Specificity of the Passive Hemagglutination Test for Antibody to Rubella Virus and the Passive Hemagglutination Response After Vaccination

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Nonspecific reactions by the passive hemagglutination test for rubella viral antibody occurred with 1.5% of sera tested. Rises in passive hemagglutination titers (≥4×) occurred with some serum samples taken 14 days after vaccination.

A passive hemagglutination test (PHA) for determination of immune status of individuals to rubella virus infection is produced by Abbott Laboratories. This report concerns two points regarding the test that have not been addressed thus far in the literature as far as we are aware: (i) the absence of control erythrocytes (RBC) in the test kit for use to confirm positive reactions and (ii) kinetics of the PHA antibody response after vaccination. The lack of any kind of control for determining the specificity of positive reactions on individual serum samples was of concern to us. Abbott Laboratories therefore provided us with control RBC (nonsensitized Dur-acytes cells) and have now also made them commercially available. With regard to the second point, it is reported that antibody reactive with the antigen used in the kit arises too late in natural infection for use in serodiagnosis of current infection (2; Abbott Laboratories, Rubacell diagnostic test package insert). It was important, therefore, to know whether the PHA antibody response was also delayed after vaccination.

A total of 676 sera submitted to our diagnostic serology laboratory for determination of immune status of individuals to rubella virus infection were screened at a dilution of 1:13.5 with PHA kits as outlined in the package insert. All tests were done in duplicate with antigen-sensitized and nonsensitized control RBC. Results were compared with hemagglutination inhibition (HI) results obtained by a standardized method with heparin manganous chloride for removal of inhibitors (1). Results are shown in Table 1. There was excellent agreement (97.5%) in results between the two tests.

Of the 676 sera, 10 showed agglutination with the control cells. Thus, if the PHA test were used on a routine basis, only a small percentage (1.5%) of the sera would have to be tested by an alternate procedure. Although the number of potentially false reactions with the test was small, we feel the use of control cells is warranted as a check on the specificity of the reaction. Another advantage in using control cells is the increased ability of the operator to discriminate between weakly positive and negative reactions when tests and controls are placed in adjacent wells.

Sera from 45 children, 12 to 18 years of age (mean 14), vaccinated with a combination of measles and rubella virus vaccines, were titrated by HI and PHA by the doubling dilution method, starting at a dilution of 1:8. Thirty of the children were bled 4 weeks postvaccination, and 15 children were bled 2 weeks postvaccination. Of the 45 serum pairs, 6 could not be evaluated for a significant rise in antibody titer (≥4-fold) by PHA because of the combination of agglutination with the control RBC at the 1:8 serum dilution and low antibody titers (1:16 to 1:32) with the antigen-sensitized RBC. Overall, 16 of the 90 sera (45 serum pairs) showed agglutination at a 1:8 dilution with the control RBC. The higher percentage of nonspecific reactions (17.7%) with these sera as compared with that in the immunity status study may reflect the difference in starting dilutions used in the two studies, i.e., 1:8 versus 1:13.5. All serum samples, except one, were negative with control RBC at a 1:16 serum dilution.

By HI, 43 of the 45 (95.5%) serum pairs showed a fourfold or higher rise in antibody titer between the pre- and postvaccination samples. Of the 39 serum pairs that could be evaluated by PHA, 28 showed a similar rise in titer in the postvaccination sample. Rises in PHA titers occurred in both 14- and 28-day serum samples. The results by PHA were unexpected because of the lack of such a response during natural infection until approximately 48 days after onset of rash (2). The probability that PHA antibody arises earlier if the response is anamnestic was therefore considered.
Review of the clinical history of each child showed that 26 of the children had a previous exposure to rubella virus, either through natural infection or by vaccination. Nineteen children had either no known exposure or no information was available (Table 2). The data on clinical history came from hospital records or parents' memory, and the reported "no known exposure" in some cases was probably not accurate. This view was substantiated in some cases by the demonstration of antibody in the prevaccination serum sample of some children with no known previous exposure to rubella virus. Although there was a greater tendency for a rise in antibody titer to be present in the postvaccination serum if antibody was already present in the prevaccination sample (21/23), rises in antibody titer were also noted when antibody was not detected (at a dilution of 1:8) in the prevaccination specimen (7/16).

Because of the late appearance of PHA antibody in natural infection, it has been suggested that serodiagnosis may be made from PHA and HI results on a single serum sample collected during the first 2 or 3 weeks of infection. Thus, if antibody were present by HI and absent by PHA, it would be indicative of a current infection.

Our data indicate, however, that in an anamnestic reaction resulting from vaccination, antibody titers by both methods may be elevated by 2 weeks after exposure to rubella virus. The possibility thus exists that in the event of natural reinfection, the use of PHA in combination with the HI test on a single serum sample may lead to an erroneous diagnosis.

We thank Abbott Laboratories for the reagents to evaluate their test.

**LITERATURE CITED**
