

Radial Immunodiffusion

- 1 The assay buffer provided was diluted by 100 to 12 assay dilution water.



- 2 10 milliliters of 1.0 percent agarose (0.1 mg/10 ml) was made in the 12 assay buffer by heating slowly until the agarose was completely dissolved, with the intention not to cause warping or cracks in the gelatin. The molten agarose was left to cool down to 55°C.



- 3 120 ml of antibody (the antiserum) had been reconstituted using 2 ml of assay buffer (12) were added to the 6 milliliters of agarose solution. The mixture was stirred gently to ensure the homogeneous distribution of the antibody.



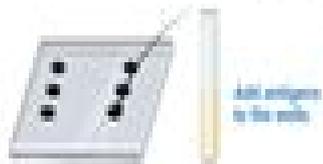
- 4 Glass plates had to be then cleaned thoroughly with alcohol, making them clean and grease free to allow for the even spread of the molten cooled agarose. The agarose solution that contained the antiserum was poured on the glass plate and placed on a flat surface and left to sit for 15 minutes without interruption.



- 5 Gel punches were employed to precisely cut the wells without rough edges with the aid of a template.



- 6 20 μl each of the antigens (standard and test) was added into the holes. The antigen disks were reconstituted by adding 0.25 milliliters of 12 assay buffer. Following are the concentrations of the standard antigens.



- 7 The plate of gel was placed in a moist humid chamber (box with wet cotton) and then incubated for a night at a room temperature.

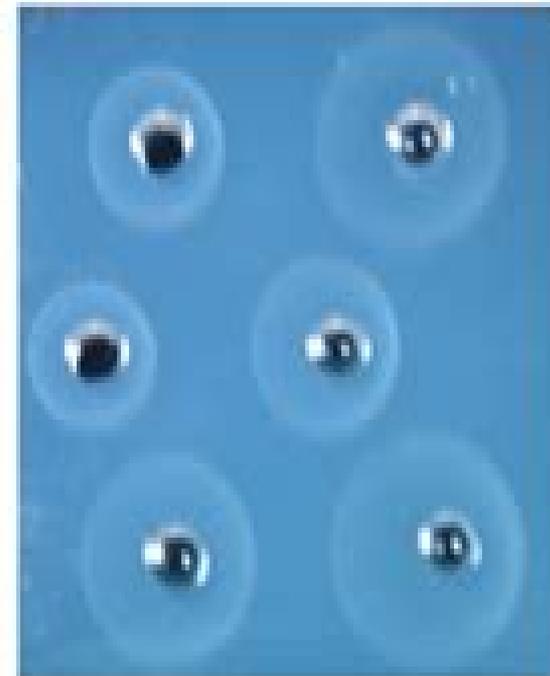
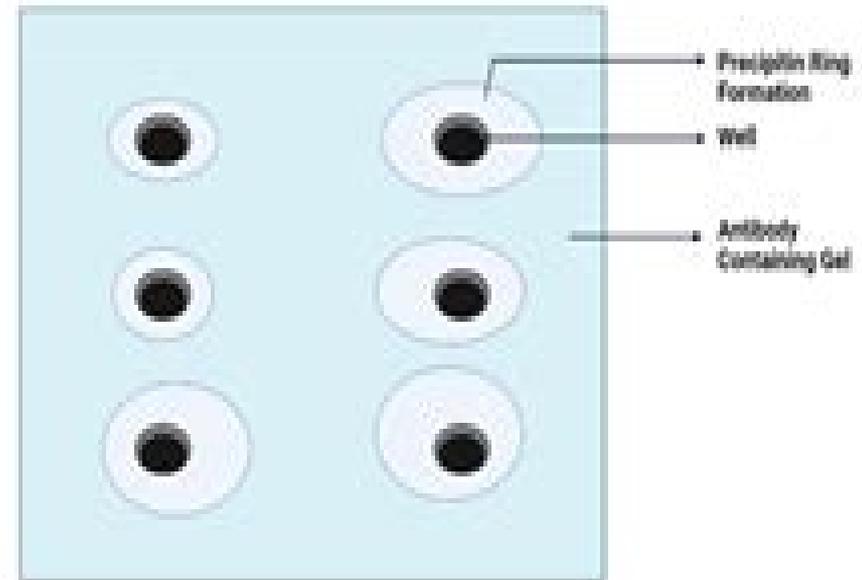


- 8 The next day, the edges that were part of the circular (if there were any) were marked, and the diameters of the rings were determined.



- 9 A graph was created with the information from the observation (if there was any) using the diameter of the circles on the y-axis, and the concentration of antigen on the x-axis (done on an excel sheet).

- 10 The concentration of the unknown was determined by measuring the concentration against the ring diameter from the graph.



Practical Immunology

Michael Seilmaier



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