Evaluation of a Rubella Hemagglutination Inhibition Test System

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Two systems for measurement of rubella hemagglutination inhibition antibodies were compared. One had chick erythrocytes as indicator and the other, which is available in kit form ("Rubindex," Ortho-Diagnostics), uses human group O erythrocytes. Correlation to within one dilution of each other was noted in 92% of the tests. The Rubindex system has the advantage of longer shelf life of reagents and also does not require one of the absorption steps necessary for the other method, thus saving technologist's time.

The importance of determining immunity to rubella virus and the diagnosis of active infection have gained considerable importance since recognition of the teratogenicity of this infection (2). The demand for rubella hemagglutination inhibition antibody testing has increased greatly in recent years and now constitutes a major portion of the work load of the diagnostic serology section of our hospital. The test is one that demands rapidity of performance, together with reproducibility and reliability of results, and with this in mind it was decided to compare the existing method of serological testing in our hospital with a new hemagglutination inhibition test system, "Rubindex" (Ortho-Diagnostics).

MATERIALS AND METHODS

Preexisting system. For convenience, the preexisting system will be referred to throughout this report as the "chick method."

The rubella hemagglutinin was obtained from Connaught Laboratories Limited and was prepared from an HPV-77 strain of rubella virus supplied in freeze-dried form. Each sample of hemagglutinin was reconstituted with 1.0 ml of distilled water, was used immediately, and was not stored beyond the day of the test.

Erythrocytes from 1- to 3-day-old unfed chicks were obtained from Connaught Laboratories. These were supplied in Alsever solution and stored at 4 C up to a maximum of 2 weeks. These cells were used as the indicator system. Before use, all erythrocytes were washed three times in dextrose-gelatin-veronal buffer and a final dilution of 0.25% based on packed-cell volume was made up in *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer at pH 6.2.

All antigen and serum dilutions were made in HEPES buffer. Serum samples for testing were prepared as follows: 0.05 ml of a solution containing 5,000 U/ml of sodium heparin and 0.05 ml of 1 M manganous chloride were added to 0.15 ml of the

serum sample. The mixture was gently agitated and centrifuged at 2,000 rpm ($900 \times g$) for 15 min at 4 C in a refrigerated centrifuge. Then, 0.1 ml of a 50% suspension of mature chick erythrocytes was added. The tubes were gently agitated to disperse the erythrocytes and incubated for 1 h at 4 C. Thereafter, 0.4 ml of HEPES buffer was added, and the tubes were gently mixed and centrifuged at 2,000 rpm for 20 min. The supernatant was removed from the tubes, taking care not to disturb the precipitate and pellet of erythrocytes. The resulting fluid was then a 1:8 dilution of treated serum sample.

Rubindex. Four kits of 100 tests each, manufactured over a period of 3 months, were used in the study.

The hemagglutinin was supplied in liquid form and was diluted before use to 1:16 in Rubindex diluent. The concentrations of sodium heparin and manganous chloride in the kit solutions were the same as in the method described above.

The indicator erythrocytes in the Rubindex system were human group O Rh-negative erythrocytes selected by the manufacturer from a panel of donors and pretreated with trypsin and dispensed as a 4% solution. These cells were diluted 1:16 in Rubindex diluent. The patients' sera were treated with sodium heparin and manganous chloride solutions as in the chick method; test sera did not require absorption with erythrocytes. After treatment with manganous chloride and sodium heparin, sera were centrifuged at $900 \times g$ for 15 min, after which the supernatants were removed, taking care not to disturb the precipitate.

Test procedures. Hemagglutinin was titrated, and sera were diluted as described in the procedural guide issued by the U.S. Department of Health (1). V-bottom microtitration plates (Cooke Engineering Inc.) were used in both test systems. The only difference between the two procedures was that the Rubindex tests, after addition of the indicator cells, were incubated at room temperature for 1 h, whereas our conventional method required incubation at 4 C for 1 h followed by 15 min at room temperature before results could be read.

Controls. Serum, cell, and antigen controls,

negative serum controls, and low- (1:16) and high-titer-positive controls (1:128) were incorporated in the test.

End points. In both test systems, complete inhibition of agglutination was taken as the end point of the titration.

Test sera for evaluation. The 275 sera that were tested were from two main sources: 125 routine test sera entering the diagnostic serology laboratory were tested as they arrived by both test systems. In addition, 150 sera previously tested for rubella antibody and stored at -70°C were retrieved, thawed, and retested by both systems. All sera were less than 6 months old.

The person reading the results of one test system was not aware of the titers obtained by the person using the other test method until completion of the study, when comparison of data, including previously obtained titers, was made.

Discrepant results. Since we anticipated that some tests might give discrepant results in determining immunity, it was decided to repeat such tests and to refer persistently discrepant sera to another laboratory for arbitration by fluorescent antibody testing.

RESULTS

Of the sera tested, 18 had to be retested because of poor serum controls or erratic patterns of agglutination. Of these, five were excluded from further study because of persistent agglutination in the serum controls with the chick erythrocytes. Of the remaining 13 specimens, there was no change in titer with the Rubindex system. On the other hand, six of those tested by the chick method showed no change in titer, six changed by one dilution, and one changed by two dilutions (Table 1).

In 142 (54.2%) of 262 sera (Table 2), the titers obtained with the two test methods were identi-

cal. The titers observed with the Rubindex system exceeded those noted in the chick method by one dilution in 86 (32.8%) test sera, by two dilutions in 18 (6.9%), and by three dilutions in three (1.1%). There were 13 sera (5%) in which the Rubindex titer was less by one dilution than that seen in the chick erythrocytes method; in no case was there a greater difference. Thus, the two systems gave results within one dilution of each other in 92% of cases.

In distinguishing immune from nonimmune patients based on a titer of less than 1:16, there were two sera in which the Rubindex method indicated immunity, and the chick method disagreed. On repeat testing, however, the chick method titer changed to match that of Rubindex. The titers obtained with both methods are shown in Fig. 1.

DISCUSSION

The titers obtained with the two methods described were fairly similar. However, there appear to be distinct advantages to the use of Rubindex. Some of the advantages of human erythrocytes over chick erythrocytes, as described by others (3), were confirmed, notably their stability for 4 weeks compared with a maximum of 2 weeks for chick erythrocytes. A second advantage was the elimination from Rubindex of the absorption of test sera with mature chick erythrocytes. Pretitration of antigen and its stability over 4 weeks resulted in time saving in the laboratory.

The test systems behaved in an almost identical fashion in determining immunity of patients based on hemagglutination inhibition titers of 1:16 or greater. The costs of the two systems were approximately the same.

The use of Rh-negative erythrocytes eliminated the possibility of nonspecific hemagglutination. Cold agglutinins could also give rise to nonspecific hemagglutination, and some workers (3) have suggested that this could be avoided by conducting the final phase of incubation at 37°C . However, we chose to rely on the likelihood of cold agglutinins absorbing onto erythrocytes as the blood was allowed to clot at 4°C before removing the serum, together with incubation of the test at room temperature. Perhaps future studies of the use of the Rubindex system may involve incubation at 37°C in the final phase as an added safeguard.

Further studies will be conducted to test the stability and dependability of the Rubindex kit over a more prolonged period of time.

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TABLE 1. *Changes in titer observed when tests were repeated*

Change	Rubindex	Chick
None	13	6
By one dilution	0	6
By two dilutions	0	1

TABLE 2. *Comparison of titers obtained with the Rubindex and chick methods*

Titer differences	Rubindex less than chick	Rubindex equal to chick	Rubindex more than chick
By one dilution	13	142	86
By two dilutions	0		18
By more than two dilutions	0		3

FIG. 1. Hemagglutination-inhibition titers obtained with the Rubindex and chick methods.

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