T. Springer, July 1982

QUANTITATIVE SINGLE RADIAL IMMUNODIFFUSION

1. Make 1% agar (Difco) in 0.01 M Tris-HCl, pH 7.8, 0.14 M NaCl, 0.1% NaN_3 (TSA), dissolve in boiling water and then transfer to 55° water bath. Or use a 5% agar stock, dissolve, and dilute into buffer held at 55°.

2. Clean 4 x 5 inch glass slides and moisten a Kim-wipe with molten agar and coat the surface of the slide with a thin agar film. Allow to dry. The coating helps the antibody-containing gel to adhere to the slide.

3. Place tubes in the water bath and add 20 ml agar/slide.

4. When cooled to 55°, add correct amount of antibody (usually 100 µl of rabbit anti-rat Fab 12-12-78 per 20 ml agar or 300 µl of rabbit anti-mouse P9 myeloma Fab 20-8-75, D'1,1,3). Mix thoroughly.

5. Heat a glass slide using a hair dryer. Place on a level lab bench surface (find perfectly level areas with a leveling bubble).

6. Immediately pour 19 ml of the antibody-containing gel onto the slide using a plastic pipette. Spread evenly over the surface and remove any bubbles using the pipette tip. Allow to set. Plates may be stored (usually unpunched) for at least a year at 4° in a sealed tupperware box over towels moistened with TSA.

7. Punch 2 mm diameter holes 1.5 cm apart and 2 cm from edges (template is available).

8. Apply samples (8 µl). Include standards of the appropriate IgG at 200, 150, 100, 50, 20, and 10 µg/ml.

9. Place slide on moist paper towels in air-tight container and allow 2 days at room temperature for diffusion (longer if IgM is the antigen).

10. Quantitate the diameter of the precipitates in 2 directions (at 90° to one another). Plot the product of the two diameters versus antigen concentration. A straight line should be obtained. The precipitates may also be measured at 18 hours but diffusion will not be complete and standards will fall on a curved line.