Radial Immunodiffusion

Aim:

To study the immunodiffusion technique by Single Radial Immunodiffusion.

Introduction:

Single Radial Immunodiffusion, also known as Mancini technique, is a quantitative immunodiffusion technique used to detect the concentration of antigen by measuring the diameter of the precipitin ring formed by the interaction of the antigen and the antibody at optimal concentration. In this method the antibody is incorporated into the agarose gel whereas the antigen diffuses into it in a radial pattern. Thus, the antibody is uniformly distributed throughout the gel.

Principle:

Single Radial Immunodiffusion is used extensively for the quantitative estimation of antigen. Here the antigen-antibody reaction is made more sensitive by the addition of antiserum into the agarose gel and loading the antigen sample in the well. As the antigen diffuses into the agarose radially in all directions, it's concentration continuously falls until the equivalence point is reached at which the antigen concentration is in equal proportion to that of the antibody present in the agarose gel. At this point ring of precipitation ('precipitin ring') is formed around the well. The diameter of the precipitin ring is proportional to the concentration of antigen. With increasing concentration of antigen, precipitin rings with larger diameter are formed.

The size of the precipitin rings depends on:

- Antigen concentration in the sample well
- Antibody concentration in the agarose gel
- Size of the sample well
- Volume of the sample

Thus, by having various concentrations of a standard antigen, standard curve can be obtained from which one can determine the amount of an antigen in an unknown sample. Thus, this is a quantitative test. If more than one ring appears in the test, more than one antigen/antibody reaction may have occurred. This could be due to a mixture of antigens or antibodies.

This test is commonly used in the clinical laboratory for the determination of immunoglobulin levels in patient samples.

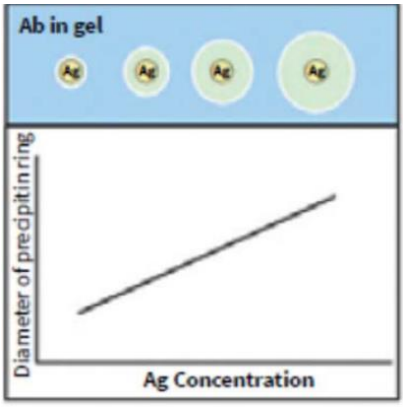

Fig 1: In Single Radial Immunodiffusion assay the diameter of the precipitin ring increases with increasing concentration of the antigen

Requirements:

1. Agarose
2. 10X Assay buffer
3. Antiserum
4. Standard Antigen A
5. Standard Antigen B
6. Standard Antigen C
7. Standard Antigen D
8. Test Antigen 1
9. Test Antigen 2
10. Glass plate 1 Nos.
11. Gel puncher 1 No.
12. Template 1 Nos
13. **Glass wares**: Measuring cylinder, Beaker
14. **Reagents**: Distilled water, ethanol/isopropanol
15. **Other requirements**: Incubator (37ºC), Microwave, spatula, Micropipettes, Tips.

Important Instructions:

1. Before starting the experiment the entire procedure has to be read carefully.
2. Always wear gloves while performing the experiment.
3. **Preparation of 1X Assay Buffer:** To prepare 10 ml of 1X Assay Buffer, add 1 ml of 10X Assay
buffer to 9 ml of sterile distilled water.
4. **Preparation of 1% Agarose gel:** To prepare 10 ml of Agarose gel, add 0.1 g of Agarose powder
to 10 ml of 1X Assay Buffer, boil to dissolve the agarose completely.
5. Wipe the glass plates with cotton; make it grease free using alcohol for even spreading of agarose.

6. Cut the wells neatly without rugged margins.
7. Add the antiserum to agarose only after it cools down to 55°C as higher temperature will inactivate
the antibody.
8. Ensure that the moist chamber has enough wet cotton to keep the atmosphere humid.
9. Ensure that the slide is grease free before pouring the gel.

* Molecular biology grade double distilled water is recommended.

Procedure:

1. Prepare 10 ml of 1% agarose gel (as give in the important instructions). Take 6 ml of this gel solution in a clean test tube.
2. Allow the solution to cool down to 55-60ºC and add 80 µl of antiserum to 6 ml of agarose solution. Mix well for uniform distribution of the antibody.
3. Pour agarose solution containing the antiserum on to a 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. Punch wells with the help of gel puncher corresponding to the markings on the template. Use gentle suction to avoid forming rugged wells.
6. Add 10 µl of the given standard antigen and test antigen samples to the wells.
A. Standard Antigen A (3.75 mg/ml)
B. Standard Antigen B (7.5 mg/ml)
C. Standard Antigen C (15 mg/ml)
D. Standard Antigen D (30 mg/ml)
E. Test Antigen 1
F. Test Antigen 2

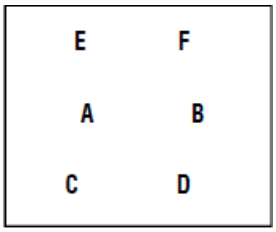

Fig 2: Template of **pattern of wells for loading of standard and test antigens**

7. Incubate the glass plate in a moist chamber overnight at 37ºC.

Troubleshooting Guide:

Sr.No	Problem	Probable Cause	Solution
1	No precipitin ring observed	Inadequate filling of the wells	Sample should be loaded directly into the well without spilling to the sides
		Drying of the agarose gel during incubation	Ensure that the moist chamber has enough moist cotton to avoid drying of the gel
		Inactivation of antiserum	Antiserum should be added to the agarose gel only after the temperature reaches to 55-60°C
2	Blur precipitin ring observed	Inactivation of antiserum	Antiserum should be added to the agarose gel only after the temperature reaches to 55-60°C
		Uneven pouring of gel	Place the glass plate on a flat surface while pouring the gel. Do not move the plate once the gel is poured