

Serological Diagnosis

Done by:

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Serological Diagnosis

Ag:

It is a foreign substance that stimulate the immune system

Ab:

It is an immunoglobulin that produce against specific Ag

*type of Ab :

IgG, igA, IgM ,IgE, IgD

Most common serological test :

1-D.I.D

2- C.I.E

3- Latex agglutination

Duple Immuno Diffusion (Ouchterlony)

DID:

- ◆ it is used widely for the qualitative estimation (view) of antigen.
- ◆ technique involving diffusion of antigen or antibody through a semisolid medium resulting in a precipitin reaction.

Material:

- 1- Agarose in H₂O 0.75%
- 2- Sodium azide or merthiolate 0.2% >>>> to prevent contamination of bacteria or fungi
- 3- small Petridish
- 4- Ag and Ab(known Ab)
- 5- hole cutter

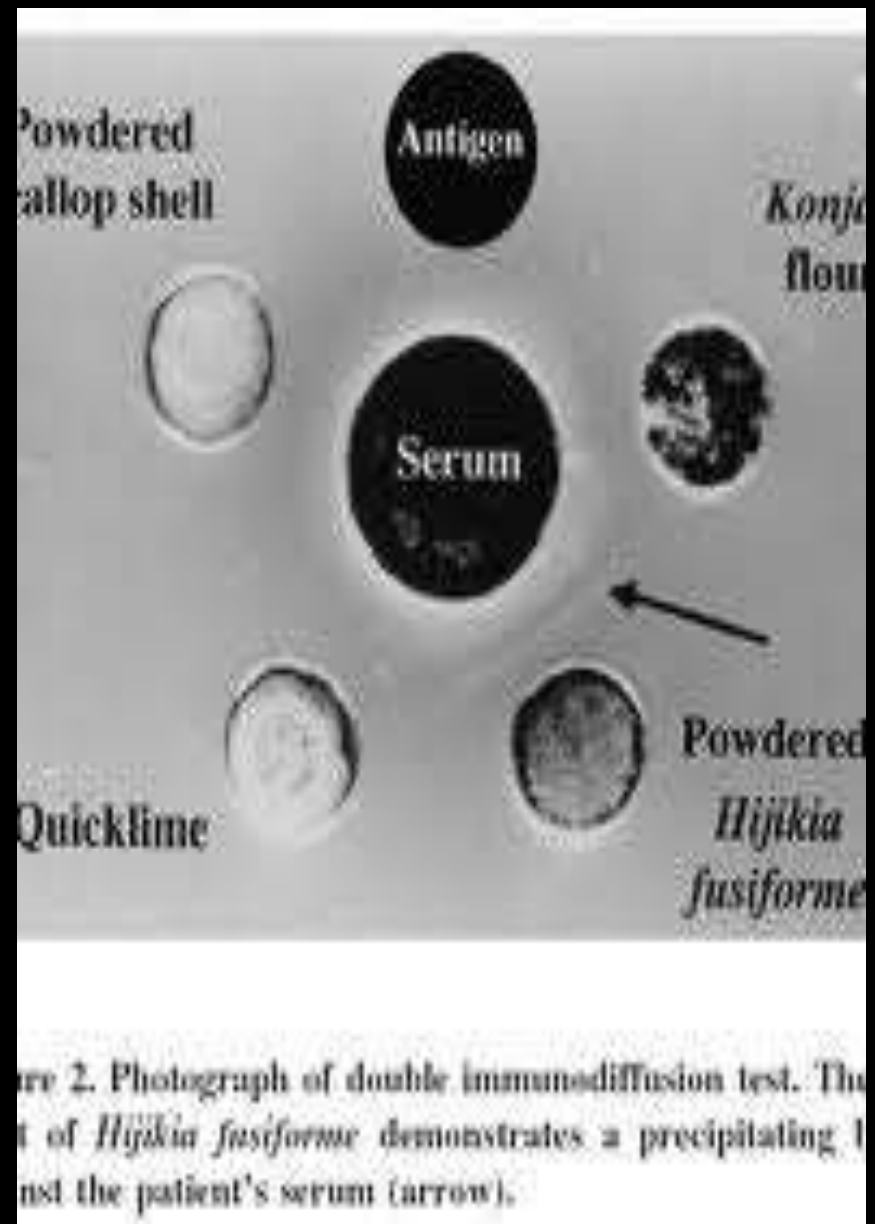
Duple Immuno Diffusion (Ouchterlony)

*Procedure:

- 1- in the small Petridish , add 6 ml of agarose , then keep it to solidify
- 2-punch the agarose in to 5 well
- 3- on the peripheral poor's we will add different Ag(25Ml from patient specimen) and in the center poor we will add known Ab
- 4- then leave the petridish for 24-72hr to 1 week at RT
- 5-after that, Ab will diffuse and Ag will diffuse

***Resulte:**

- In the optimal concentration we will see white line called line precipitation
- Line precipitation indicate that in the optimal concentration Ag is binding to the specific Ab



Duple Immuno Diffusion (Ouchterlony)

*Disadvantage:

1- it is less sensitive because Ab and Ag will diffuse in all direction

*Advantage:

It is specific

Counter Immuno Electrophoresis

*C.I.E:

It is a technique in which Ag and Ab move in opposite directions and form precipitation where they meet in optimal concentration

*Material:

- 1-Electrophoresis chamber
- 2- parpiton buffer PH= 8.6 (alkaline)

Counter Immuno Electrophoresis

*Material:

3- Slid

4- filter paper

5- Agarose 1%

6- power supply (P.S)

7- Ag and Ab

8- hole cutter

Counter Immuno Electrophoresis

Procedure:

1. Prepare agarose (1%)
2. Mark the end of a glass slide as +ve and -ve, so that when placed the glass slide in electrophoresis apparatus, the +ve mark faced towards anode and the negative mark faced towards cathode.
3. Place the glass slide on a horizontal surface. Pipette and spread 5 ml of 1.0% Agarose onto slide, spreading while releasing the agarose. Allow to solidify for 15 minutes.
4. Cut wells >>distance between the two wells should not be more than 0.5 cm.

Counter Immuno Electrophoresis

Procedure:

6. Transfer the slide containing the gel to the electrophoresis apparatus
- 7-Take required amount buffer (about 200 ml) in electrophoresis chamber. Place the filter paper at both end of glass slide, so that it's one end touch to the end of glass slide and the other end in the buffer.
- 8-Add the Ag) and Ab () to the well
- 9-Turn on the power

Counter Immuno Electrophoresis

- *When we open the power supply :
- *Ag in 8.9 pH is are strongly negatively charged and low M.W so will migrate rapidly the electric field toward the anode.
- *Ab in such a medium is less negatively charged and high M.W will migrate in an opposite or 'counter' direction toward the cathode according to electro endosmosis . If the antigen and antibody are specific for each other at the optimal concentration will form precipitin line.

CIE



Counter Immuno Electrophoresis

The time to get the result >line of precipitation is 1,5 hr to 2 hr.

*Result:

If Ag is specific to Ab at the optimal concentration >>>> we will see line ppt

*Advanteg:

Sensitive(because Ag AND Ab move in one direction) , fast ,specific

3-Latex Agglutination Test

- ◆ The latex agglutination test is a laboratory method to check for certain antibodies or antigens in a variety of body fluid eg: cerebrospinal fluid.
- ◆ Procedure :
- ◆ The sample is sent to a lab, where it is mixed with latex beads coated with a specific antibody or antigen. If the suspected substance is present, the latex beads will clump together (agglutinate).

Result



3-Latex Agglutination Test

◆ Advantage:

This simple, sensitive and rapid