Haemagglutination kinetics using a continuous-flow system

PH ROUGER, P GANE, AND CH SALMON

From the National Blood Group Reference Laboratory, 53 Boulevard Diderot, 75571 Paris Citédex 12, France

SUMMARY An automated technique for measuring haemagglutination kinetics is described. Equipment used in this test is very simple and already present in most blood transfusion centres. This method may be used with advantage to differentiate homozygous and heterozygous subjects in Rhesus, MNSs, and Kell systems, and to study antigen variations, especially in genetic investigations.

Material and methods

The measurement of agglutination kinetics was undertaken using an AutoAnalyzer. The manifold is shown in Figure 1. It is derived partly from the manifolds used for red cell typing or antibody screening according to the method previously described by Rosenfield and Haber.6

REAGENTS

The cell samples, freshly collected in ACD or thawed, were washed in an isotonic solution and were then bromelin (or trypsin) treated as follows: one volume of bromelin solution (2.5 g/1000 ml) was added to one volume of packed red cells. After 10 minutes’ incubation at 37°C the tube was filled with saline and then centrifuged, and the red blood cells were washed three times in saline. A red cell suspension (4.5 x 10^12/l) was prepared.

All the antibodies (6 anti-D, 2 anti-E, 2 anti-e

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2 anti-C, 2 anti-\(\tilde{\varepsilon}\), 2 anti-M, 2 anti-N, 3 anti-S, 3 anti-s, 3 anti-K) used were selected by preliminary tests of haemagglutination kinetics. Most of the antisera were commercial reagents used routinely. The most important problem was the choice of the appropriate dilution.

**PROCEDURE**
At time 0, 50 \(\mu l\) of the diluted antiserum were mixed with the red cell suspension (2 ml). The mixture was immediately pumped into a reaction coil. The reaction was performed in 150 seconds. After a double decantation and lysis of the residual red cells, the haemoglobin concentration was determined in a colorimeter at continuous flow (SC Colorimeter-Techicon). The corresponding curve is shown in Figure 2. The same procedure was performed for each kinetic study. To determine the zygosity of one or more subjects, we studied in the same experiment four 'homozygous' controls, four 'heterozygous' controls, and one 'negative' control.

**Results**
All the curves were drawn on the same chart paper, and the corresponding 'homozygous zone' and 'heterozygous zone' were outlined (Figs 3 and 4). Thus, it may be clearly determined whether the curve obtained with unknown red cells corresponds to one of these two zones.

**Rhesus System**
A dose effect was demonstrated by this technique with anti-D, anti-C, anti-\(\tilde{\varepsilon}\), anti-E, and anti-e. However, all the antibodies used did not give a dose effect. For 30 different samples the zygosity \(D/\tilde{D}\) or \(D/\tilde{d}\) was estimated, according to the most
probable genotype obtained using anti-C, anti-c, anti-E, anti-e, and antibodies against compound antigens, or by family studies. No discrepancy was noted.

In a comparative study of some samples with the method described by Lopez, no discrepancy was found. In both techniques some Rr samples can give a curve almost identical with R_sR_s.

**MNSS system**

With this procedure, only some commercial anti-N, anti-M, anti-S, and anti-s give a dose effect.

**KELL system**

The interpretation was very simple. With this method one can determine the heterozygous individuals (k/- or K/-) of a Ko family (Fig. 5).

**Fig. 5** Agglutination kinetics at continuous flow with anti-k.

**Discussion**

This method may be used to follow the first steps of an immunological agglutination reaction. The continuous recording makes it possible to detect fleeting phenomena which could not be observed using repeated punctual titrations.

It is very suitable for studying the zygosity of a red cell character: it is thus possible to determine if a D+ subject is genetically D/^D or D/^d (or D/^d). The method is very practical and fast in the study of a father's rhésus genotype in cases of haemolytic disease of the newborn. It is also more sensitive and remarkably more reproducible than the classical method of agglutination scoring. In our type of study, four controls of the same and genetically known phenotype must be used.

Other important applications of this technique could be the study of various parameters involved in the first step of the agglutination mechanism, mainly pH, ionic strength, temperature, macromolecules, enzymes, and antibody affinity.

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Requests for reprints to: Dr Ph Rouger, National Blood Group Reference Laboratory, 53 Boulevard Diderot, 75571 Paris Cedex 12, France.
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