PRO EXPERIMENTIS

Rapid Titration of Viral Hemagglutinins and Hemagglutination Inhibition Antibodies with the Aid of a Fragiligraph

A fragiligraph[1] is an instrument for determining and automatically recording the osmotic fragility of red blood cells[2,3]. A sample of blood diluted in isotonic NaCl solution is put into a container cell, two walls of which consist of dialysing membrane; the cell is introduced into a test-tube of distilled water, and placed in the optical path of an automatically recording colorimeter. Dialysis through the membrane results in continuous decrease in the salt concentration of the medium surrounding the erythrocytes; the resulting gradual hemolysis is recorded as an increase of light transmission through the red cell suspension.

The very small volume of erythrocyte suspension required in this method together with the high sensitivity of the instrument to slight changes in the light transmission, suggested that the fragiligraph might conveniently be used for the quantitative determination of viral agglutinins. Viral hemagglutinins are routinely measured either by the pattern method[3] or more accurately by the photoelectric method developed by HIRST and PICKLES[4] and later improved by DRESCHER[5,6].

In this photoelectric method the concentration of virus (or viral hemagglutinin) that causes 50% of red blood cells to clump and sediment out of the optical field is taken as one unit of hemagglutination (CCA unit)[4]. For titration, virus dilutions are introduced into numerous tubes, containing 4–6 ml of erythrocytes in saline. After incubation for 80–100 min the reduction in optical density due to hemagglutination is determined. The same degree of accuracy may be obtained with a fragiligraph using a single sample of 0.075 ml of a virus and red cell mixture; the results are obtained within 5 to 30 min. The procedure is as follows: equal volumes of virus suspension and a 10% suspension of red blood cells from a suitable animal are mixed in a test-tube and immediately introduced into the container cell with the aid of a syringe (Figure 1). The container cell is then inserted into a test-tube containing isotonic NaCl (or any other suitable *isotonic* diluent); the recorder of the instrument is switched on at the moment of mixing of the virus and the cells, so as to account for the time used for mixing and filling the cell. As the agglutination of the red blood cells by the virus progresses, causing accelerated sedimentation of the clumps, the light transmission through the suspension increases until a plateau is reached when only non-agglutinated erythrocytes remain in the optical path. The light transmission of a suspension of erythrocytes as single cells stays unaltered for a period as long as 40 min.

A representative set of agglutination-sedimentation curves for increasing concentrations of Newcastle disease virus and a 10% suspension of chick erythrocytes is shown in Figure 2. When the slopes of the straight portion of the sigmoid-type curves are plotted against the virus concentration, a practically straight line is obtained which is standard for a given virus (Figure 3). When the agglutination-sedimentation curve of a virus sample is recorded on the fragiligraph, the slope obtained can be

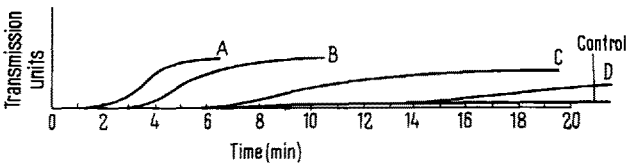

Fig. 2. Agglutination-sedimentation curves of chick red blood cells treated with Newcastle disease virus. A = 220 hemagglutination units; B = 110 HA; C = 55 HA; D = 27 HA.

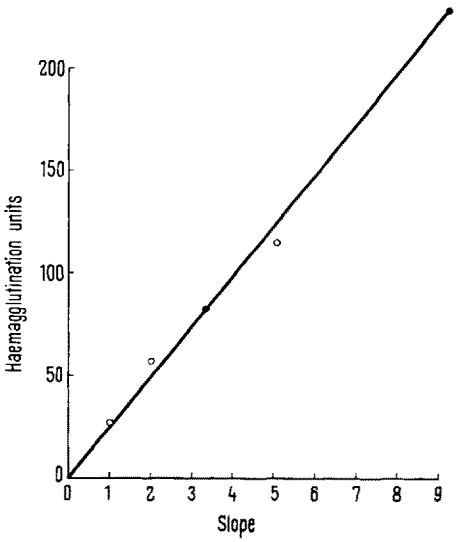

Fig. 3. Standard curve for NDV obtained from experiments such as depicted in Figure 2.

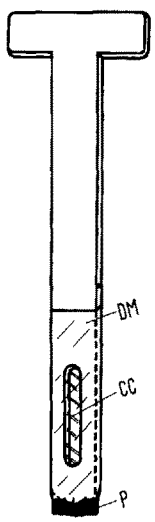

Fig. 1. Container cell for fragiligraph. DM = dialysis membrane; CC = container cell; P = seal.

[1] Available from Elron Electronic Industries Ltd., Haifa (Israel).
[2] D. DANON, J. clin. Path. *16*, 377 (1963).
[3] WHO Expert Committee on Influenza, Influenza Techn. rep. Ser. 64 (1953).
[4] G. K. HIRST and E. G. PICKLES, J. Immunol. *45*, 273 (1942).
[5] G. L. MILLER and W. M. STANLEY, J. exp. Med. *79*, 185 (1944).
[6] J. DRESCHER, Zbl. Bakt. Orig. *169*, 314 (1957).

translated into concentration of hemagglutinins on the standard curve.

The agglutination-sedimentation curves not only vary in slope as a function of the concentration of hemagglutinins; the point at which the curve starts to rise depends also on the concentration of the agglutinating virus, the more dilute it is the later the point at which the curve starts to rise (Table).

The hemagglutinins of 6 different viruses were estimated by this method: Newcastle disease virus (NDV), influenza A, encephalomyelocarditis (EMC), Semliki Forest virus (SFV), adenovirus 3 and polyoma. The method was found to be satisfactory for all the viruses tested except polyoma virus. Chick erythrocytes were employed in the test for NDV and influenza, sheep erythrocytes for EMC, duck erythrocytes for SFV, and rhesus erythrocytes for adenovirus 3.

Hemagglutination inhibiting antibodies may also be titrated with the help of the fragiligraph, by determining the antibody dilution which decreases the slope of the agglutination-sedimentation curve of a control virus to predetermined value.

Relationship between the form of the agglutination sedimentation curves and NDV concentration

Virus concentration HA units	Time at which curve starts to rise[a], min	Slope
27	13	1.0
55	4	2.0
110	3	4.8
220	1.8	8.8

[a] Time elapsed (in minutes) between mixing of virus and red cells and the point at which the curve starts to rise.

Résumé. Une méthode est décrite pour la mesure des hémagglutinines virales et des anticorps inhibiteurs des hémagglutinines au moyen d'un système photoélectrique et enregistreur automatique – le fragiligraphe. Une suspension des érythrocytes et du virus est introduite dans la microcuvette du fragiligraphe. L'agglutination et la sédimentation des érythrocytes causent l'augmentation de la transmission de la lumière à travers la suspension. Le temps jusqu'au début de l'inflexion de la courbe et son angle permettent d'évaluer la concentration du virus.

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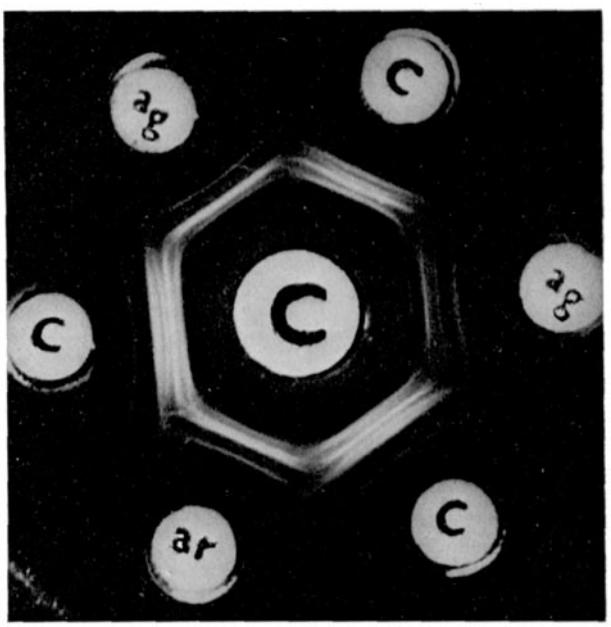
The Agar Gel-Diffusion Technique as a Method of Differentiating Mosquito Larvae

The gel-diffusion technique has been previously used by us for the antigenic analysis of mosquito eggs (ZAMAN and CHELLAPPAH[1]). This is a report of the antigenic analysis of mosquitoes using 4th instar larvae. The three Culicines reared in our laboratory, *Culex pipiens fatigans, Aedes aegypti* and *Armigeres subalbatus* have been investigated.

The antigens were a saline extract of 4th instar larvae. Approximately 100 larvae were macerated in 1 ml isotonic saline in a glass tissue grinder. During the maceration the grinder was kept immersed in an ice-bath. The suspension was then left overnight at 5° C with a magnetic stirrer. Next morning, it was cleared by centrifugation and standardized to contain approximately 3 mg of proteins/ml.

The rabbits were immunized with 4th instar *Culex* antigen injected subcutaneously along with Freund's adjuvant (Difco) at weekly intervals. Each rabbit received approximately a total of 2000–3000 larvae in 8–10 doses. The rabbits were held one week after the last injection and the serum collected. The gel-diffusion plates were made with Ion agar No. 2 (Oxoid). The concentration of agar was 1% in distilled water. To this 0.01% Na merthiolate was added as a bacteriostatic agent. The reaction was allowed to take place at room temperature in a moist chamber. The antigens and the sera were used undiluted. The lines were photographed after 7–10 days.

The Figure shows the reaction obtained with *Culex* antiserum when used against *Culex, Armigeres* and *Aedes* antigens. With the homologous system a single distinct line was seen near the antigen well. No corresponding line was observed with *Aedes* and *Armigeres* antigens. The line



C, Central well = *Culex* antiserum. C, Outside wells = *Culex* antigen. ag = *Aedes* antigen. ar = *Armigeres* antigen.

[1] V. ZAMAN and W. T. CHELLAPPAH, Exper. *20*, 429 (1964).