TECHNICAL METHODS

A SIMPLE AND PRECISE TECHNIQUE FOR THE TITRATION OF HAEMAGGLUTININS

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(RECEIVED FOR PUBLICATION, JULY 23, 1949)

The need for a standard technique for the titration of haemagglutinins has frequently been stressed. There is little doubt that among the causes of error in this test the difficulty of evaluating exactly the degree of agglutination and thus the endpoint of the reaction is the most important (Carlinfanti and Cavalli, 1947). A simple technique has therefore been designed (Carlinfanti and Molla, 1947), the essential feature of which, as in the Hirst method (1942) for virus titration, is the comparison of the sedimentation rates of agglutinated and unagglutinated red cells.

The test can be read objectively without special equipment by observing the column of red cell suspension at a fixed interval of time (four hours) after mixing it with the serum dilutions and comparing it with a standard tube prepared by diluting the same suspension. By means of a number of investigations on the influence of temperature, time, concentration of erythrocytes, etc., the procedure has been developed to give the maximum accuracy and simplicity of performance.

Technique

Small test-tubes of an internal diameter of 8 mm. and approximately 7 cm. long are used; in the rack they should be held vertically in order to prevent red cells from clustering unevenly at one side of the tube. An empty hole is left between each tube and the next, so that the standard can be placed beside any tube during the reading of the test. Serial twofold dilutions of the serum are prepared in 1 ml. saline and then 1 ml. of a 1% suspension of washed red cells* is added with a thin 1-ml. pipette in such a way that by expelling the liquid from the pipette into the tube the suspension is well mixed with the diluted serum.

The tubes are now set aside at ordinary room temperature (between 17° and 25° C.) for exactly four hours. In the meantime a standard tube is prepared by

* This suspension can be standardized by checking with haematocrit the density of a more concentrated suspension and correcting the error in carrying out further dilution to 1%. But this correction does not seem to be necessary, since errors of up to $\pm 50\%$ of red cell density do not affect appreciably the result of the test, provided, of course, the standard is prepared from the same suspension used for the test.

adding three volumes of saline to one volume of the suspension used for the test; 2 ml. of this 0.25% cell suspension are placed in a tube and carefully shaken just before use. The reading is performed by comparing its colour and opacity with those of the intermediate zone of the column of liquid contained in the various tubes of the titration. A paper sheet with a rectangular hole 1 cm. high and some 5–6 cm. long will be used for hiding the upper part of the liquid, which is clear, and the lower part of the tube where the sediment has settled (Fig. 1).

FIG. 1.—Diagram showing method of reading titres by the use of a standard tube.

The endpoint of the titration is that at which 50% of the cells involved remain suspended after four hours of settling. Accordingly the titre assigned to the serum corresponds to that dilution giving a cell density equal to the standard in the middle part of the tube. In case none of the tubes corresponds to the standard, the titre will be determined by interpolating one or two values between two twofold dilutions.

This technique has been in use in our own and other laboratories for over two years and has proved to be very satisfactory. Its precision is far greater than that of the usual technique in which the reading is performed by observing the presence and size of the red cell aggregates; it can be further enhanced, if necessary, by adopting a photoelectric colorimeter. The reciprocal of the titre obtained can be considered as the number of "haemagglutinating units" contained in 1 ml. of the serum on test. This method, however, does not obviate the need for standard sera to which this arbitrary unit can be referred. In the titration performed by this method A_2 red cells give markedly lower titres than A_1 cells. Furthermore, by measuring with appropriate standard suspensions the cell density in the tubes where the serum concentration is higher than the endpoint, a curve can be drawn, whereby the agglutinating properties of the serum can be more exactly featured (Grifoni, 1948).

REFERENCES

- Carlinfanti, E., and Cavalli, L. L. (1947). *Riv. Ist. Sieroter. Italiano*, **22** (N.3), 129.
 — and Molla, W. (1947). *Un metodo preciso e semplice per la titolazione delle emoagglutinine*.
 Congrès international de la transfusion sanguine, Turin.
 Grifoni, V. (1948). Personal communication.
 Hirst, G. K. (1942). *J. exp. Med.*, **76**, 195.