



451 MBIO
Immunology

Aljawharah F. Alabbad

2017



Lab 5

IMMUNODIFFUSION

❖ Immunodiffusion

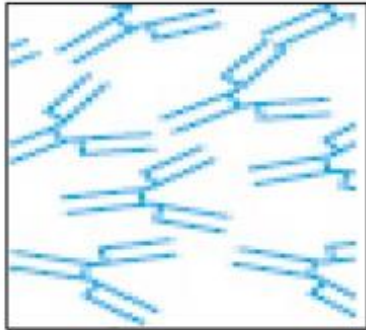
- A technique for the identification and quantification of any of the immunoglobulins.
- It is based on the presence of a visible precipitate that results from an antigen-antibody combination under certain circumstances.

■ Principle

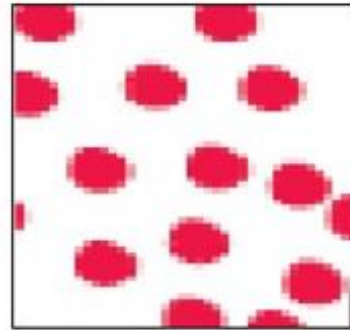
- An immobile precipitate, visible as a band (precipitin line) in the gel, develops if specific antibody-antigen binding takes place, and if antibody-antigen components are present at optimal proportions.

■ Types of Precipitation Method

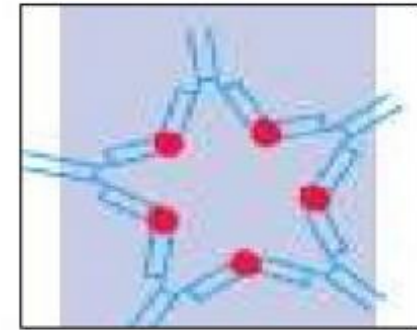
- **Single diffusion (Radial immunodiffusion).**
- **Double diffusion (Ouchterlony).**
- Immunoelectrophoresis.
- Immunofixation.



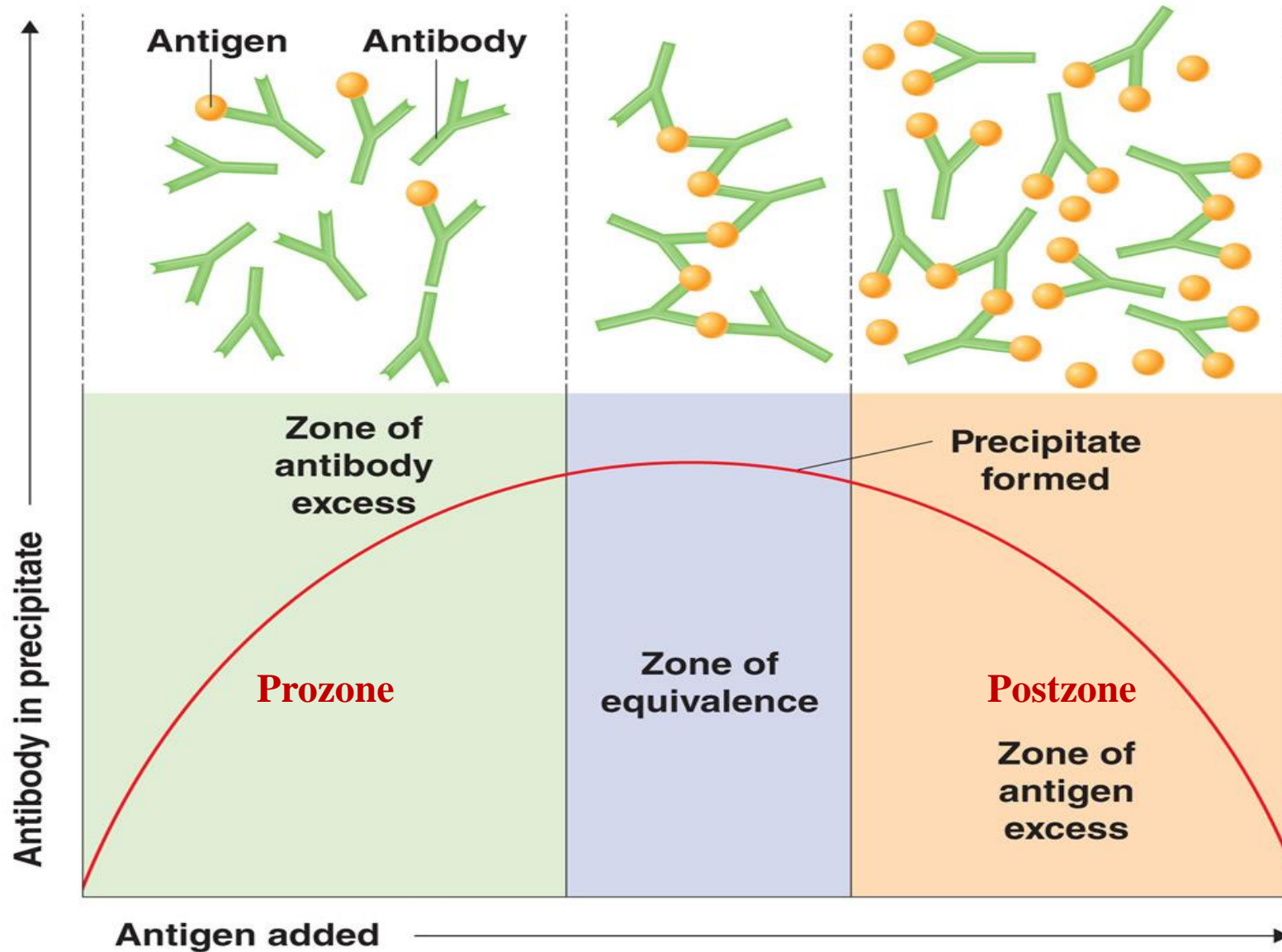
Antibodies



Antigens



Ag-Ab complex



❖ Used Medium

1. **Agar** : contain high molecular weight complex of polysaccharide, addition of 0.3 – 1.5 % agar will diffuse most of reactants.
- Agar has strong negative charge.

2. Agarose : purified agar used to help stabilize the diffusion process and allow visualization of precipitation bands.

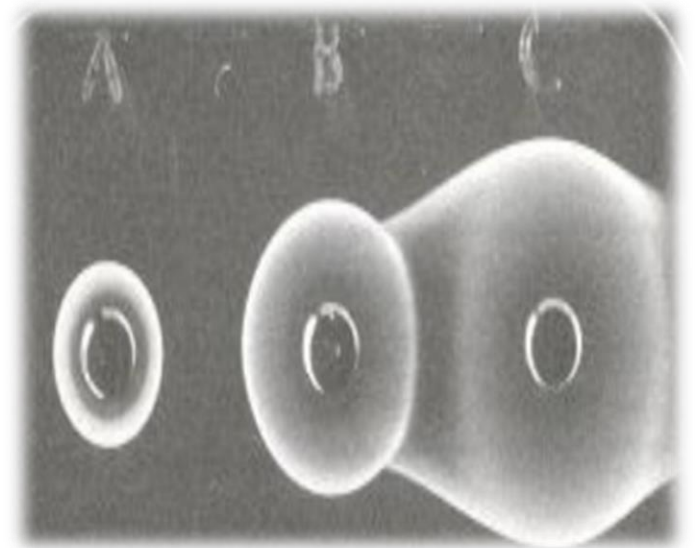
- Agarose more preferred than agar, because agarose has no charge. Therefore, interaction between gel and reactants are minimized.

1. Radial Immunodiffusion

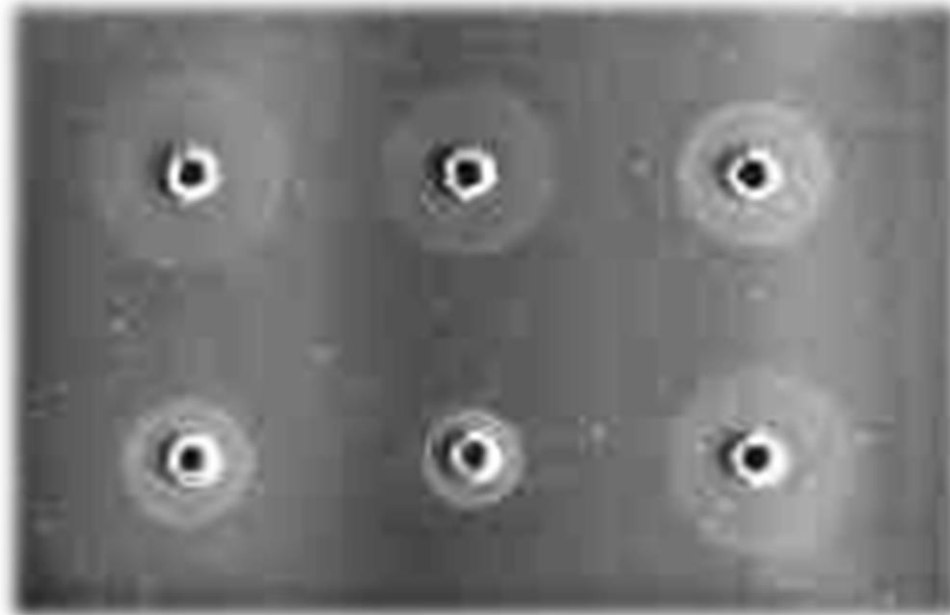
(Single)



- A single diffusion technique where Ab is put into gel and Ag is measured by the size of a precipitation ring formed when it diffused out in all directions from a well cut into the gel.



- This technique is quantitative is based upon the reaction between an Ag, and a specific Ab during a diffusion period.



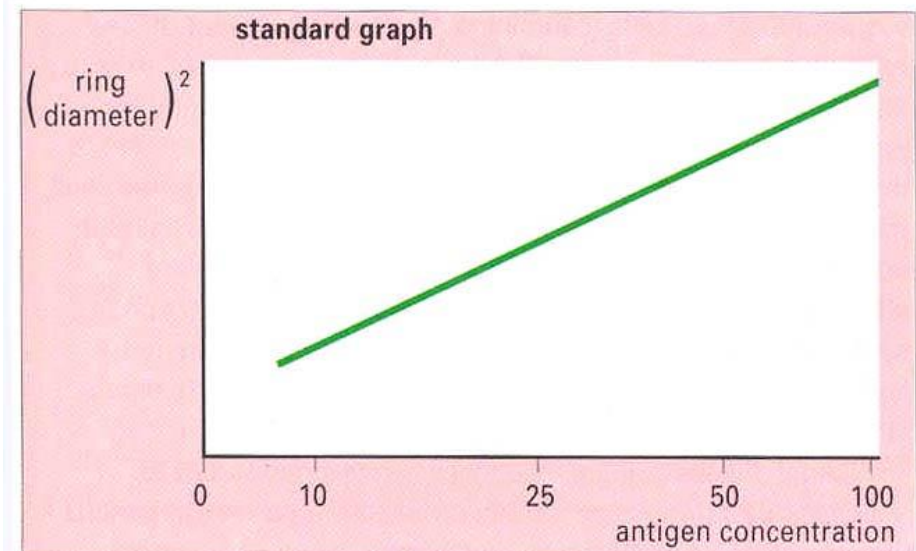
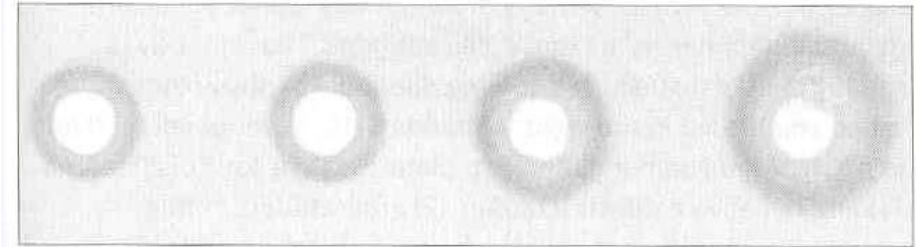
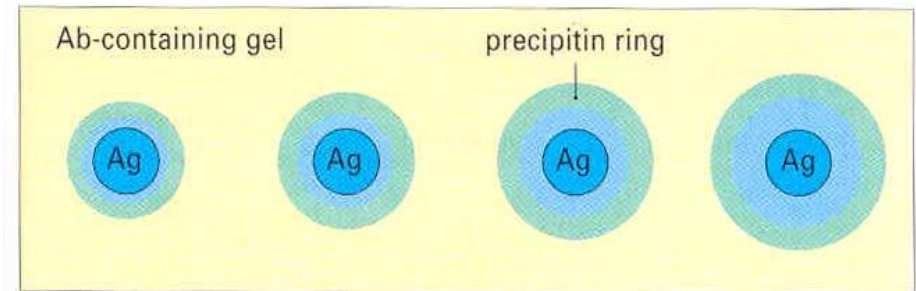
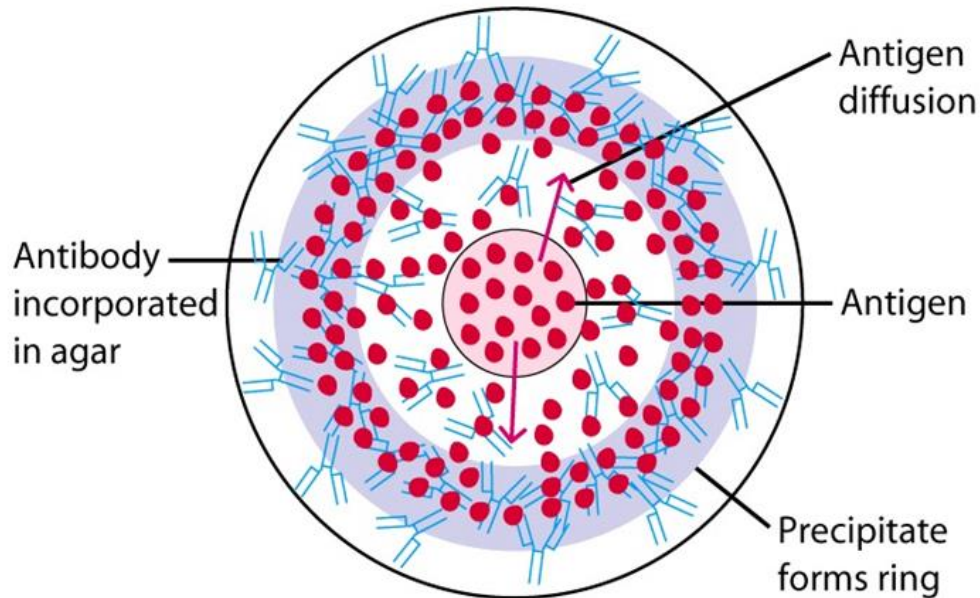
■ Method

- Ab is put into a gel and Ag is put in a well cut into the gel and a precipitin ring formed when Ag diffuses out in all directions.
- The Ag-Ab interaction is manifested by a well-defined ring of precipitation around the Ag well.

■ Result

- Ag diffuses radially from well and form a ring shaped band of precipitation.
- The halo of precipitation diameter gives the estimate of concentration of antigen.

RADIAL IMMUNODIFFUSION



- **Interpretation** : Diameter of ring is proportional to the antigen concentration. (quantitative Ig levels).

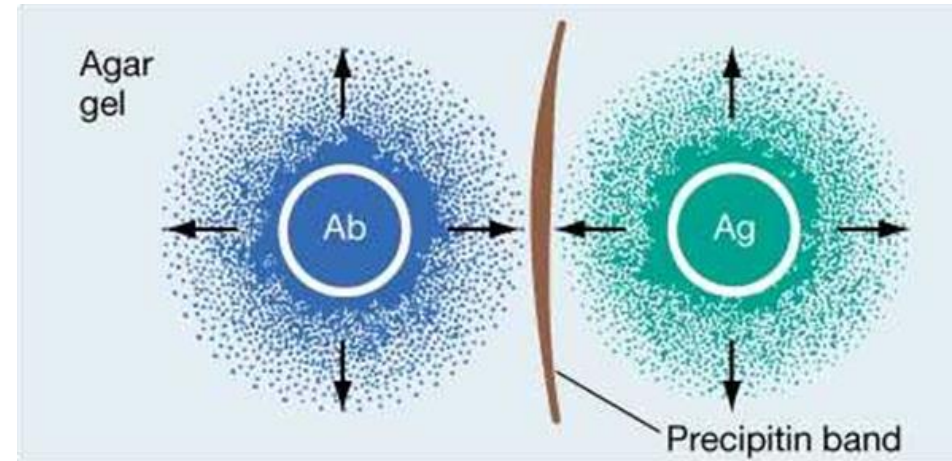
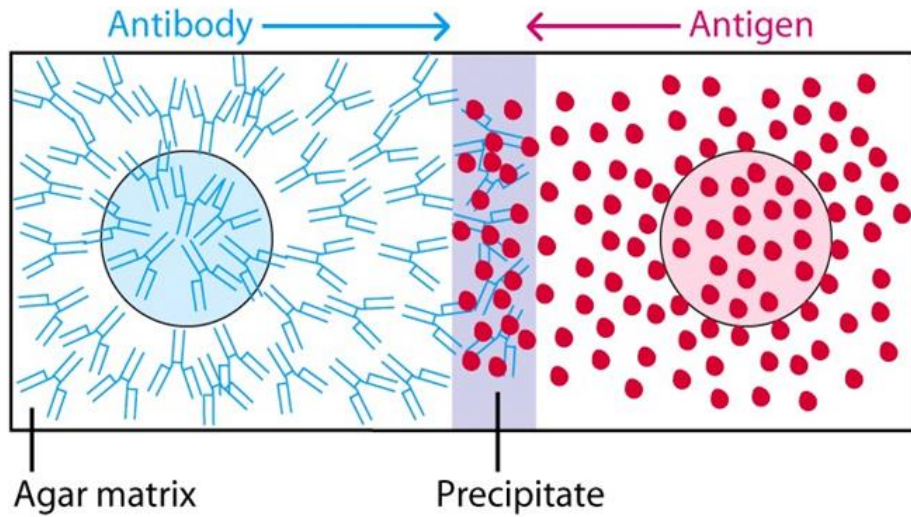


2. Ouchterlony (Double diffusion)

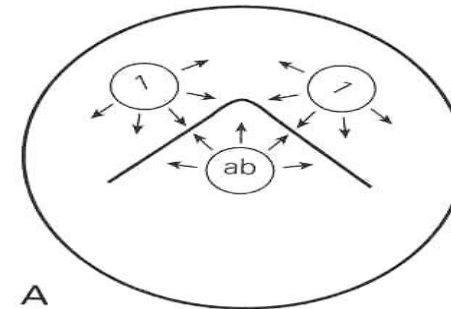


- Both antigen and antibody can diffuse independently.
- It is based upon the simultaneous application of Ag and Ab in separate but adjacent wells of an agar plate.
- As the materials diffuse toward one another, lines form resulting from the Ag-Ab interactions

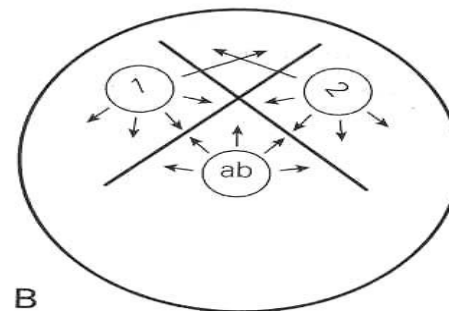
DOUBLE IMMUNODIFFUSION



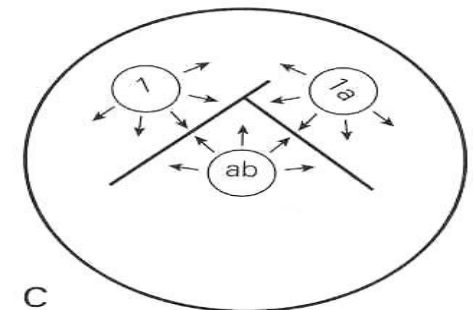
- If multiple wells of Ag are positioned around an Ab well on the same plate, several patterns of reactivity may be observed, as following :



A
Serological identity
Ag=ag1, Ab=anti-1

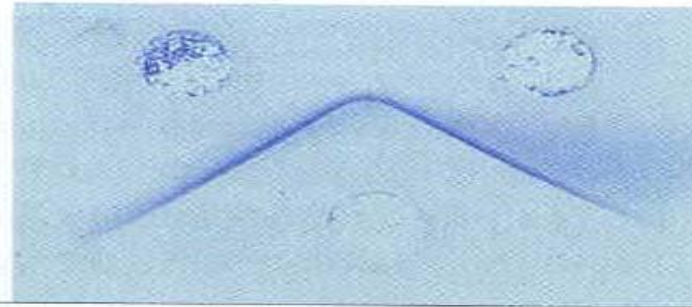
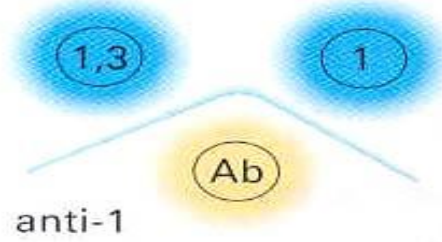


B
Non identity
Ag=ag1, ag2
Ab=anti-1, anti-2

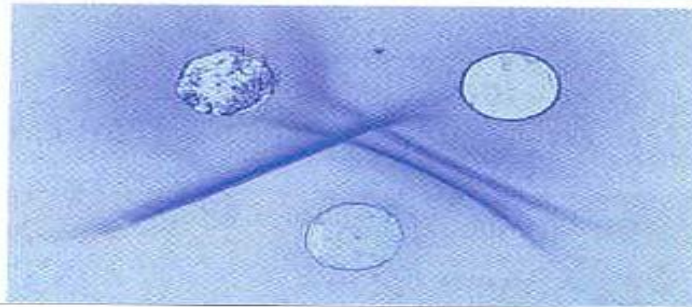
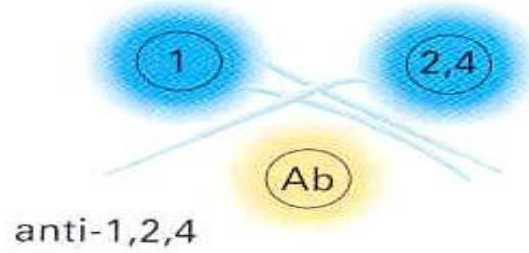


C
Partial identity
Ag=1, 1a; Ab=anti-1
Ag1a is a part of ag1,
but is the simpler ag

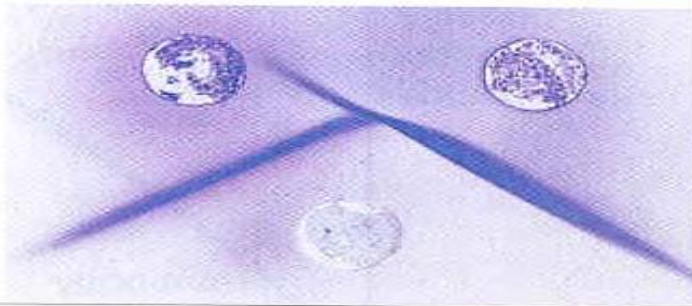
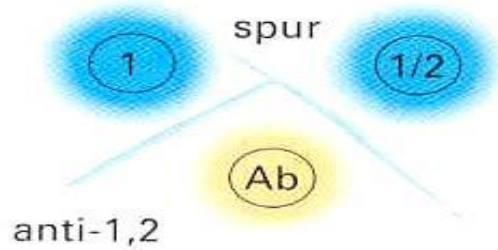
1. identity



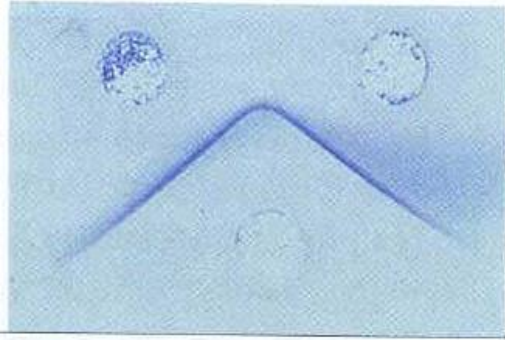
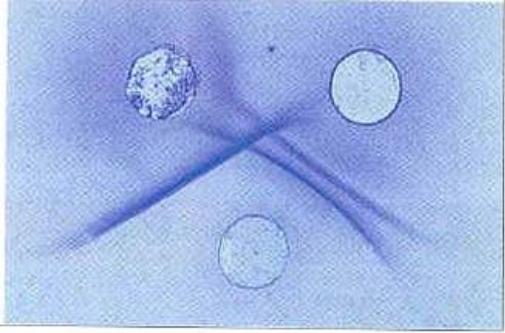
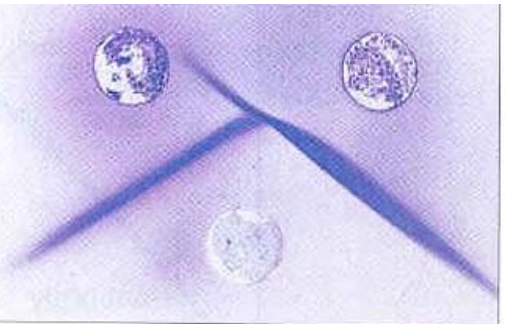
2. non-identity



3. partial identity



Ouchterlony Pattern

Pattern	Description	
1. Identity	If the Ag A (patient) is the same as the Ag B (control), the reaction with the Ab will be the same and the result is a <u>solid, continuous, smooth</u> line of identity between the Ag wells and the Ab well.	
2. Non-identity	If Ag A (patient) is different from Ag B (control), and both react with the Abs to A & B, <u>the precipitin lines cross and a double spur is formed</u> ; this is a line of nonidentity.	
3. Partial identity	If Ag A (patient) and Ag B (control) share a common element but are not exactly the same (Abs to A), <u>a single spur is formed</u> . This is the line of partial identity.	

❖ The Experiment

- **Aim :** To understand Immunodiffusion technique
 - **Material :**
 - Human IgG, IgA, and IgM “NL” “Bindarit TM
 - Radial immnodiffusion kit
 - Human serum
 - Micropipettte

■ Procedure

1

- Wells are cut in the gel.

2

- Reactants are added in the well (5 μ l).

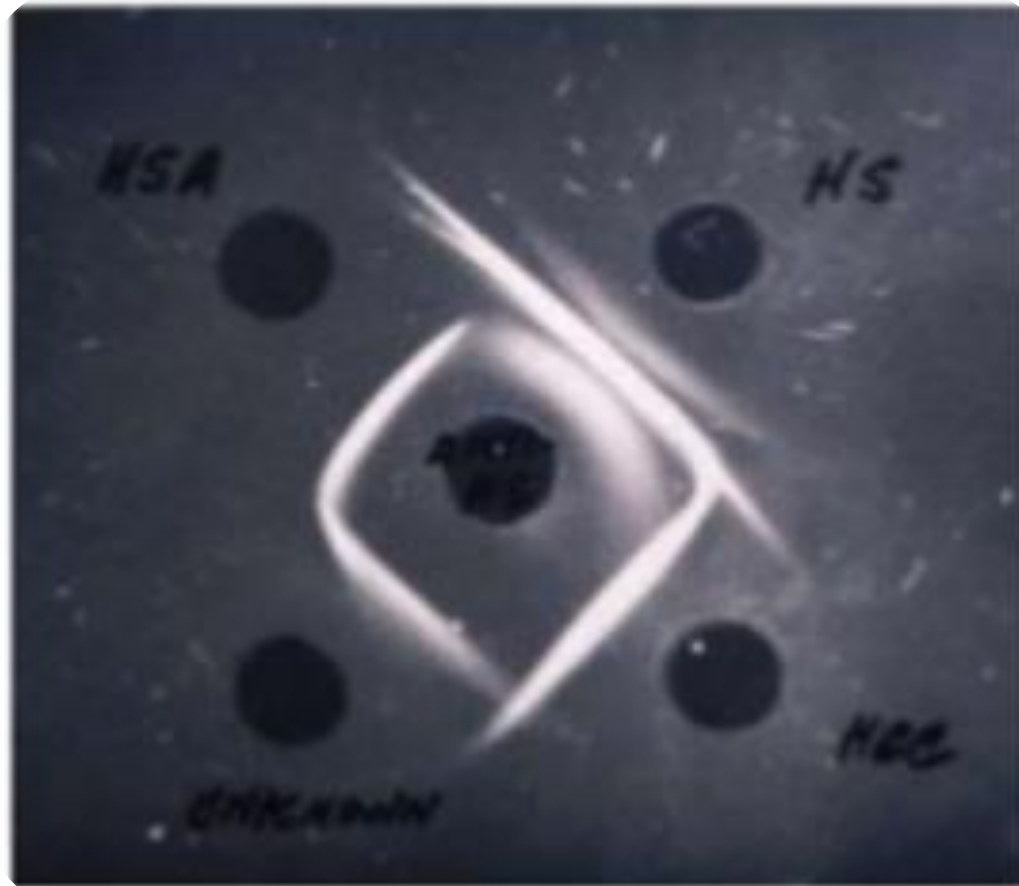
3

- Incubate for 12 – 48 H in a moist chamber.

4

- Precipitin lines will form, stained if required.

■ Result





Any Questions

alalabbad@ksu.edu.sa

