Gel Diffusion Method for Determining the Titer of Duck Hepatitis Virus

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Apparatus necessary for a quantitative gel diffusion and precipitation assay of duck hepatitis virus was designed and constructed.

Duck hepatitis virus (4), like many noncytopathic avian viruses, is normally quantitated with the Reed and Muench method (5) by using embryo lethality as the end point. Unfortunately, this has disadvantages in regard to time, expense, and reliability (3).

We have developed a precipitation and gel diffusion assay designed to eliminate these problems. It has a high degree of sensitivity and requires a minimum of time and manipulations. Other quantitative gel diffusion assays have been proposed (2), but this system is unique because of the high ratio of reactant volume to agar volume.

A plexiglass diffusion block, described in detail elsewhere (M. G. Gabridge, M.S. Thesis, Michigan State Univ., East Lansing, 1966), contains eight separate reaction wells. Each consists of two circular reservoirs connected by a narrow slot which contains agar and serves as the reaction site (Fig. 1). Once the agar strip is cut to the desired length, the wells are swabbed with alcohol and the block is placed under an ultraviolet lamp until dry. The wells are filled with the reactants, and the block is covered and incubated. The distance of the precipitate bands in the agar strip is then measured and compared to that obtained with known virus titers.

Duck hepatitis virus (DHV) stocks were in the form of allantoic fluid from infected chicken embryos. A DHV reference assay used a standard quantal assay employing five embryos per dilution, with embryo death at 24 hr being indicative of infection. Antiserum was prepared in rabbits (Gabridge, M.S. Thesis).

It was determined that optimal precipitation occurred when the reactants were in the form of a gel. Dilutions were therefore prepared in Ionagar so that the final concentration matched that in the strip (0.85% in saline). The reaction strip was normally 0.5 inch (1.27 cm) in length, and incubation was at 37°C for 24 to 48 hr.

When various concentrations of virus were allowed to diffuse against a constant amount of antiserum, precipitate formed at various points along the agar strip. When the distance of the leading edge of precipitate was measured at different time intervals and plotted against titer, a linear relationship was observed (Fig. 2). When one sample was tested 15 times, the mean distance was 9.6 mm, with a standard deviation of 0.4 mm.

Controls indicated that normal allantoic fluid, the vehicle in the DHV immunization, precipitated 5.5 mm from the antiserum well in a 12.5-mm strip. Since the leading edge of precipitate was always at a greater distance than this, nonspecific bands should not interfere in this titration.

In the graph of titer versus distance, the area between any two curves simultaneously represents a distance and a time interval. The vertical distance between any two curves is thus equivalent to velocity and, when plotted against titer, yields another straight-line relationship (Fig. 3). Any antigen could thus be titrated through a sequence of dilution, diffusion, precipitation, measurement of distance or velocity, and comparison to a standard curve.

The sensitivity of a gel diffusion and precipitation system is directly proportional to the amount of reactant which can be fed into the immediate reaction area (1). This, in turn, is related to the ratio of the initial reactant volume to the volume of agar occupied at the first sign of a reaction—the higher the ratio, the higher the sensitivity which may be expected.

In a block titration with 1.0 ml of antigen and 0.25 ml of agar in a 0.5-inch strip, the ratio would be 1:1.12 or 0.89. In a standard Ouchterlony test, the value is about 0.2. By eliminating most
FIG. 1. Diagram of one well from the diffusion block (measurements in inches).

FIG. 2. Relationship of virus concentration to distance of leading edge of precipitate from antigen well. [Double diffusion in a 0.5-inch (1.27 cm) agar strip at 37 C.]

FIG. 3. Relationship of velocity of leading edge of precipitate to virus concentration. [Double diffusion in a 0.5-inch (1.27 cm) agar strip at 37 C.]

of the agar which is not critical in determining band position, the amount of virus in the reaction zone, and hence the sensitivity, has been increased.

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