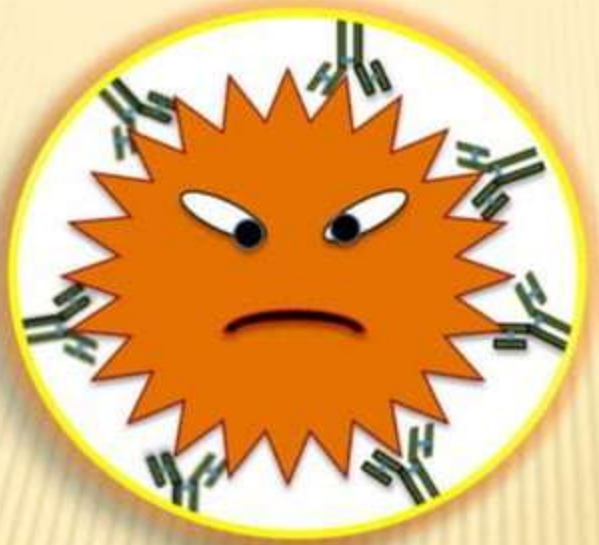
Part :02



"ANTIGEN–ANTIBODY REACTIONS"

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TYPES OF ANTIGEN – ANTIBODY REACTION

The types of antigen – antibody reactions are:

- **Precipitation Reaction.**
- **Agglutination Reaction.**
- **Complement Fixation.**
- **Immunodiffusion.**
- **Immuno-electrophoresis**

COMPLEMENT FIXATION TEST (CFT)

COMPLEMENT FIXATION TEST (CFT)

- The complement fixation test (CFT) was extensively used in **syphilis serology (wassermann test)** after being introduced by **Wasserman** in **1909.**
- Lysis of RBC or bacteria requires some non-specific unstable components of fresh serum which are called "*complement.*"
- Complement is a **protein** (globulin) present in normal serum.
- This complement system comprises of **11 proteins** and are present in every individual. They bind to **Fc component of Ab** involved in *Ag-Ab complex.*
- This ability of the *Ag-Ab complex* to fix complement is used in **complement Fixation tests.**

PRINCIPLE OF CFT

- The CFT is a technique that has been used over many years to **detect and quantify antibody that Serology does not agglutinate or precipitate** when reacted with its antigen, but can be demonstrated by its use or fixation of complement.
- Complement takes part in many of the immunological reactions. It gets absorbed during the combination of antigens and antibody.
- This property of **Ag-Ab complex** to fix the complement is used in **complement fixation test** for the **identification of specific antibodies.**
- The **hemolytic system** containing **sheep erythrocytes (RBC)** and its **corresponding antibody (amboceptor)** is used as an **indicator** which shows the **utilization** or **availability of the complement**.
- If the **complement is fixed** then there will be **no lysis of sheep erythrocytes**, thus denoting a **positive test.**
- If the **complement is available** then there will be **haemolysis** which is a property of complement, denoting a **negative test.**

COMPONENTS OF CFT

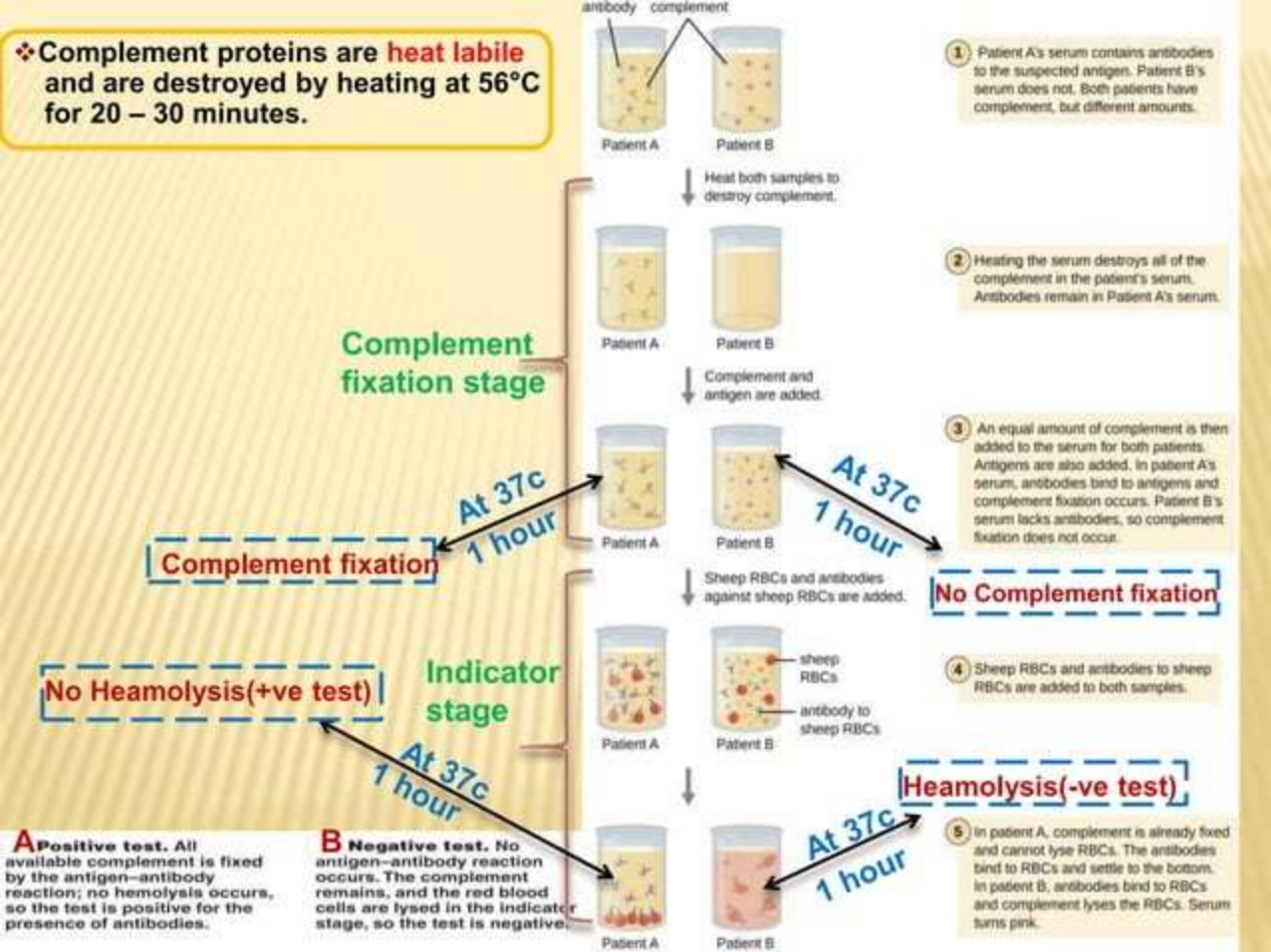
The test requires five reagents and is carried out in two steps.

Test System

- **Antigen:** It may be soluble or particulate.
- **Antibody:** Human serum (May or may not contain Antibody towards specific Antigen)
- **Complement:** It is pooled serum obtained from 4 to 5 guinea pigs. It should be fresh or specially preserved as the complement activity is heat labile (stored at -30 °C in small fractions). The complement activity should be initially standardized before using in the test.

Indicator System (Hemolytic system)

- **Erythrocytes:** Sheep RBC
- **Amboceptor (Hemolysins):** Rabbit antibody to sheep red cells prepared by inoculating sheep erythrocytes into rabbit under standard immunization protocol.


Complement proteins are heat labile and are destroyed by heating at 56°C for 20 – 30 minutes.
antibody
complement
Patient A
Patient B
1 Patient A's serum contains antibodies to the suspected antigen. Patient B's serum does not. Both patients have complement, but different amounts.
Heat both samples to destroy complement.
2 Heating the serum destroys all of the complement in the patient's serum. Antibodies remain in Patient A's serum.
Complement fixation stage
Complement and antigen are added.
3 An equal amount of complement is then added to the serum for both patients. Antigens are also added. In patient A's serum, antibodies bind to antigens and complement fixation occurs. Patient B's serum lacks antibodies, so complement fixation does not occur.
At 37c 1 hour
Complement fixation
No Complement fixation
Sheep RBCs and antibodies against sheep RBCs are added.
sheep RBCs
antibody to sheep RBCs
4 Sheep RBCs and antibodies to sheep RBCs are added to both samples.
No Heamolysis(+ve test)
Indicator stage
Heamolysis(-ve test)
5 In patient A, complement is already fixed and cannot lyse RBCs. The antibodies bind to RBCs and settle to the bottom. In patient B, antibodies bind to RBCs and complement lyses the RBCs. Serum turns pink.
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.

COMPLEMENT FIXATION TEST (CFT)

Advantages of CFT:

- Ability to screen against a large number of viral and bacterial infections at the same time.
- Economically Cheap.

Disadvantages of CFT:

- Not sensitive - cannot be used for immunity screening.
- Time consuming and labor intensive.
- Often non-specific e.g. cross-reactivity between HSV .

“PRECIPITATION IN AGAR”

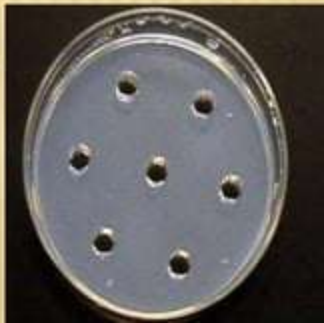
IMMUNODIFFFUSION

"In this method the specific antigens and antibodies are allowed to combine in a semi solid or gel media."

- Immunodiffusion is performed by using agar or agarose gel.

Advantages:

- The reaction formed from this method is **stable** and can be preserved for staining.
- It can be used to detect identity, non-identity & cross-reaction b/w antigens in a mixture.



IMMUNODIFFUSION

- Immunodiffusion reactions are classified on the basis of:

(a) number of reactants diffusing.

(b) direaction of diffusion.

Single diffusion in one dimension	Double diffusion in one dimension	Single diffusion in two dimension	Double diffusion in two dimension.

1. SINGLE DIFFUSION IN ONE DIMENSION

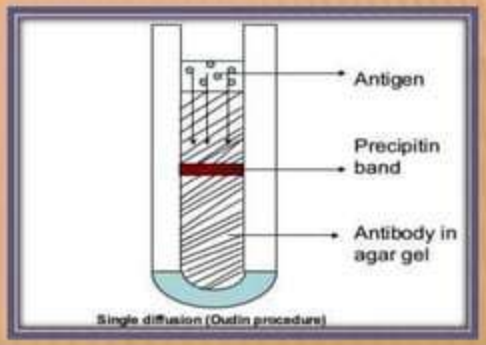
"*Single diffusion of antigen in agar in one dimension called single diffusion in one dimension.*"

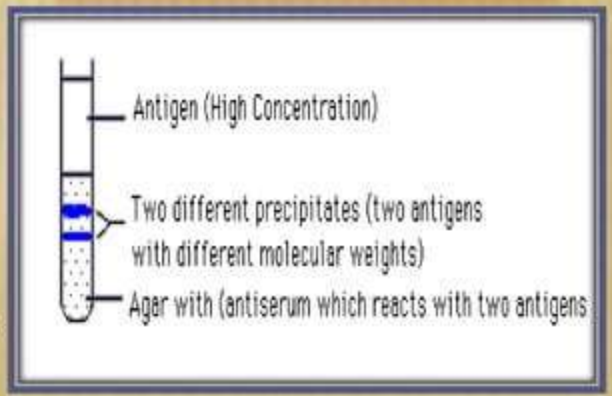
- It is also called *oudin procedure*, because this technique was introduced by **oudin**. Who for the first time used gels for precipitation reaction.

SINGLE DIFFUSION IN ONE DIMENSION

Oudin Procedure:

- The antibody is incorporated in a agar gel in a test tube and then the antigen is layered over it.
- The antigen diffuses downwards through the gel, forming a line of precipitation.
- Precipitate dissolves as the concentration of the antigen increases.
- The number of bands indicates the number of different antigens

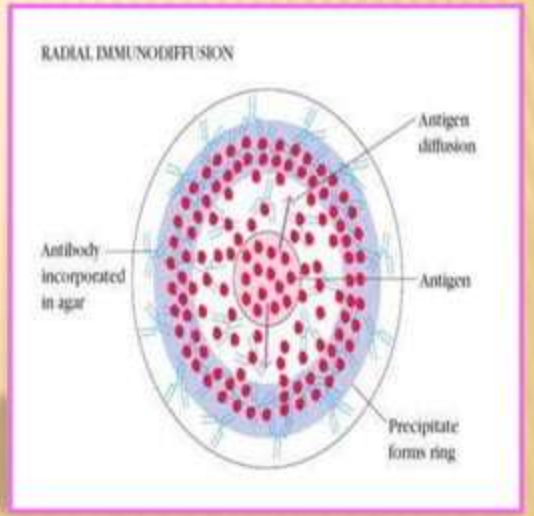




2. SINGLE DIFFUSION IN TWO DIMENSION

"Single diffusion in two dimension is also called ***Radial immunodiffusion*** **(Mancini test)** *"*

- RID has been used for the quantitative estimation of antibodies and antigens in the serum.

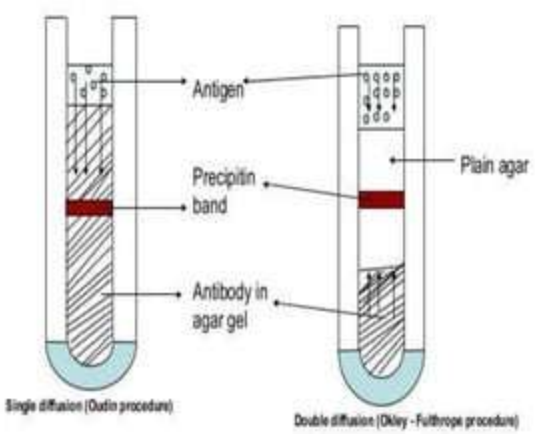


3.DOUBLE DIFFUSION IN ONE DIMENSION

*This method is also called **"Oakley-fulthorpe procedure"**. In this method **plain agar** is also used.*

Oakley-fulthorpe procedure.

- Ab is incorporated in a gel, above which a column of plain agar
- The antigen is layered on top of the agar medium.
- The Ag and Ab move towards each other through the intervening column of plain agar.
- This will result in **the formation of precipitate** when they meet at optimum proportion.



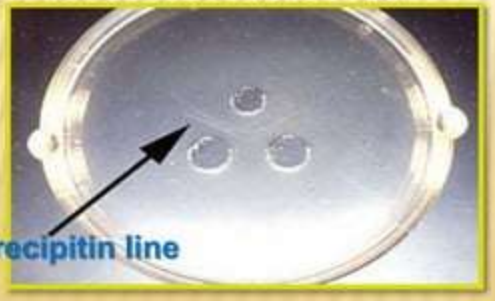
Single and double diffusion in one dimension

4. DOUBLE DIFFUSION IN TWO DIMENSION

*"This method is also called 'Ouchterlony's procedure'.(also known as **agar gel immunodiffusion** or **passive double immunodiffusion**)*

"In this method both Ag & Ab diffuse independently through agar gel in 2 dimensions, horizontally & vertically."

- The technique is named for **Örjan Ouchterlony**, the Swedish physician who invented the test in **1948.**



Ouchterlony's procedure:

- The test is performed by cutting wells in the agar gel poured on a glass slide or in a petri-dish.
- The antibodies is placed in a center well and different antigens are added to the well surrounding the center well.
- After an incubation period of 12-48 hours in a moisture chamber, the lines precipitins are formed at the site of the combination of antigen and antibody.

DOUBLE DIFFUSION IN TWO DIMENSION

Three types of reactions can be demonstrated as follows:

- **Fusion of lines at their junction to form an arc.**

-Serologic identity / presence of common epitope.(two identical Ag's are present)

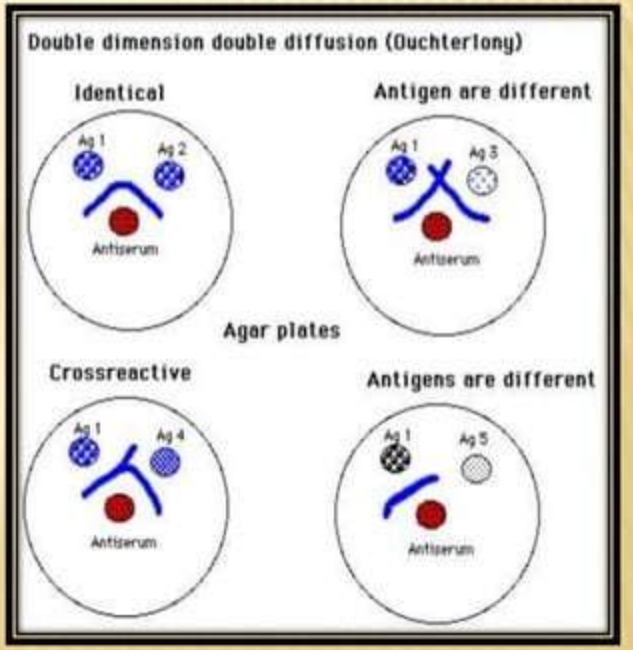
- **Crossed lines.**

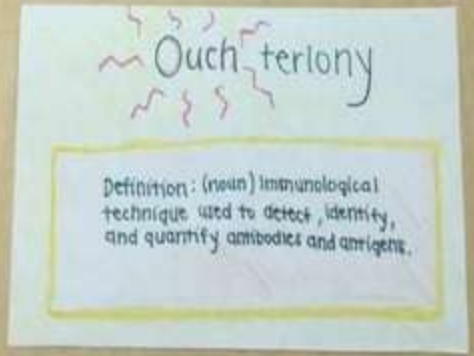
-Demonstrates 2 separate reactions.

-Compared Ag's shared no common epitopes.

- **Fusion of 2 lines with spur.**

-Indicates cross-reaction or Partial identity




Ouchterlony
Definition: (noun) Immunological technique used to detect, identify, and quantify antibodies and antigens.

DOUBLE DIFFUSION IN TWO DIMENSION

Uses:

- It is an immunological technique used in the detection, identification and quantification of antibodies and antigens, such as immunoglobulins and extractable nuclear antigens.
- Demonstration of Antibodies in the serodiagnosis of small pox.
- This technique is also used for the Identification of fungal antigens.

“PRECIPITATION IN AGAR WITH AN ELECTRIC FIELD”

IMMUNO-ELECTROPHORESIS

"It is a method in which different antigens in serum are separated according to their charge under an electric field."

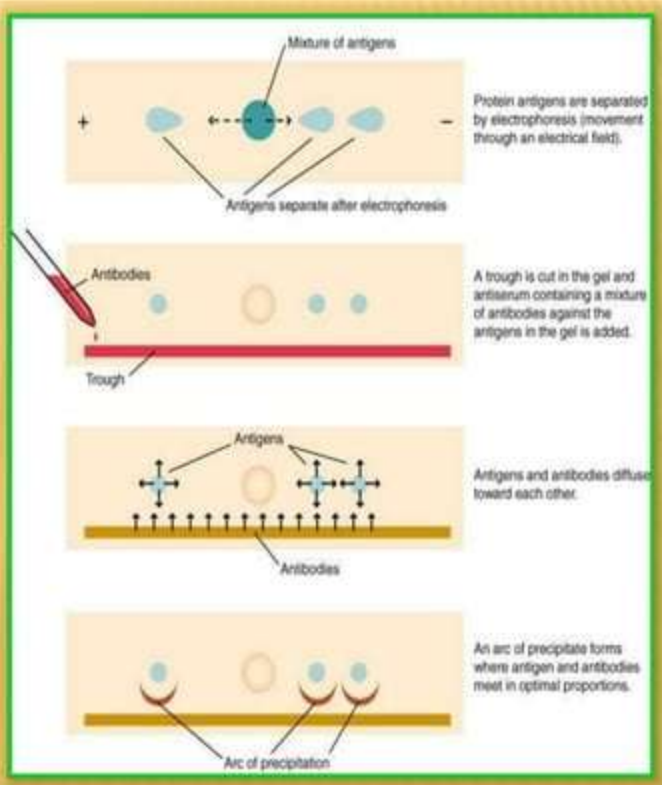
- It's a process of combination of **immunodiffusion** and **electrophoresis.**

Advantages:

- The main advantage of immunoelectrophoresis is that a no. of antigens can be identified in serum.

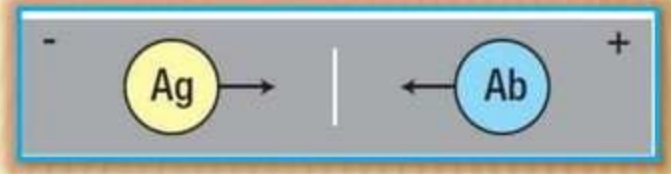
PROCEDURE:

- In this method, a drop of Ag is placed into a well in agar on glass slide.
- An electric current is then passed through the agar.
- During electrophoresis, Antigens move in the electric field according to their charge & size.
- A trough is cut into agar and is filled with the antibody and diffusion is allowed to occur.
- As the Ag and Ab diffuse towards each other, they form a series of lines of precipitation.



COUNTER CURRENT IMMUNO-ELECTROPHORESIS

"It depends on movement of antigen towards the anode (positive charge) & antibody towards the cathode (negative charge) through the agar in the electric field."



Advanteges:

- This method is a highly specific for the detection of both antigen & antibodies in the serum, CSF and other body fluids in diagnosis of many infectious diseases including **bacterial, fungal, viral & parasitic.**

- This method is commonly used for **HBsAg**

PROCEDURE:

- The test is performed on a glass slide with agarose in which pair of wells are punched out.
- One well is filled with Ag & the other with Ab.
- Electric current is then passed through the gel.
- The migration of Ag and Ab is greatly facilitated under electric field, & the line of precipitation is made visible in 30-60 mins.

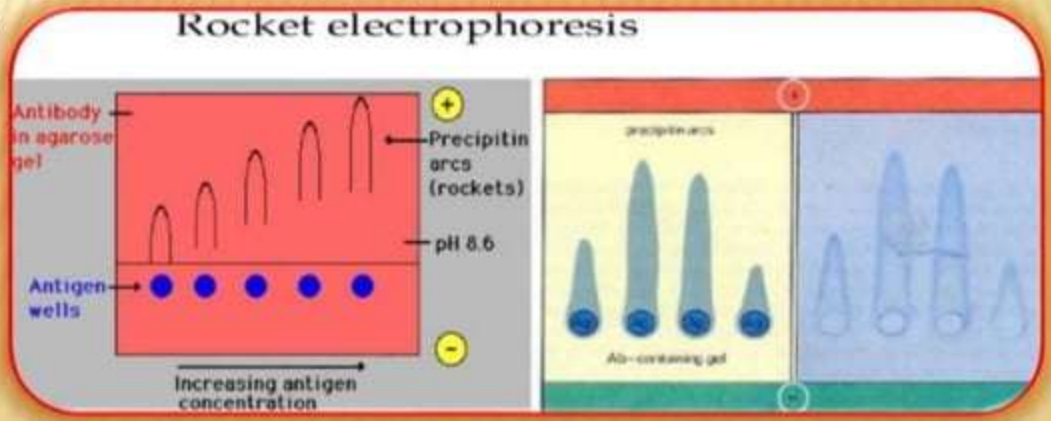
ROCKET ELECTROPHORESIS

"This technique is an adaptation of radial immunodiffusion developed by ***'Laurell'.*** *It is called due to the appearance of precipitin bands in the shape of cone-like structures* ***(rocket appearance)*** *as the end reaction."*

- Rocket electrophoresis is used mainly for quantitative estimation of antigen in serum

PROCEDURE:

- In this method Ab is incorporated in the gel, & antigens are placed in wells cut in gel.
- Electric current is then passed through the gel, which facilitated the migration of Ag into the agar.
- This results in formation of a **precipitin line(conical in shape),** resembling a rocket.
- The **height of rocket is directly proportional to concentration of antigen in the sample.**

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