet in optimal proportions for precipitation.
5- **Rocket Electroimmunodiffusion (EID):** The rocket immunoelectrophoresis technique or electroimmunodiffusion (EID) is a simple, fast, and reproducible technique for quantitation of a single protein, and is also applicable in a protein mixture. Several unknown samples can be analyzed on a single plate. Known reference solutions have to be included in each plate. To obtain an accurate quantitation, the proteins in the reference solutions and in the unknown samples have to be physicochemical and immunologically identical.

6- **Counterimmunoelectrophoresis (CIEP): is another modification of the basic immunoprecipitation technique that utilizes electrophoresis to enhance the rate of migration of antigen and antibodies in a gel matrix.**

Factors affecting rate of diffusion: Size of the particles, temperature, gel viscosity and hydration, interaction of reactants with gel

2- Agglutination: the antigen is suspended 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 result in forming aggregate or agglutinate.

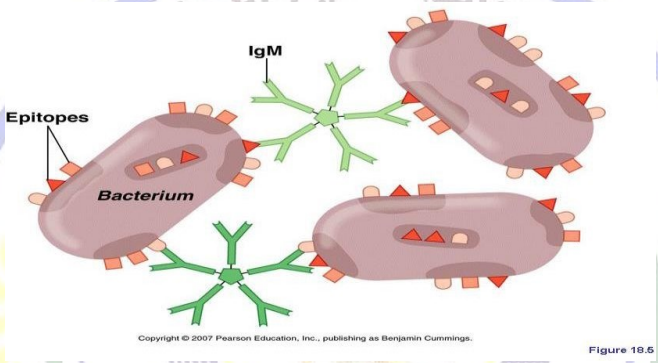
Stage of agglutination reaction:

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

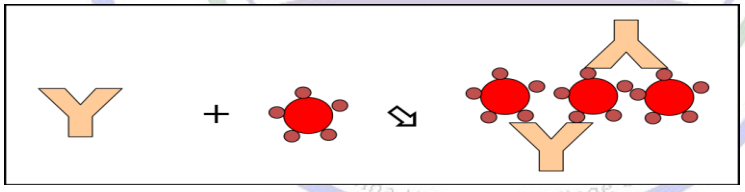
2- Two phases: A single antibody molecule binds to antigenic determinants on adjacent particles. The visible reaction occurs under appropriate conditions and over time, particles remain connected and interconnected by antibody bridge.

Types of agglutination reaction:

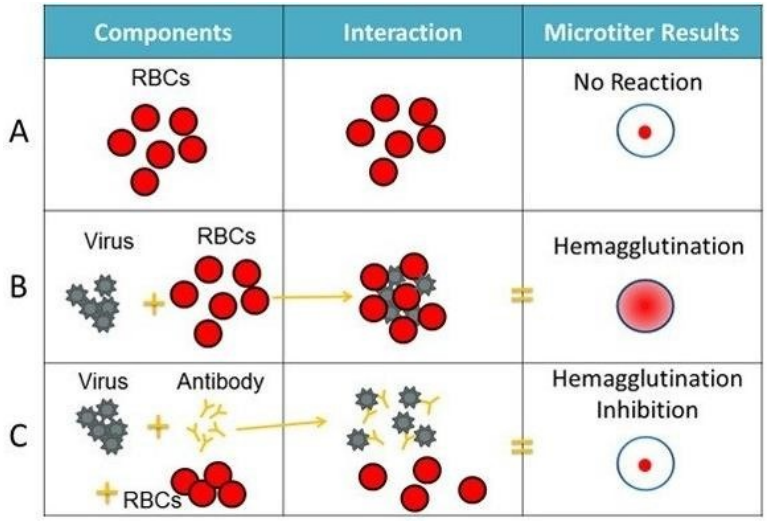
A- Active or Direct Agglutination: To test patient's sera (contain antibody) against large antigen. Direct agglutination can be used to determine antibody titer such as Widal test.



B- Hemagglutination: clumping blood to determine blood type.

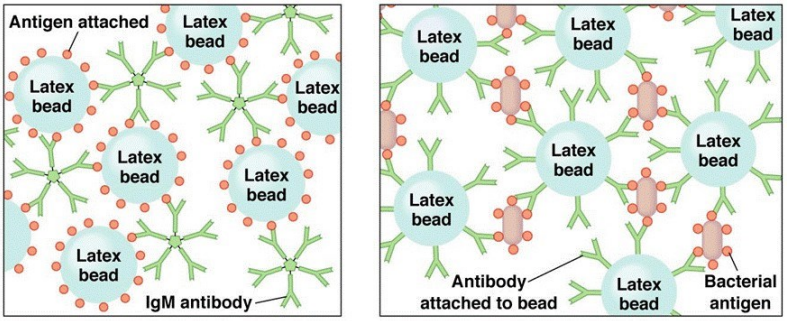


C-Hemagglutination Inhibition: inhibition of clumping of blood and is used in the diagnosis of viral infections.



D- Agglutination Passive: is used in the diagnosis of some types of bacteria and yeasts.

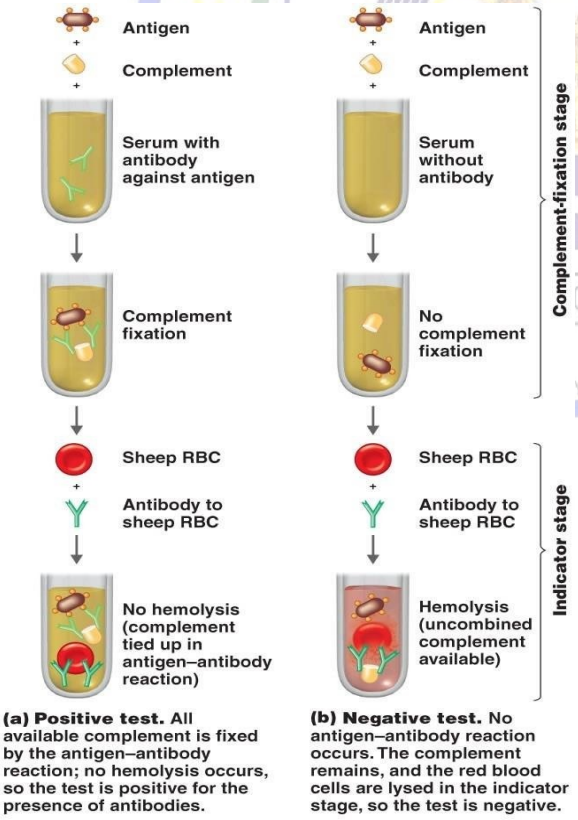
2. Indirect agglutination: serum is mixed with latex spheres (inert substance) with the soluble antigens attached. Antibodies will then cause visible agglutination of the latex spheres with the soluble- antigens-attached such as CRP test.



(a) Reaction in a positive indirect test for antibodies. When particles are coated with antigens, agglutination indicates the presence of antibodies, such as the IgM shown here.

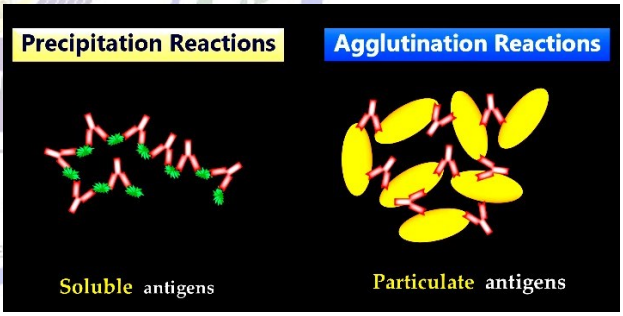
(b) Reaction in a positive indirect test for antigens. When particles are coated with monoclonal antibodies, agglutination indicates the presence of antigens.

3- Complement fixation test: is used in the diagnosis of syphilis. In addition to tuberculosis caused by bacteria *Mycobacterium tuberculosis*.



(a) Positive test. All available complement is fixed by the antigen–antibody reaction; no hemolysis occurs, so the test is positive for the presence of antibodies.

(b) Negative test. No antigen–antibody reaction occurs. The complement remains, and the red blood cells are lysed in the indicator stage, so the test is negative.



Serological tests for autoimmune diseases

1-Rheumatoid Arthritis

Chronic inflammatory reaction affects the joints and surrounding tissues. It has infected with the disease of abnormal proteins in the synovial fluid when the affected joint and round of these proteins in the blood of the patient and called Rheumatoid factor (RF).

These proteins are characterized as large and similar in characteristics of antibodies Igm type and capacity of the union piece to the type of IgG antibody which leads to an inflammatory reaction in the joint area, so these proteins are the RF antibody knows against anti-antibodies.

RF Test: examine the interaction of RF in the serum of the patient, where the test material is consisting with balls of latex loaded natural antibodies of kind IgG so interaction is generating as positive (the presence of infection) complicated see with the naked eye (Latex agglutination), a common reaction in the laboratory.

2-C-Reactive Protein C.R.P

Protein occurs naturally in the blood serum with low concentration, it has the ability to bind with carbohydrates that enter the structure of wall *Pnemococci* bacteria and raises the concentration in serum patients with rheumatoid arthritis and rheumatic fever of more than 1000 times of concentration in the serum normal individuals. So this test is an indication of the existence of such injuries and infections. Being in two ways:

1- Precipitation test on slide or tube:

Serum (CRP of Ab) + anti-CRP (Ag) ⟶ precipitation

2- Latex fixation test:

Serum (CRP of Ab) +Latex coated with anti-CRP ⟶ agglutination

2(Min)

- **Serological tests for infectious diseases**

1- Widal test

Used to detect infection with the bacterium *Salmonella* cause fever (Typhoid fever) is test agglutination between the antibodies in the serum of the patient (as who suffering from high up standard antibodies) and the somatic antigen (OAg) and flagellar antigen (HAg). If the level of antibodies to the patient (OAg 1/160) and (Hag 1/160) where the patient is infected typhoid fever.

- **Disadvantages of test:**

1- Event crosses reaction which gives a false positive result.
2 - Not distinguishes examination between the old and new infection.
3 - Not diagnosed infection early.

2- Wrights agglutination test or Rose Bengal

Interaction of agglutination used for the diagnosis of brucellosis fever or undulating fever caused by *Brucella abortus* bacteria as this examination diagnosed the case of the first week.

3- Antistreptolysin test (A.S.O.T)

Streptolysin O (SLO) enzyme analyst of blood product from the bacteria *Streptococcus pyogenes* him the ability to stimulate the immune system and production of antibodies unite with and stop its effectiveness in the analysis of (WBC, RBCs) and present in blood, the measurement of the level of this type of antibodies (Anti SLO) in the serum of the patient as an indicator of infection this type of bacteria, such as fever, rheumatic arthritis or renal tubules defect (glomerulonephritis).

- Test method:

ASO (serum Ab)+Latex coated Ag (SLO) ⟶ Ag-Ab complex

Measured concentration of antibodies titer in units like Todd units (IU) and the concentration in the case of injury, 400 IU a sign of infection with the bacterium *Streptococcus pyogenes*.

Immunodiagnostic methods: A laboratory technique that makes use of the binding between an antigen and its homologous antibody in order to identify and quantify the specific antigen or antibody in a sample.

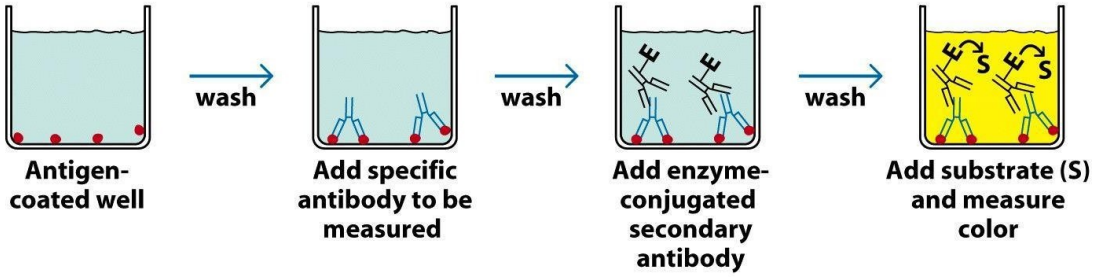
1- Enzyme-Linked Immunosorbent Assay: ELISA – primary immunological test, using an enzyme as a label to determine presence of target protein, is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample. The ELISA has been used as a diagnostic tool in medicine and plant pathology, as well as a quality control check in various industries. The enzyme linkage or labeling allows you to follow your target protein and if present (qualify) and at what amounts (quantify). An enzyme conjugate is an enzyme bound or joined with an antibody which binds with your target protein. This enzyme labeling is a safe and effective way to track your antibody. Literally hundreds of ELISA kits are manufactured for: 1- research 2- human and veterinary diagnosis

Types: 1- Indirect ELISA 2- sandwich ELISA 3- competitive ELISA

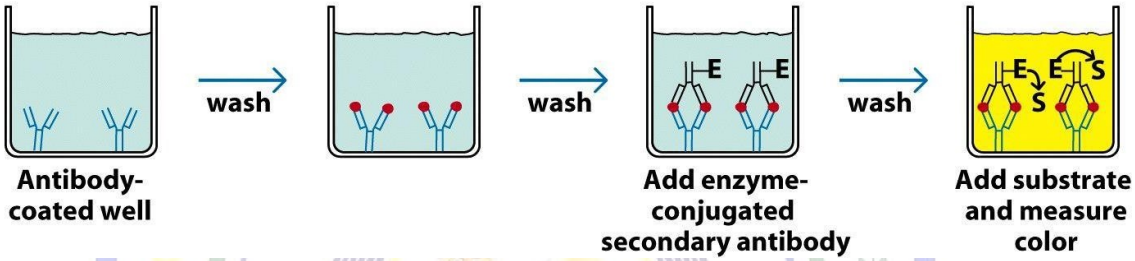
Components of reaction: 1-Antigen: The antigen is your target protein which comes from your sample extract. **2-Antibody**: binds only to the specific wanted antigen. **3-Enzyme Conjugate** horseradish peroixdase or alkaline phosphatase: An enzyme conjugate (EC) is an antibody joined with an enzyme. Enzyme labeling allows the researcher to follow the antibody. This joining of the enzyme to antibody is often called conjugation. **4-Substrate**: ELISAs traditionally utilize chromogenic substrates, though newer assays employ fluorogenic and electrochemiluminescent substrates enabling much higher sensitivity. **5- Solution of Stop reaction** (HCL, H2SO4).

Advantages of the ELISA: 1-Can detect either antibody or antigen.2-Can quantify amounts of antigen or antibody. 3-Easy to perform, inexpensive, and can test many samples quickly. 4-Plates coated with antigen and gelatin can be stored for later testing.

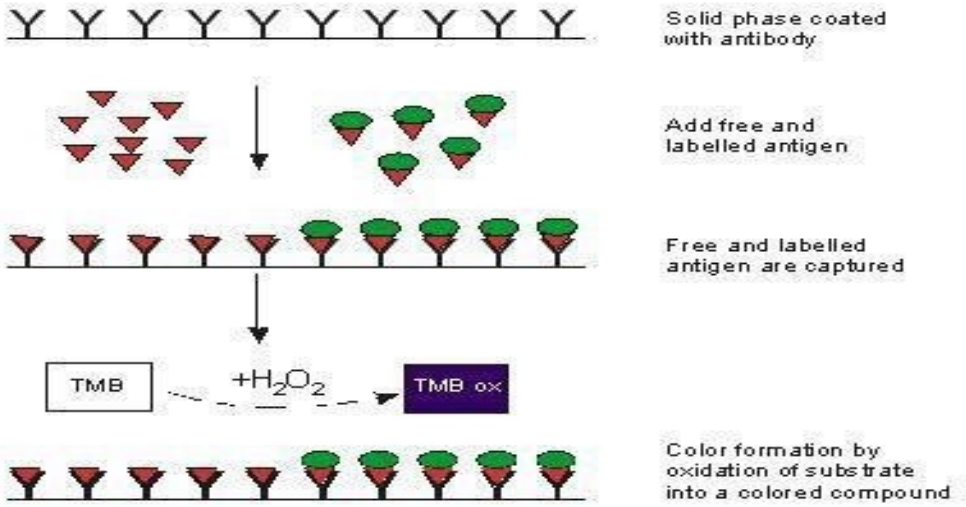
Indirect ELISA



Sandwich ELISA



Competitive Enzyme Immunoassay



A very wide variety of substances can be detected: **1. Proteins:** Plant and animal proteins (hormones, enzymes, cytokines); Microbial proteins (viral, bacterial, fungal, bacterial). **2. Other Bio-molecules- small to large**: *Peptides, Polysaccharides, Gycolipids, Steroids, Organic Toxins; *Drugs (aminoglycosides, cocaine)

Radioimmunoassay: A highly sensitive and specific assay method that uses the competition between radiolabeled and unlabeled substances in an antigen-antibody reaction to determine the concentration of the unlabeled substance; it can be used to determine antibody concentrations or to determine the concentration of any substance against which specific antibody can be produced.

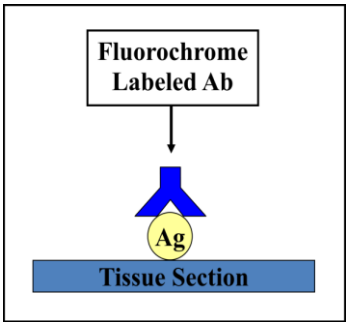
Radioimmunoassay (RIA) is a very sensitive technique used to measure concentrations of antigens (for example, hormone levels in the blood) without the need to use a bioassay. Although the RIA technique is extremely sensitive and extremely specific, it requires specialized equipment and is costly. It also requires special precautions, since radioactive substances are used. Therefore, today it has been largely supplanted by the ELISA method, where the antigen-antibody reaction is measured using colorimetric signals instead of a radioactive signal. The RAST test (radioallergosorbent test) is an example of radioimmunoassay. It is used to detect the causative allergen for an allergy.

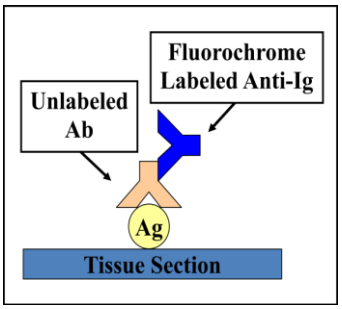
Immunofluorescence (IF): Immunofluorescence is a powerful technique that utilizes fluorescentlabeled antibodies to detect specific target antigens. It is used widely in both scientific research and clinical laboratories, this technique used fluorescence microscope and is used primarily on microbiological samples.

This technique uses the specificity of antibodies to their antigen to target fluorescent dyes to specific biomolecule targets within a cell, and therefore allows visualization of the distribution of the target molecule through the sample. Immunofluorescence is a widely used example of immunostaining and is a specific example of immunohistochemistry that makes use of fluorophores to visualize the location of the antibodies.

Immunofluorescence can be used on tissue sections, cultured cell lines, or individual cells, and may be used to analyze the distribution of proteins, glycans, and small biological and non-biological molecules. Several microscope designs can be used for analysis of immunofluorescence samples; the simplest is the epifluorescence microscope, and the confocal microscope is also widely used. Various super-resolution microscope designs that are capable of much higher resolution can also be used.

Direct Immunofluorescence **Indirect Immunofluorescence.**





Polymerase chain reaction: polymerase chain reaction, (PCR), a technique used to make numerous copies of a specific segment of DNA quickly and accurately. The polymerase chain reaction enables investigators to obtain the large quantities of DNA that are required for various experiments and procedures in molecular biology, forensic analysis, evolutionary biology, and medical diagnostics.

PCR (polymerase chain reaction) is a method to analyze a short sequence of DNA (or RNA) even in samples containing only minute quantities of DNA or RNA. PCR is used to reproduce (amplify) selected sections of DNA or RNA. Previously, amplification of DNA involved cloning the segments of interest into vectors for expression in bacteria, and took weeks. But now, with PCR done in test tubes, it takes only a few hours. PCR is highly efficient in that untold numbers of copies can be made of the DNA. Moreover, PCR uses the same molecules that nature uses for copying DNA.PCR specifically targets and amplifies a single sequence from within a complex mixture of. Most PCR methods typically amplify DNA fragments of between 0.1 and 10 kilo base pairs (kb), although some techniques allow for amplification of fragments up to 40 kb in size.

History: PCR was first conceived in April, **1983** by Kary Mullis. Mullis and Faloona, 1987. Specific synthesis of DNA in vitro via a polymerase- catalyzed chain reaction. In **1989** PCR was selected as the major scientific development and Taq DNA polymerase as molecule of the year by the science magazine. In **1993** Kary Mullis was awarded the Nobel Prize in chemistry for this achievement.

1. DNA template that contains the DNA region (target) to be amplified.

2. Two primers that are complementary to the 3' (three prime) ends of each of the sense and anti-sense strand of the DNA target.

3. Taq polymerase or another DNA polymerase with a temperature optimum at around 70 °C.

4. Deoxynucleoside triphosphates (dNTPs, sometimes called "deoxynucleotide triphosphates"; nucleotides containing triphosphate groups), the building-blocks from which the DNA polymerase synthesizes a new DNA strand.

5. Buffer solution, providing a suitable chemical environment for optimum activity and stability of the DNA polymerase.

6. Divalent cations, magnesium or manganese ions; generally, Mg2+ is used, but Mn2+ can be utilized for PCR-mediated DNA mutagenesis, as higher Mn2+ concentration increases the error rate during DNA synthesis

7. Monovalent cation potassium ions.

8. The PCR is commonly carried out in a reaction volume of 10–200 µl in small reaction tubes (0.2–0.5 ml volumes) in a **thermal cycler**. **The thermal cycler** heats and cools the reaction tubes to achieve the temperatures required at each step of the reaction. (**Machine** that automatically changes the temperature at the correct time for each of the stages and can be programmed to carry out a set number of cycles) is used for a PCR reaction.

PCR stages 1- Thermal denaturation: Initial denaturation temperature of 94ºC for 8 min. For subsequent cycles, 94ºC for 1-2 min is usually adequate.

2- Primer annealing: The temperature and length of time required for primer annealing depends on the base composition and the length and concentration of the primers.

3- Primer Extension: Primer Extension is typically carried out at 72ºC, which is close to the temperature optimum of the Taq polymerase.

Notes: 1- Most thermal cyclers have heated lids to prevent condensation at the top of the reaction tube. Older thermocyclers lacking a heated lid

require a layer of oil on top of the reaction mixture or a ball of wax inside the tube.

2- The temperatures used and the length of time they are applied in each cycle depend on a variety of parameters. These include the enzyme used for DNA synthesis, the concentration of divalent ions and dNTPs in the reaction, and the melting temperature (Tm) of the primers.

3- Typically the annealing temperature is about 3–5 °C below the Tm of the primers used. Stable DNA–DNA hydrogen bonds are only formed when the primer sequence very closely matches the template sequence. **4-** The extension time depends both on the DNA polymerase used and on the length of the DNA fragment to be amplified.

5- The amplified product can be detected using gel electrophoresis to view the band containing DNA fragments of a particular size containing the gene of interest in the original starter DNA sample.

6-DNA fragments separate according to size, and the dye ethidium bromide forms a brightly fluorescent adduct as it binds to DNA, Standard low-molecular weight marker is used (DNA ladder).

Applications of PCR:

1. Genetic fingerprinting (A- Forensic analysis at scene of crime to identify a person who suspected of committing a crime by comparing his or her DNA with a given sample (blood, hair, semen…. etc) obtained from a crime scene. B- Paternity testing).

2. Analysis of ancient DNA.

3. Cloning genes.

4. Genetic diagnosis, Mutation detection (PCR facilitates the advancement of

prenatal diagnosis of genetic defects such as: Cystic fibrosis, Duchenne muscular dystrophy, Haemoglobinopathies).

5. Mutagenesis to investigate protein function.

6. Quantitative differences in gene expression by Reverse Transcription (RT)-PCR.

7. Detection of pathogens especially when applied to those which are: (difficult or costly to culture, slow growing, present in low concentration, hazardous topropagate in the lab.

Real-time PCR: is a laboratory technique of molecular biology based on the polymerase chain reaction (PCR), which is used to amplify and simultaneously quantify a targeted DNA molecule. For one or more specific sequences in a DNA sample, quantitative PCR enables both detection and quantification. The quantity can be either an absolute number of copies or a relative amount when normalized to DNA input or additional normalizing genes. The procedure follows the general principle of polymerase chain reaction; its key feature is that the amplified DNA is detected as the reaction progresses in "real time".

This is a new approach compared to standard PCR, where the product of the reaction is detected at its end. Two common methods for the detection of products in quantitative PCR are:
(1) non-specific fluorescent dyes that intercalate with any double-stranded DNA, and
(2) sequence- specific DNA probes consisting of oligonucleotides that are labelled with a fluorescent reporter which permits detection only after hybridization of the probe with its complementary sequence to quantify messenger RNA (mRNA) and non-coding RNA in cells or tissues.

There are numerous applications for quantitative polymerase chain reaction in the laboratory. It is commonly used for both diagnostic and basic research. Uses of the technique in industry include the quantification of microbial load in foods or on vegetable matter, the detection of GMOs (Genetically modified organisms) and the quantification and genotyping of human viral pathogens.