Determination of Rubella Hemagglutination-Inhibiting Antibody in Whole Blood

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A technique is described for determination of rubella hemagglutination-inhibiting antibody in only 50 to 100 μlitters of whole blood.

The rubella hemagglutination-inhibition (HI) test (6) is widely used for determination of seroimmunity and for the diagnosis of recent rubella infection. By and large, however, the test has been used on serum samples only. Efforts to extend this test to whole blood by absorbing the blood on filter paper discs, eluting with buffers, and performing the HI test on the eluates (1, 3) are hampered by hemolysis in the eluates and by the difficulty of ascertaining the initial dilution of the disc-eluates with respect to whole blood. A technique used by Plotkin and co-workers (5) to express diluted plasma from whole blood with heparin-manganous chloride has been extended by us. In the present work, heparin-manganous chloride and formalized sheep red blood cells (SRBC) are used in a single step for removal of nonspecific inhibitors and hemagglutinins, respectively. The perfectly clear supernatant fluids obtained after such processing of whole blood are used for antibody titration. Doubling the HI titer of these supernatant fluids to allow approximately for hematocrit is shown to give excellent correlation with the titers of serum antibody.

A 0.1-ml amount of either venous blood collected in heparin or blood collected by fingerprick in a capillary pipette was added to 0.2 ml of heparin-manganous chloride mixture. This reagent, used for the removal of nonspecific inhibitors of agglutination, was prepared by mixing 0.4 ml of heparin (5,000 units/ml), 0.8 ml of 0.8 M manganous chloride, and 4.8 ml of complete HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer, pH 6.2 (4). The mixture was held in the cold with occasional shaking. After 30 min, 0.2 ml of a 12.5% suspension in HEPES buffer of formalized SRBC, prepared as described by Gupta and Harley (3), was added to absorb hemagglutinins. The mixture was again held in the cold for at least 1 hr with intermittent shaking and was then centrifuged. The supernatant fluid was carefully collected and then heated for 30 min at 56 C. As a slight white precipitate sometimes formed in the supernatant fluid after heating, it was necessary to centrifuge again for proper agglutination and settling patterns in the antibody titration. The supernatant fluid from blood now at a 1:5 dilution was used for antibody titration as described by Gupta and Peterson (4), with 4 units of rubella hemagglutination antigen (Flow Laboratories, Inc., Rockville, Md.) and 0.20% formalinized SRBC in HEPES at pH 6.2. The test plates were read after being kept overnight in the cold. Antibody titer was expressed as the reciprocal of that dilution which completely inhibited the hemagglutination by the antigen.

To assess the reliability of the method, whole blood and the corresponding serum from 58 subjects were tested in parallel. Capillary blood samples from 16 subjects were also compared with the corresponding sera. The serum samples were treated and tested in exactly the same way as the whole blood samples, with the exception that they were diluted 1:1 with HEPES before heating at 56 C so that they were initially diluted 1:10 before antibody titration. The antibody titer of the serum was plotted against twice the antibody titer of the whole blood sample. Figure 1 shows that 41 of the 58 blood samples had identical titers, 6 had twofold higher titers, and 11 had twofold lower titers than the serum. This result is equivalent to 100% correlation, as the experimental error in the rubella HI test is taken to be a twofold dilution. On the other hand, direct comparison of the whole blood titers with the serum titers yielded only 6 samples with identical titers, whereas 29 serum samples had twofold (excluding 12 negatives) and 11 had fourfold higher titers than the whole blood. It appears, therefore, that doubling the HI titers obtained with whole blood gives better correlation with serum titers, as would be ex-
samples of sera. The middle of the three diagonal lines is the line of identical titers, representing samples whose titers are the same under the conditions described above. The other two lines represent samples with titers one dilution higher or lower.

FIG. 1. Comparison of double the HI titers of 58 samples of whole blood with the titers of corresponding sera. The middle of the three diagonal lines is the line of identical titers, representing samples whose titers are the same under the conditions described above. The other two lines represent samples with titers one dilution higher or lower.

pected from the approximately 1:1 ratio of cells to fluid in whole blood.

The doubled titers of 16 capillary blood samples when compared with corresponding serum titers were also found to be equivalent (i.e., within a twofold dilution). With 0.1 ml of blood, about 0.3 ml of supernatant fluid was obtained after processing, allowing antibody determination in triplicate with adequate controls. Starting with 50 μl of whole blood, sufficient supernatant fluid can be obtained at a 1:5 dilution for the titration of antibody.

When fresh pigeon erythrocytes were substituted for formalinized SRBC, it was found necessary to use HEPES buffer at pH 7.0 to 7.2 as pigeon cells were agglutinated at the lower pH. At this higher pH, however, sensitivity of the erythrocytes to agglutination by rubella hemagglutination antigen was decreased.

Lastly, it was found that no loss of antibody occurred during storage when processed whole blood supernatant fluids were kept frozen for more than 1 month. Stored supernatant fluids may therefore be compared with a second sample to demonstrate a possible rise in titer in either the pregnant rubella contact or the patient with a rubelliform illness.

This modified test may thus be used in any situation in which the HI test is required. In view of the simplicity and applicability to small volumes of whole blood, it is particularly appropriate for mass screening programmes and for use in infants and small children.

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LITERATURE CITED