

## 6. OUCHTERLONY DOUBLE IMMUNODIFFUSION TEST

### Aim:

To study the reaction pattern of an antigen with a set of antibodies by Ouchterlony Double Diffusion method.

### Introduction:

Immunodiffusion in gels encompasses a variety of techniques, which are useful for the analysis of antigens and antibodies. Gel immunodiffusion can be classified into two groups:

1. Single Immunodiffusion
2. Double Immunodiffusion

In the Ouchterlony double diffusion, both the antigen and the antibody diffuse toward each other in a semisolid medium to a point till their optimum concentration is reached. A band of precipitation occurs at this point. The qualitative Ouchterlony Test can simultaneously monitor multiple Antibody-Antigen system and can be used to identify particular antigens in a preparation. This procedure was developed by Örjan Ouchterlony.

### Principle:

When soluble antigen and antibody samples are placed in adjacent wells in agarose gel, they diffuse radially into the agarose gel and set up two opposing concentration gradients between the wells. Once the gradients reach to an optimal proportion, interactions of the corresponding molecules occur and a line of precipitation will form. Using such a technique, the antigenic relationship between two antigens can be analyzed. Distinct precipitation line patterns are formed against the same anti-sera depending on whether two antigens share all antigenic epitopes or partially share their antigenic epitopes or do not share their antigenic epitopes at all. The Ouchterlony test also can be used to estimate the relative concentration of antigens. When an antigen has a relatively higher concentration, the equivalent zone will be formed a little bit away from the antigen well. When an antigen has a relatively lower concentration, the equivalent zone will be formed a little bit closer the antigen well. The pattern of lines that form can be interpreted to determine the relationship between the antigens and antibodies.

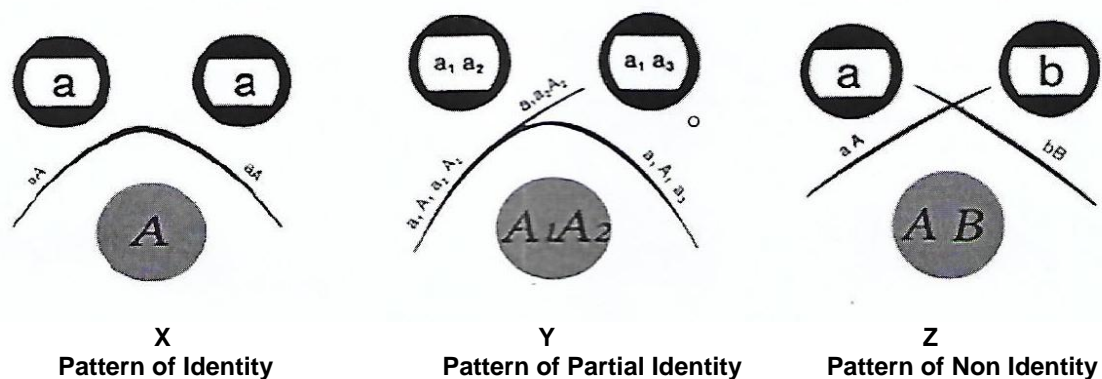


Fig 1: Antigen- Antibody Patterns formed in Ouchterlony Double Diffusion

**Pattern of Identity: X**

Pattern of identity occurs when the antigens in the two wells are identical and specific for the antibody in the antiserum present in the third well. The concentration of the two antigens been the same, they will diffuse at the same rate resulting in a smooth line of precipitate. The antibodies cannot distinguish between the two antigens i.e. the two antigens are immunologically identical as shown in Fig 1.

**Pattern of Partial Identity: Y**

Pattern of partial identity occurs when the antigens in the two wells share some epitopes which are same for both, yet each of the two antigens also have unique epitopes. In this case antiserum contains polyclonal antibodies specific for each epitope. When one of the antigens has some of the same epitopes compared to other, the polyclonal antibody population will respond differently to the two antigens and the precipitin line formed for each antigen will be different. The 'spur' is thought to result from the determinants present in one antigen but lacking in the other antigen (refer to Fig 1). A similar pattern of partial identity is observed if the antibodies are cross reactive with an epitope on one of the antigen that is similar, but not identical to that present on the other antigen.

**Pattern of Non-Identity: Z**

Pattern of non-identity occurs when the antigens in the two wells are totally different. They are neither cross reactive, nor do they have any epitopes which are same. In this case the antiserum containing the antibodies is heterogeneous as some of the antibodies react with antigen in one well while some react with antigen present in the other well. So the two antigens are immunologically unrelated as far as that antiserum is concerned (refer to Fig 1).

**Materials required:**

Sl No.	Materials	Remarks
1	Agarose	All the materials were provided in HiPer® Ouchterlony Double Diffusion Testing Kit
2	Assay buffer	
3	Antiserum X	
4	Antiserum Y	
5	Antiserum Z	
6	Antigen X1	
7	Antigen X2	
8	Antigen Y1	
9	Antigen Y2	
10	Antigen Z1	
11	Antigen Z2	
12	Glass plate	
13	Gel puncher	
14	Template	
15	Measuring cylinder	
16	Beaker	
17	Alcohol	
18	Incubator (37°C)	
19	Micropipettes and Tips	
20	Moist chamber	

## Procedure:

1. Prepare 10 ml of 1% agarose (as given in important instructions).
2. Cool the solution to 55-60°C and pour 5 ml/plate on to grease free glass plates placed on a horizontal surface. Allow the gel to set for 30 minutes.
3. Place the glass plate on the template provided.
4. Punch wells with the help of the gel puncher corresponding to the markings on the template. Use gentle suction to avoid forming of rugged wells.
5. Add 10 ml each of the antiserum and the corresponding antigens to the wells as shown in fig 2.
6. Keep the glass plate in a moist chamber overnight at 37°C.
7. After incubation, observe for opaque precipitin lines between the antigen and antiserum wells.

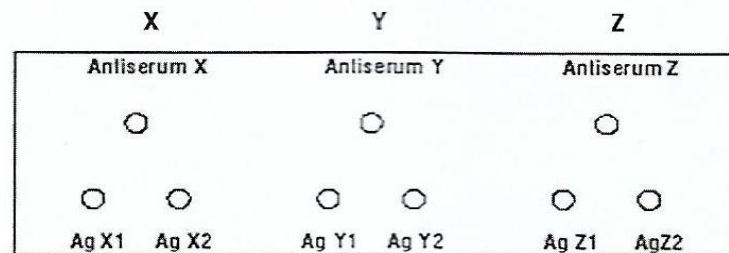


Fig 2: Template for addition of antiserum and antigen to their respective wells

## Observation and Result:

Observe for presence of precipitin lines between antigen and antisera wells. Note the pattern of precipitin line observed in each case.



X Pattern of Identity      Y Pattern of Partial Identity      Z Pattern of Non Identity

Fig 3: Diagram showing pattern of precipitin lines

## Interpretation:

When antigen and antibody meet in optimal proportions a precipitation line is formed. In Ouchterlony Double Diffusion (Antigen Antibody Pattern), three patterns of precipitin lines can be observed.

1. Pattern X or pattern of identity is observed between the antigens and the antiserum, it indicates that the antigens are immunologically identical.
2. Pattern Y or pattern of partial identity is observed, it indicates that the antigens are partially similar or cross-reactive.
3. Pattern Z or pattern of non-identity is observed, it indicates that there is no cross-reaction between the antigens. i.e. the two antigens are immunologically unrelated.

**Observation and Result:**