Antibody-mediated vs. cell-mediated immunity

Antibody-mediated immunity is the branch of the acquired immune system that is mediated by B-cell-antibody production. The antibody-production pathway begins when the B cell's antigen-binding receptor recognizes and binds to antigen in its native form. Local Th cells secrete cytokines that help the B cell multiply and direct the type of antibody that will be subsequently produced. Some cytokines, such as IL-6, help B-cells to mature into antibody-secreting plasma cells. The secreted antibodies bind to antigens on the surface of pathogens, flagging them for destruction through complement activation, opsonin promotion of phagocytosis and pathogen elimination by immune effector cells. Upon elimination of the pathogen, the antigen–antibody complexes are cleared by the complement cascade (see Fig. 2) [2].

Five major types of antibodies are produced by B cells: IgA, IgD, IgE, IgG and IgM. IgG antibodies can be further subdivided into structurally distinct subclasses with differing abilities to fix complement, act as opsonins, etc. The major classes of antibodies have substantially different biological functions and recognize and neutralize specific pathogens. Table 2 summarizes the various functions of the five Ig antibodies [5].

Antibodies play an important role in containing virus proliferation during the acute phase of infection. However, they are not generally capable of eliminating a virus once infection has occurred. Once an infection is established, cell-mediated immune mechanisms are most important in host defense against most intracellular pathogens.

Cell-mediated immunity does not involve antibodies, but rather protects an organism through [2]:

- The activation of antigen-specific cytotoxic T cells that induce apoptosis of cells displaying foreign antigens or derived peptides on their surface, such as virus-infected cells, cells with intracellular bacteria, and cancer cells displaying tumour antigens;
- The activation of macrophages and NK cells, enabling them to destroy intracellular pathogens; and
- The stimulation of cytokine (such as IFNγ) production that further mediates the effective immune response.

Cell-mediated immunity is directed primarily at microbes that survive in phagocytes as well as those that infect non-phagocytic cells. This type of immunity is most effective in eliminating virus-infected cells and cancer cells, but can also participate in defending against fungi, protozoa, cancers, and intracellular bacteria. Cell-mediated immunity also plays a major role in transplant rejection.

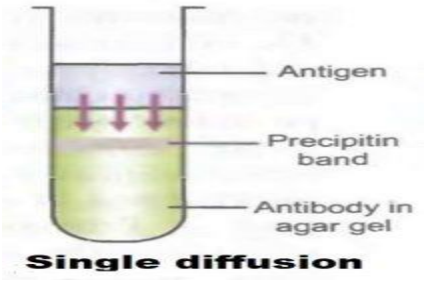
Immunodiffusion reaction

- The precipitation test in a agar gel is termed as immunodiffusion test.
- In this test, reactant are added to the gel and antigen-antibody combination occurs by the means of diffusion.
- The rate of diffusion is affected by the size of the particle, temperature, gel viscosity, amount of hydration and interaction between the matrix and reactants.
- An agar concentration of 0.3-1.5% allows for diffusion of the most of the reactants.
- One of the major advantages of immunodiffusion reaction is that the line of precipitation is visible as a band which can also be stained for preservation.
- Another advantage is that it can be used to detect, identify the cross reaction and non-identify between different antigens in a reacting mixture.

Types of immunodiffusion reaction

Immunodiffusion reaction are classified based on the ;

- Number of reactant diffusing and Direction of diffusion, as follows;



Single diffusion in one dimension:

- As the name suggests, it is the single diffusion of antigen in a agar in one dimension.
- In this method, the diffusing reactant only diffuses in one direction. This includes the oudin tube method. This test involves laying a solution or gel containing the antigens onto a layer of antiserum-containing agar in a tube. Because of gravity/ concentration gradient, the antigen diffuses into an antibody-containing gel region. Initially, the antigen concentration becomes higher as it moves. The antigen concentration reaches equilibrium or is equal to the antibody concentration, at which point precipitation takes place. The precipitation is used to detect the presence or absence of antigen. Qualitative analysis.
- In this method, antibody is incorporated into agar gel in a test tube and the antigen solution is poured over it.
- During the course of time, the antibody in agar gel and a line of precipitation is formed.
- The number of precipitate band shows the number of different antigen present in antigen solution.

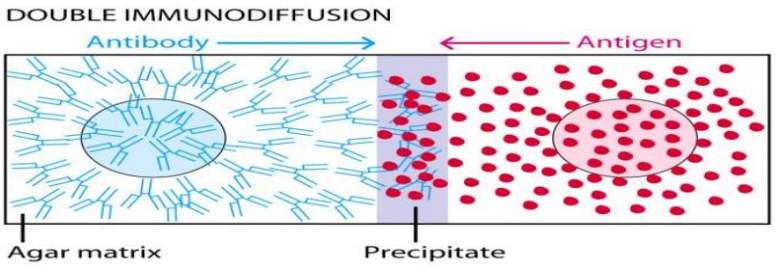
Single diffusion in two dimension:

- It is also called radial immunodiffusion.
- This is the single radial immunodiffusion method (SRID), also known as the Mancini method. This method incorporates the antibody against the known antigen into the gel. Wells are drilled into these gel layers, to add the antigen of your choice. The concentration gradient is formed again as the antigen diffuses radially out of the well. The precipitation takes place around the well at the point of equivalence.
- In this method, antiserum solution containing antibody is incorporated in agar gel on a slide or petridish.
- The wells are cut on the surface of the gel.
- The antigen is then applied to the well cut into the gel.
- When antibody already present in gel reacts with antigen which diffuses out of the well, a ring of precipitation is formed around the wells.
- The diameter of the ring is directly proportional to the concentration of the antigen.
- The greater the amount of antigen in the well, the farther the ring will be from the well.

- Radial immunodiffusion has been used for the quantitative estimation of antibody and antigen in serum.
  - RID can be used to quantify the amount of antigen. The wells are dug into the antibody-incorporated gel, and the antigen of different concentrations are added to each of the wells. Each antigen concentration will be assigned a zone of equivalence at various distance.
  - A graph showing the antigen concentration against the diameter or ring area is drawn. You can find the unknown concentration by calculating the diameter of the antigen sample or the ring area, and then plotting it in the graph along with the calibration curve.
- It is used to
  - measure IgG, IgM, IgA and complement components in the serum
  - measure antibodies to influenza virus in the sera.

Double diffusion in one dimension:

- This is similar to single diffusion in one dimension except that there is only a layer of agar between layers of antigen or antibody. Both reactants then diffuse toward one another and the precipitation rings are formed in the middle layer. Due to the concentration gradient, each reactant diffuses in one direction: either upwards or downwards. This is why diffusion occurs in one dimension.
- In this method, the antibody is incorporated in agar gel in a test tube above which a layer of plain agar is placed.
- The antigen is then layered on the top of this plain.
- During the course of time, the antigen and antibody move toward each other through the intervening layer of plain agar.
- In this zone of plain agar, both antigen and antibody react with each other to form a band of precipitation at their optimum concentration.



Double diffusion in two dimension:

- This is also known as the Ouchterlony method and is the most commonly used immunochemical technique. This is used to determine if two antigens have similarity or not. The Ouchterlony method can be used on a petri-dish, or even a microslide that is overlaid with agar. The antigen and antibody solutions are then added to the wells. This can be used to perform both quantitative and qualitative analysis.

- **Qualitative Ouchterlony technique:**

- To compare antigens against identical or cross-reacting determinants, the qualitative Ouchterlony method is used. The two antigen solutions are placed in adjacent wells, and the homologous antibodies is placed in the middle well.

- The precipitation line will formed because the antigens present in both wells fuse are in the same concentration, and these Ag are identical. This reaction is called a reaction of identification and the pattern is called a pattern of identity.

When two antigens of unrelated nature are added in the two wells, and a central well is filled up with antibodies for each antigen, immunoprecipitations can occur separately with the specific antibodies. These precipitation lines will cross one another following their respective zones of equivalence, and create a cross-shaped structure. This is called a pattern of non-identity.

- A spur is formed when two related antigens are added to the wells with the antisera in the center well. The spur is a sign that one antigen determinant is present in each sample, while the other is only in one. The precipitation line of an additional antigen that projects toward the antigen sample containing only the common determinant is called the spur. This is known as a reaction with partial identity.
- **Quantitative Ouchterlony technique**

- Similar to SRID, this allows semi-quantitative estimation of antigen concentrations by using the relative position of the precipitation line. The precipitation line would be farther away if the antigen concentration is higher than the average concentration.

- The concentration of antigen solution in the well will determine the distance of the precipitation lines. A graph showing the distance to the concentration can then be plotted. The calibration curve can be used to determine the concentration of unknown antigen samples.

-
- In this method, both the antigen and antibody diffuse independently through agar gel in two dimensions, horizontally and vertically.
- The test is performed by cutting wells in the agar gel poured on a glass slide or in a petri dish.
- The antiserum consisting of antibody is placed in the central well and different antigens are added to the wells surrounding the center well.
- After an incubation period of 12-48 hours in a moist chamber, the lines of precipitin are formed at the sites of combination of antigen and antibody.

Advantages

- The major advantage of immunodiffusion reaction over other methods is the ability to see the line of precipitation as a band that can be stained for preservation.

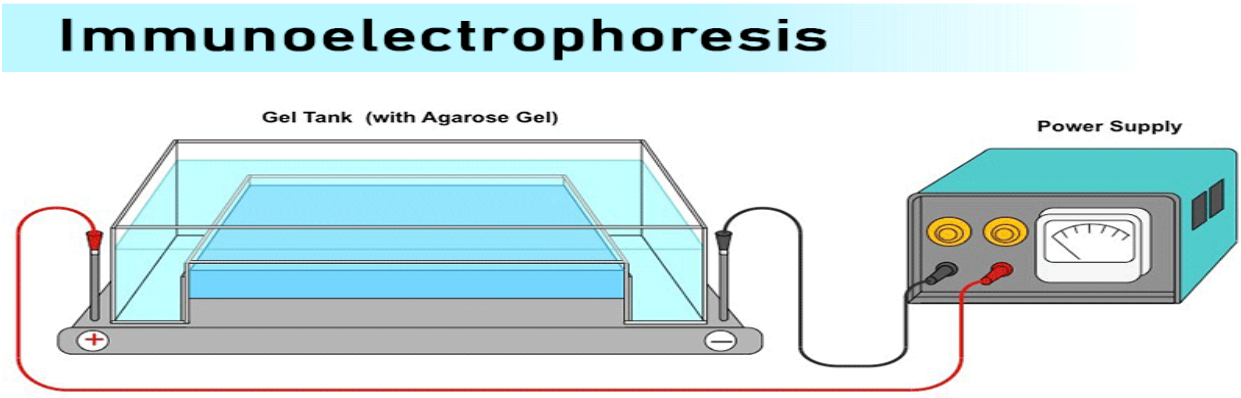
It can also be used to identify cross reactions and non-identify antigens within a mixture

Applications

- To determine the relative concentrations Antibodies/Antigens.
- To compare Antigens or
- To determine the purity of an Antigen preparation.
- To diagnose disease.
- Surveys of serology.

Immunoelectrophoresis

- Immunoelectrophoresis refers to precipitation in agar under an electric field.
- It is a process of a combination of immuno-diffusion and electrophoresis.
- An antigen mixture is first separated into its component parts by electrophoresis and then tested by double immuno-diffusion.
- Antigens are placed into wells cut in a gel (without antibody) and electrophoresed. A trough is then cut in the gel into which antibodies are placed.
- The antibodies diffuse laterally to meet diffusing antigens, and lattice formation and precipitation occur permitting determination of the nature of the antigens.
- The term "immunoelectrophoresis" was first coined by Grabar and Williams in 1953.



Principle of Immunoelectrophoresis

When an electric current is applied to a slide layered with gel, the antigen mixture placed in wells is separated into individual antigen components according to their charge and size. Following electrophoresis, the separated antigens are reacted with specific antisera placed in troughs parallel to the electrophoretic migration and diffusion is allowed to occur. Antiserum present in the trough moves toward the antigen components resulting in the formation of separate precipitin lines in 18-24

hrs, each indicating reaction between individual proteins with its antibody.

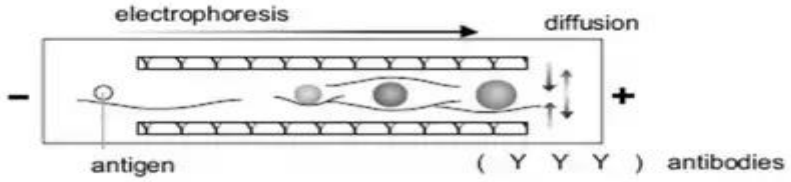


Fig 1: Immunoelectrophoresis technique

Procedure of Immunoelectrophoresis

1. Agarose gel is prepared on a glass slide put in a horizontal position. Prepare 10 ml of 1.5% agarose
2. Mark the side of the glass plate that will be towards negative electrode during electrophoresis.
3. Cool the solution to 55-60oC and pour 6 ml/plate on to grease free glass plate placed on a horizontal surface. Allow the gel to set for 30 minutes.
4. Place the glass plate on the template provided
5. Using the sample template, wells are borne on the application zone carefully. Punch a well with the help of the gel puncher corresponding to the markings on the template at the negative end. Use gentle suction to avoid forming rugged wells.
6. Cut two troughs with the help of the gel cutter, but do not remove the gel from the troughs.
7. Add 10 l of the antigen to the well and place the glass plate in the electrophoresis tank such that the antigen well is at the cathode/negative electrode.
8. The sample is diluted 2:3 with protein diluent solution (20µl antigen solution +10 µl diluent).
9. Using a 5 µl pipette, 5 µl of control and sample is applied across each corresponding slit (Control slit and Sample slit).
10. The gel is placed into the electrophoresis chamber with the samples on the cathodic side, and electrophoresis runs for 20 mins/ 100 volts.
11. Electrophorese at 80-120 volts and 60-70 mA, until the blue dye travels 3-4 cms from the well. Do not electrophorese beyond 3 hours, as it is likely to generate heat.
12. After electrophoresis, remove the gel from both the troughs and keep the plate at room temperature for 15min. Add 80 l of antiserum A in one of the trough and antiserum B in the other.
13. Incubated for 18- 20 hours at room temperature in a horizontal position.
14. The agarose gel is placed on a horizontal position and dried with blotter sheets.
15. The gel in saline solution is soaked for 10 minutes and the drying and washing repeated twice again.
16. The gel is dried at a temperature less than 70°C and may be stained with protein staining solution for about 3 minutes followed by decolorizing the gel for 5 minutes in distaining solution baths.
17. The gel is dried and results evaluated.

Glass plate showing precipitin lines following immunoelectrophoresis

Results of Immunoelectrophoresis

1. The presence of elliptical precipitin arcs represents antigen-antibody interaction.

2. The absence of the formation of precipitate suggests no reaction.
3. Different antigens (proteins) can be identified based on the intensity, shape, and position of the precipitation lines.

Applications of Immunoelectrophoresis

1. The test helps in the identification and approximate quantization of various proteins present in the serum. Immunoelectrophoresis created a breakthrough in protein identification and in immunology.
2. Immunoelectrophoresis is used in patients with suspected monoclonal and polyclonal gammopathies.
3. The method is used to detect normal as well as abnormal proteins, such as myeloma proteins in human serum.
4. Used to analyze complex protein mixtures containing different antigens.
5. The medical diagnostic use is of value where certain proteins are suspected of being absent (e.g., hypogammaglobulinemia) or overproduced (e.g., multiple myeloma).
6. This method is useful to monitor antigen and antigen-antibody purity and to identify a single antigen in a mixture of antigens.
7. Immunoelectrophoresis is an older method for qualitative analysis of M-proteins in serum and urine.
8. Immunoelectrophoresis aids in the diagnosis and evaluation of the therapeutic response in many disease states affecting the immune system.

Advantages of Immunoelectrophoresis

1. It is an important analytical procedure with high resolving power as it connects the departure of antigens by electrophoresis with immunodiffusion against an antiserum.
2. The main benefit of immunoelectrophoresis is that a number of antigens can be recognized in serum.

Disadvantages of Immunoelectrophoresis

- It is a slower, less sensitive process, and more challenging to perform than Immunofixation electrophoresis.
- It is unable to detect some minute monoclonal M-proteins because the most rapidly emigrating immunoglobulins present in the highest concentrations may obscure the presence of small M-proteins.
- In food, analysis the use of immunoelectrophoresis is limited by the availability of specific antibodies.

Immunoassay

Immunoassay is an analytical technique used for the quantification of an analyte based on the antigen-antibody reaction.

Types of immunoassay:

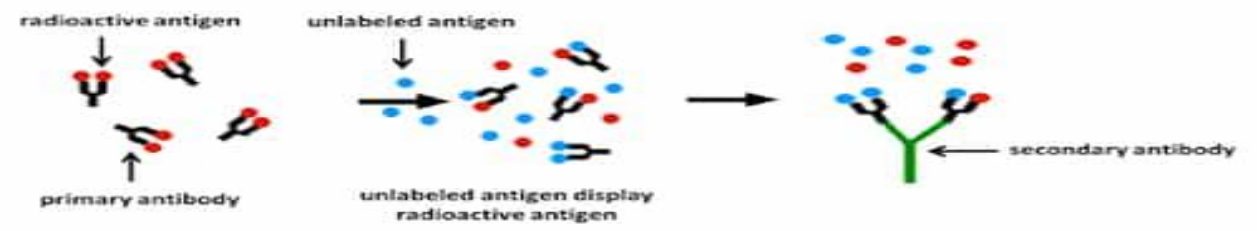
¬Radioimmunoassay (RIA)
¬Enzyme-linked immunosorbent assays (ELISA)
¬Fluoroimmunoassay (FIA)
¬Chemiluminescence immunoassay (CLIA)

RIA-Principle:radioimmunological assay (**RIA**) is based on the principle of all immunological assays, which is the recognition of an antigen present in a sample by antibodies directed against this antigen. The radioimmunoassay principle is very similar to that of competitive ELISA and quantifies small molecules, peptides, and proteins in biological samples.

Radioimmunoassay technique (RIA) is very sensitive in vitro technique used to measure the concentration of antigens (eg, hormone levels in the blood) through the use of antibodies directed against these antigens. Radioimmunoassay (RIA) is based on the principle of all immunoassays which is the recognition of an antigen present in a sample by antibodies directed against this antigen. The principle of radioimmunoassays is very similar to that of competitive ELISA and allows quantification of small molecules, peptides and proteins in biological samples.

Principles and Technique of RIA

RIA is performed by using antibody-antigen binding and radioactive antigen. The basic principle of RIA is a competitive binding reaction, where the analyte (for example, antigen) competes with radio-labelled antigen for binding to the fixed antibody or the binding sites of the receptor. The binding of the unlabeled antigen to the fixed and limited amount of antibody causes displacement of radio-labelled antigen and results in decreasing the radioactivity of the antigen-antibody complex

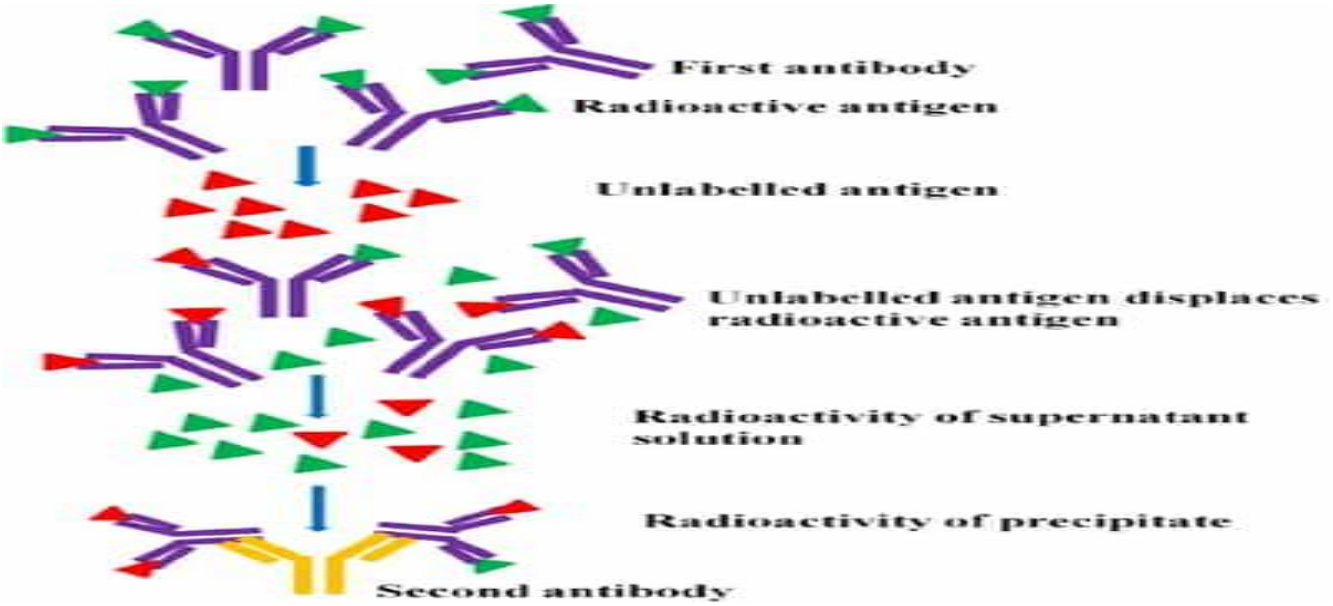


1. A radio-labelled antigen (e.gInsulin labelled with I^{125}) is made to compete with an unstable antigen for a limited number of binding sites of a specific antibody raised against insulin.
2. The antigen binds to the antibody. Owing to inadequate binding sites, some of the antigens will be free and will include radio-labelled antigens also.
3. After equilibrium, the antigen-antibody complex is precipitated by using suitable reagents. The supernatant is separated from the precipitate by centrifugation.
4. The supernatant is separated from the precipitate by centrifugation.
5. Both the precipitate (the bound antigen, B-form) and the supernatant (the free antigen, F) will have radioactivity since they have I^{125} – insulin.
6. The extent of the radioactivity of the two forms is measured in gamma-ray well type scintillation counters.
7. The magnitude of the radioactivity of the free form may be related to the concentration of the un-labelled antigen.
8. Alternatively, the radioactivity of the sound form or the ratio of B/F is also related to the concentration of the un-labelled antigen.
9. Different concentrations of the un-labelled insulin standard are used separately with the same concentration of the labelled insulin.
10. The assay is very sensitive since the labels used for RIA have high specific activity.
11. Normally, an antibody is raised for any antigen to be estimated. The technique is said to be radio immuno-assay since it couples radioactivity and immune function (antigen binding to antibody).

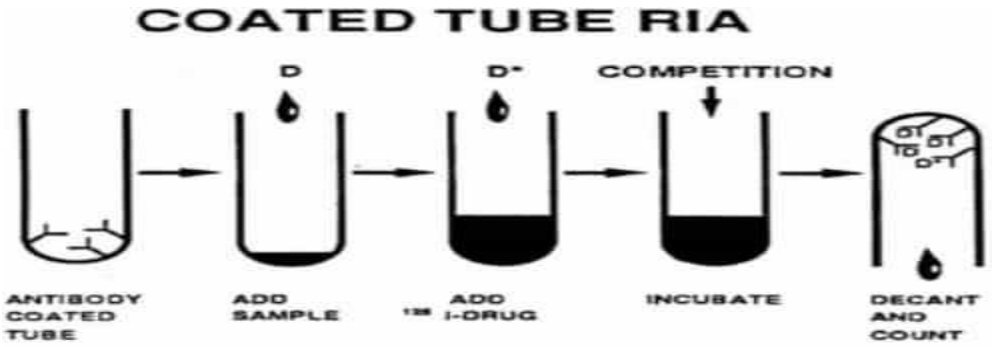
Types of RIA

There are two different methods of RIA that are commonly employed for drug detection in biological matrices, ***double-antibody RIA*** and ***coated-tube RIA***.

(a) Double-Antibody RIA - In double-antibody RIA, a second antibody is added to facilitate precipitation of the bound primary antibody. Once the primary/secondary antibody-antigen complex precipitates, the unbound labelled drug can be easily removed.



(b) Coated-tube RIA - In coated-tube RIA, the primary antibody is coated on the inside of each tube. The unbound labelled drug can be easily removed by pouring off the supernatant.



Role in Life Science

1. It has a significant role in the diagnosis of diseases.
2. Radioimmunoassay is employed for the estimation of Vitamins like B_2, and folic acid; hormones like insulin, thyroxine (T_4), triiodothyronine (T_3), Cortisol, testosterone, dihydrotestosterone, estrogens; trophic hormones like ACTH, FSH, LH; drugs like digoxin, digitoxin; antigens like the Australia antigen.

3. RIA can help to differentiate the basic biochemical lesion in endocrinology whether the increased level of a hormone is due to the production of the hormone as such or the tropic hormone.
4. This technique offers safety to the patient in the use of drugs if there is only a narrow margin between the therapeutic and toxic dosage.
5. This technique is also useful in diagnosing insulinomas, sex hormone-sensitive tumours, etc. and this facilitates proper treatment of the diseases.

ELISA

Principle:

ELISA combines the specificity of antibodies with the sensitivity of simple enzyme assays, by using antibodies or antigens coupled to an easily assayed enzyme that possesses a high turnover number.

Simply put for ELISA an antigen is adsorbed/fixed to the surface of a solid matrix which may be a well on a micro titration plate/ well strip, beads or may be a special type of paper made of Nitrocellulose. Then, a specific antibody is applied over the surface so it can bind to the antigen. This antibody is linked to an enzyme, and, in the final step, a substance containing the specific substrate for the enzyme is added. The subsequent reaction produces a detectable signal, a color change in the substrate.

ELISA technology has advanced a lot. To make this immunoassay more and more sensitive as well as specific various advances have been made and now we have different types of ELISAs. There are slight differences in the technology though basically ELISA remains an antigen antibody reaction performed as wet test using different reporter and signal systems.

Types of ELISAs

ELISAs can be divided into the following categories based on the principle used: z Indirect z Sandwich z Competitive z Antigen and antibody capture ELISA.

Indirect ELISA

HIV antigens are attached covalently to the solid phase support allowing corresponding/specific antibodies present in the specimen to bind, and these bound antibodies are subsequently detected by enzyme labelled anti-human immunoglobulin and specific substrate system. If the test specimen contained the antibodies specific to the antigen fixed on the solid phase colour reaction will take place. The colour reaction can be read with naked eye or with ELISA Reader at the specified UV light using the special filters. The result is expressed as OD (optical Density) value.

Indirect ELISA Always follow the instructions of the manufacturer of the kit which are given in the kit literature and develop a protocol/SOP. One example is given below.

Materials required: Single / multichannel pipettes with disposable tips: 5-50ul , 50-200ul ; Incubator (37+20C) ;ELISA reader with or without washer ;Powderless disposable gloves ;Absorbent paper ;Deionised water ;Discard jar with hypochlorite solution ;Wash bottles

Protocol: Add appropriate amount of diluted sample in various wells ↓ Incubate for required time at room temperature ↓ Empty plate and tap out residual liquid. Wash 3-5 times ↓ Appropriately diluted enzyme conjugate is added and incubated as specified ↓ Wash the plate (3-5 times) and tap out residual

liquid ↓ Add appropriate amount of substrate solution to each well ↓ Incubate as specified ↓ Add required amount of stop solution Sodium hypochlorite solution (5.2%) ↓ Read plate with plate reader/read colour change with naked eye.

Sandwich ELISA One of the most useful of the immunoassays is the two antibody "sandwich" ELISA. This assay is used to determine the antigen concentration in unknown samples. This ELISA is fast and accurate, and if a purified antigen standard is available, the assay can determine the absolute amount of antigen in an unknown sample.

To utilize this assay, one antibody (the "capture" antibody) is purified and bound to a solid phase typically attached to the bottom of a plate well. Specimen containing antigen is then added and allowed to complex with the bound antibody. Unbound products are then removed with a wash, and a labeled second antibody (the "detection" antibody) is allowed to bind to the antigen, thus completing the "sandwich". The assay is then quantitated by measuring the amount of labeled second antibody bound to the matrix, through the use of a colorimetric substrate.

Materials: As for Indirect ELISA test.

Protocol

The following is the general protocol for a sandwich ELISA. Follow manufacturer's instructions for precise steps.

Add required volume of sample containing antigen to the wells ↓ Incubate for required amount of time ↓ Wash the plate three to four times ↓ Add the labeled second antibody ↓ Incubate at room temperature for required amount of time ↓ Wash the plate 3-4 times ↓ Add substrate as indicated by manufacturer ↓ Read on the ELISA reader after suitable incubation time.

Competitive ELISA

In this assay the antibodies present in the specimen compete with the enzyme conjugated antibodies in the reagent for binding to the antigen on the solid phase. If the test specimen contains corresponding/specific antibodies, these will compete with the labelled antibodies in the reagent for binding to antigen. So that less or not labelled antibodies can attach to the solid phase. Hence, faint or no colour is produced on addition of substrate if specimen contains antibodies against the antigen on the solid phase.

Materials :These are same as used in case of indirect / sandwich ELISA techniques

Protocol:

Add appropriate amount of standard/ sample solution to the wells ↓ Allow to incubate for required amount of time (as in kit insert) ↓ Add appropriate amount of conjugate solution to the wells ↓ Wash the plate with wash solution 3-4 times ↓ Add substrate as indicated by manufacturer. ↓ After suggested incubation time has elapsed, ↓ Optical densities at target wavelengths can be measured on an ELISA reader

Antigen and antibody capture ELISA

Antigen capture ELISA can be based on principle of indirect or competitive ELISA, only difference being in the initial step of attaching antigen to the solid phase. A monoclonal antibody directed against an antigen is bound to the solid support. Next step is addition of antigen supplied as reagent in the test kit. This antigen is captured by the monoclonal antibody bound to the solid phase. Test specimen appropriately diluted is added next. Antibodies if present in the specimen bind to the antigen on solid

support. Remaining principle is same as indirect ELISA Only advantage of antigen capture ELISA is that it is more specific than indirect assay.

PROCESS OF ELISA

Although many variants of ELISA have been developed and used in different situations, they all depend on the same basic elements:

Coating/Capture: direct or indirect immobilization of antigens to the surface of polystyrene microplate wells.

Plate Blocking: addition of irrelevant protein or other molecule to cover all unsaturated surface-binding sites of the microplate wells.

Probing/Detection: incubation with antigen-specific antibodies that affinitybind to the antigens.

Signal Measurement: detection of the signal generated via the direct or secondary tag on the specific antibody

In a typical assay designed to detect an antigen in a sample, either the antigen is immobilized by direct adsorption or first antibody is adsorbed and then antigen is adsorbed on the well surface of the ELISA plate. The plate is blocked with albumin. The antigen is probed with a specific detection antibody. The detection antibody may be directly labeled with a signal-generating enzyme or fluorophore or it may be secondarily probed with an enzyme- or fluor-labeled secondary antibody. For enzymatic detection, the appropriate enzyme substrate is added. The signal observed is proportional to the amount of antigen in the sample. Every step of test is followed by washing to remove the un-reacted reactants, only specific (high-affinity) binding remains that causes the detection signal at the final step.

APPLICATIONS OF ELISA

ELISA can be applied to detect and measure antibody in serum against viruses, bacteria, parasites z

ELISA has also been used in home pregnancy test (rapid ELISA) z

ELISA is used in food industry to detect potential food allergens such as milk, peanuts, walnuts, almonds, and eggs.

ELISA can also be used in toxicology as a rapid presumptive screen for certain classes of drugs.

ELISA was widely used in various areas such as immunology, Biological Pharmacy, Diagnostic industry, and so on.

Monoclonal antibodies are proving to be an effective form of treatment against several diseases, like cancer and COVID-19. These imitate the function of antibodies naturally present in one's body, only with greater resistance power. This is why these antibodies serve as remedies to beat deadly viruses like SARS-CoV-2. Monoclonal antibodies are artificially engineered in laboratories by scientists as a form of medication. This is because they are characterised by their ability to help a human body combat viral infections better. These can target only one specific type of antigen.

The short-form for them is Moabs or Mabs. Here is how it works.

The body responds by producing antibodies to counteract the virus or antigens, whenever a person falls ill. These antibodies are specific to a particular antigen. Therefore, scientists can replicate these antibodies and help in the treatment of a disease.

So far, healthcare professionals have used monoclonal antibodies in the treatment of cancer.

Examples of Monoclonal Antibodies

There are four ways in which scientists prepare monoclonal antibodies. They are:

- **Murine:** Murine monoclonal antibodies consist of mouse proteins, and any treatment with these ends with the suffix -omab.
- **Human:** These are made purely from human beings, and any treatment with these ends with the suffix -umab.
- **Humanised:** Humanised monoclonal antibodies are made of parts of mouse proteins attached to human proteins. Any treatment with these ends with -zumab.
- **Chimeric:** These are also a combination of part mouse and part human. The treatment ends with -umab.

Here are some FDA approved monoclonal antibodies.

- Ustekinumab (Stelara)
- Trastuzumab (Herceptin)
- Tocilizumab (Actemra)
- Secukinumab (Cosentyx)
- Rituximab (Rituxan)

Steps in production of monoclonal antibodies:

Step I: Immunization of rabbit or rat and extraction of B-lymphocytes

- In order to isolate B-lymphocyte producing certain antibodies, rabbit or lab rat is immunized through repeated injection of specific antigen (sheep RBCs)
- A sample of B-cells is extracted from spleen of rabbit or rat

Step II: fusion of myeloma cell with B-lymphocytes:

- The extracted B-lymphocytes is added to a culture of myeloma cell from bone marrow.
- The intended result is the formation of hybridoma cells formed by fusion of B-cell and myeloma cell.
- The fusion is done by using Polyethylene glycol (PEG) or by electrophoration or by using phages.

Step III: selection of hybridoma cell

- The next step is selection of hybridoma cells.
- The B-lymphocytes contains HPRT1 gene which codes for enzyme Hypoxanthine-guanine phosphoribosyltransferase (HGPRT). The enzyme HGPRT involved in synthesis of nucleotides from Hypoxanthine present in culture medium. Therefore B- cells can grow in medium containing Hypoxanthine amonopterin thymine (HAT media).

- But myeloma cell lack HPRT1 gene so, it does not produce HGPTR enzyme and it does not grow in HAT medium.
- The myeloma cell fused with another myeloma cell or those do not fused at all die in HAT medium since they do not utilize Hypoxanthine.
- Similarly, B- cell that fuse with another B- cell or those do not fuse at all die eventually because they do not have capacity to divide indefinitely,
- So, only hybridoma cell ie. fused cell between myeloma and B-cell can survive and divide in HAT medium.
- Screening is done to select hybridoma cells which are the desired cell for monoclonal antibodies production.

Step IV: culture of Hybridoma cell:

- The selected hybridoma cells are cultured in suitable culture medium, often supplemented with insulin, transferon, ethanol, amine and other additional hormones.
- Some commonly used culture media for hybridoma cell for production of monoclonal antibodies are:
  - DMEM (Dulbecco's modified eagle medium)
  - IMDM (Iscove's Modified Dulbecco's Medium)
  - Ham's F12
  - RPMI 1640 medium (Roswell Park Memorial Institute 1640 medium)

Step V: Inoculation of hybridoma cell into suitable host

- These hybridoma cells are then injected into lab animal so that they starts to produce monoclonal antibodies.
- These hybridoma cells may be frozen and store for future use.

Step VI: extraction and purification of Monoclonal antibodies:

- Monoclonal antibodies from host animal is extracted and purified by one of the following methods;
- Ion exchange chromatography
- Antigen affinity chromatography
- Radial immunoassay
- Immune precipitation

Application of Monoclonal antibodies:

1. **Disease diagnosis:**

- ELISA to test HIV, hepatitis, Herpes etc
- RIA- to test viral infection
- Mabs to Hunam chorionic gonadotropin

2. Disease treatment

- OKT3- it is an antibody to T3 antigen of T cell which can be used to prevent acute renal allograph rejection in human.

- Different types of Mabs are used in radial immunodetection and radial immune therapy of cancer.

3. Passive immunization or disease prevention

- Monoclonal antibodies based drugs can be used to treat septic shock
- Used as vaccine

4. **Detection and purification of biomolecules**

- Mabs are very useful in determining the presence and absence of specific proteins through western blotting technique.
- Besides that, it can be used to classify strains of a single pathogen. Eg. *Neisseria gonorrhea* can be typed using Monoclonal antibodies.