A Plastic Plate for Use in Tests Involving Virus Hemagglutination and Other Similar Reactions[1]

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The purpose of this communication is to describe a plastic plate[3] that may be used as a substitute for the test tubes and racks needed in titrations of influenza virus hemagglutinin and the agglutination-inhibition antibody (*1*). Through simplification of the technical aspect of the work, chiefly by obviating the washing of tubes and setting up of racks, the use of the plate has facilitated greatly the conduct of large numbers of titrations simultaneously and has provided certain other advantages.

The plate is made from a block of clear Lucite, $7'' \times 9'' \times \frac{1}{2}''$, into which 80 shallow cavities have been drilled in 10 rows of 8 each; space between rows in each direction measures $\frac{3}{32}''$. The hollows are $\frac{3}{4}''$ in diameter, have a maximum depth of $\frac{7}{16}''$, and can hold approxi-

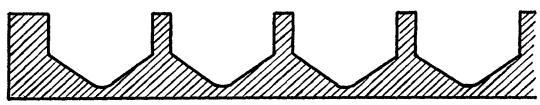

FIG. 1. Diagram of a vertical section made through the apex of the drilled cavities in the plastic plate. The thickness of 1/2" Lucite blocks may vary by as much as 1/16".

mately $2\frac{1}{2}$ cc of fluid. As shown in Fig. 1, the wall of the cavity is vertical and the bottom is conical rather than hemispherical (as is the bottom of a test tube). The inclined sides of the cone allow the unaltered cells to slide toward the apex and, at the same time, provide a large surface of gentle slope to which the "agglutinated" cells adhere as they settle out of suspension. The characteristic patterns of the sedimented cells in positive and negative reactions are the same as those seen in test tubes (*6*), but end-points of titrations, somewhat sharper.

Because of the larger surface the settled cells are required to cover in the depressions of the plastic plate, a greater number of red cells are required as compared with the concentration employed in tests done in small test tubes. In a previous report (*6*) of a procedure for titrating influenza virus hemagglutinin, a final concentration of 0.125% of chicken red blood cells in a 1-cc mixture was recommended in tests performed in 10×75-mm tubes. However, for tests done in the plastic plate twice this quantity of cells is necessary.

After the various reactants are mixed together, each plate is shaken effectively, but carefully, and set aside to be read after the cells have settled. Although the test may be read in 45 min, an hour has been selected arbitrarily as the time for recording the results. The plates are viewed from the top, preferably against a white background. Criteria for positive and negative reactions are the same as illustrated elsewhere (*6*).

As for the temperature at which the cells are allowed to settle, it has been found that equally satisfactory results are obtained in tests done at 4° C and 30° C, provided the readings are made within the prescribed time. The wide, shallow depressions permit rapid settling of the red cells; this appears largely to eliminate the variations attributable to temperature (*6*) and noted particularly in tests done in small test tubes. Absence of significant temperature effect is of considerable value in summer.

These plates have been found particularly useful in performing the agglutination-inhibition tests. The procedures followed involve the use either of a constant dilution of virus and serial dilutions of serum (*1, 2, 6, 8*) or of a constant dilution of serum and serial dilutions of virus (*5, 7*). In the presence of serum the cells settle more rapidly and the test should be read in 30–45 min.

In the performance of the agglutination-inhibition test, and also for virus titrations, frequent use is made of the spring-plunger type automatic syringe (from the B-D Cornwall pipetting unit) as a substitute for a pipette. When the automatic syringe is used for making serial dilutions, it is fitted with an 18- or 19-gauge needle that has a shaft approximately $\frac{1}{4}''$ long.

Plates are prepared for re-use by rinsing them well under tap water and then immersing them in a detergent solution or in *dilute* cleaning solution (10% sulfuric acid containing 0.5% potassium dichromate). In the routine procedure followed at present the plates are submerged in dilute dichromate-sulfuric acid solution for about 2–3 min, rinsed liberally under running tap water, and then rinsed in 3 successive pans of distilled water. Excess moisture is shaken off by brisk tapping against the hand, the plates then being allowed to dry either in the air or in front of a fan. The acid-dichromate solution recommended above appears not to be injurious to Lucite. Immersion for several hours produced no visible change, although a short period in more concentrated acid-dichromate changes the clear, smooth surface of the Lucite into an opaque, granular surface.

The plate described was drilled by machine at a cost that is not prohibitive. This could be reduced considerably, however, by using a mold prepared in the desired form. The applicability of this or similarly designed devices to other problems of a related nature is evident.

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[2] The author wishes to acknowledge the helpfulness of Robert L. Laurent in the preparation of the plates.

[3] Nartsisov (*4*) and Mulder (*3*) have suggested somewhat similar, but less convenient, devices for the same purpose.

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