

# Comparison of passive haemagglutination and haemagglutination-inhibition techniques for detection of antibodies to rubella virus

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**SUMMARY** Because of the technical problems and length of time involved in the satisfactory performance of the haemagglutination-inhibition test for detection of rubella-specific antibodies, a commercially available passive haemagglutination (PHA) kit utilising rubella antigen-sensitised human erythrocytes was tested for its suitability for use in diagnostic laboratories. The immune response to acute rubella infections as measured by PHA was considerably delayed compared to the response measured by haemagglutination-inhibition. Titres of rubella-specific antibody only became comparable six months after the infection. The commercially available PHA kit is a useful addition to diagnostic laboratories for the determination of immune status and, in conjunction with the haemagglutination-inhibition test, can be an indicator of recent rubella infection.

The haemagglutination inhibition test (HI) is the most commonly used technique for the detection of antibodies to rubella virus. However, its satisfactory performance requires many manipulations of the test serum so that a reliable and more simple method for determining rubella antibody status would be a welcome addition to any laboratory now routinely performing this test. Recently, a passive haemagglutination test (PHA) for the detection of rubella antibodies has been developed (Rubacell, Abbott Laboratories, North Chicago, Illinois).

In order to evaluate the usefulness of this PHA test for diagnostic laboratories, the antibody response in serum specimens collected from young children with recent rubella infections was compared by HI and PHA. In addition, sera routinely obtained from patients attending an antenatal clinic in Melbourne were tested by both methods. Finally, the nature of the PHA antibody response was briefly studied.

## Material and methods

### PATIENTS AND SPECIMENS

Serial blood specimens were obtained from 11 children aged 5 to 12 years involved in an outbreak

of rubella in a home for the mentally retarded. In each case the clinical diagnosis was confirmed by virus isolation. Blood samples were obtained on the day of the rash and 10, 57, and 178 days later.

In addition, 123 serum specimens were obtained from women attending an antenatal clinic in Melbourne. Of these specimens, 58 had been stored at 4°C for at least six months.

### HI TEST

The HI technique used in this study has been described in detail elsewhere (Gust, 1973). Briefly, the test involved the treatment of sera with either kaolin or heparin-manganous chloride to remove non-specific inhibitors, followed by adsorption with 50% pigeon erythrocytes. Doubling dilutions of the treated sera were incubated with 6 to 8 haemagglutinating doses of rubella antigen and 0.2% pigeon erythrocytes.

### PHA TEST

PHA antibodies were detected using a commercially available test kit (Rubacell, Abbott Laboratories, Chicago, Illinois). Stabilised human erythrocytes, sensitised with rubella-virus soluble antigen, were reacted with untreated human sera for determination of antibody levels. The exact procedure followed was according to the manufacturer's instructions except

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that the test sera were initially diluted 1:8 rather than 1:13.5, thereby allowing direct comparison of HI and PHA titres. Doubling dilutions of the sera were carried out using the buffer provided so that they were diluted between 1:8 and 1:4096 in 25  $\mu$ l volumes. 25  $\mu$ l of uniformly suspended rubella-sensitised human group O cells (Duracyte Cells) was then added to each well, and the plate was sealed and gently agitated to ensure thorough mixing of the reagents. The agglutination pattern could be read after 2 hours' incubation at room temperature or, if left undisturbed, after 24 hours' incubation.

#### COOMBS' TEST

Test sera were reacted with the rubella virus-sensitised human erythrocytes supplied with the PHA kit and incubated for 2 hours at room temperature. The cells were washed five times in normal saline and 70  $\mu$ l of Coombs' reagent (Composite Coombs, CSL, Melbourne) was added. After incubation for 5 minutes at room temperature and centrifugation at 1000 rpm for 60 seconds, the presence or absence of agglutination was observed.

#### DETECTION OF RUBELLA SPECIFIC IgM

Serum specimens were diluted 1:4 in phosphate buffered saline (PBS) and layered onto 10-40% (w/v) sucrose gradients in 5.0 ml polyallomer tubes. Sera to be tested by HI were first adsorbed with 50% pigeon erythrocytes to remove nonspecific agglutinins. Specimens were spun at 35 000 rpm for 18 hours using an SW 50.1 rotor in a Beckman L5-65 ultracentrifuge. Eight equal-volume fractions were collected from the bottom of the tubes and each was tested for the presence of antibodies by HI and/or PHA. Previous studies in this laboratory have shown that the majority of antibody in fractions 2 and 3 is IgM, and antibody detected in fractions 5 and 6 is invariably IgG (C. Birch—unpublished results).

#### 2-MERCAPTOETHANOL TREATMENT OF SERA

Sera were pretreated with 25% kaolin and 50% pigeon erythrocytes (Gust, 1973). Nine volumes of treated serum were incubated with one volume of 0.5 M 2-mercaptoethanol for one hour at 37°C. As a control, PBS replaced the 0.5 M 2-mercaptoethanol.

#### Results

##### CONVENIENCE OF HI COMPARED WITH PHA

In the HI test, the removal of nonspecific inhibitors and agglutinins from test sera is a procedure requiring several manipulations and at least one fairly lengthy incubation period, so that in most laboratories the test is performed over two days. In contrast, the use of rubella-sensitised human

erythrocytes in the PHA test alleviates treatment of serum and, depending on the number of sera to be tested, can be completed in less than 3 hours. In our hands, the HI test was considerably easier to read than the PHA, although, with practice, PHA end-points could be read with confidence.

#### HI ANTIBODY RESPONSE

In each of the 11 patients with acute rubella, HI antibodies rose rapidly after the onset of the rash and reached maximum titre by day 10; 178 days post-infection these levels had decreased to approximately half the maximum level. At day 10 high levels of rubella-specific IgM were detectable in all sera, but at 57 days post-infection IgM antibody remained detectable in very low levels in only two of the children. The typical HI response of three children is shown in Figure 1.

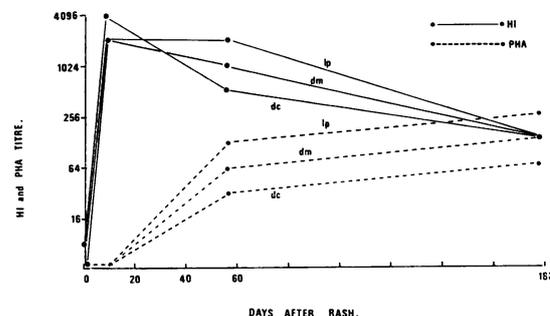


Fig. 1 HI and PHA antibody response of three children (lp, dm, and dc).

#### PHA ANTIBODY RESPONSE

The PHA antibody response was delayed compared to the HI response. Ten days after the appearance of the rash no antibodies were detectable by PHA. Antibody first detectable by PHA appeared between day 10 and day 57 and continued to rise slowly up to day 178, at which time the titres were comparable to those obtained by HI. The PHA responses of the same three children are also shown in Fig. 1, although it should be noted that the slope of the PHA curves between day 10 and day 57 may not necessarily reflect the exact nature of the antibody response.

#### COOMBS' TEST

Four sera, obtained 10 days after the appearance of the rash and having high titres (1:1024 to 1:2048) by HI but negative (< 1:8) by PHA, were incubated with the sensitised erythrocytes provided in the Rubacell kit and antihuman immunoglobulin. No agglutination occurred in any of the four specimens tested.

#### CLASS OF IMMUNOGLOBULIN DETECTED BY PHA AND HI

Two of the 11 serum specimens collected at day 57 had low levels of rubella-specific IgM detectable by HI, but neither had specific IgM detectable by PHA. Both methods detected antibody in the IgG-containing fractions of all 11 sera.

#### SENSITIVITY OF PHA COMPARED WITH HI

Sixty-five fresh sera were obtained from women having no clinical history of recent rubella infection or known recent exposure to rubella virus. Comparison of HI and PHA titres showed the two methods to be in close agreement for immune status determination (Fig. 2). Of the 64 sera tested, 51 (80%) had titres within one twofold dilution of each other. All five sera negative by HI were also negative by PHA. HI and PHA antibody titres in 58 sera stored at 4°C for at least six months also showed close correlation. Of 58 sera tested, 48 (82.7%) were within one twofold dilution of each other, and, again, sera negative by HI were also negative by PHA. Of the 123 sera tested, 21 (18.6%) were not within one twofold dilution of each other (Fig. 2). However, in all such sera rubella-specific antibody was detectable by both methods.

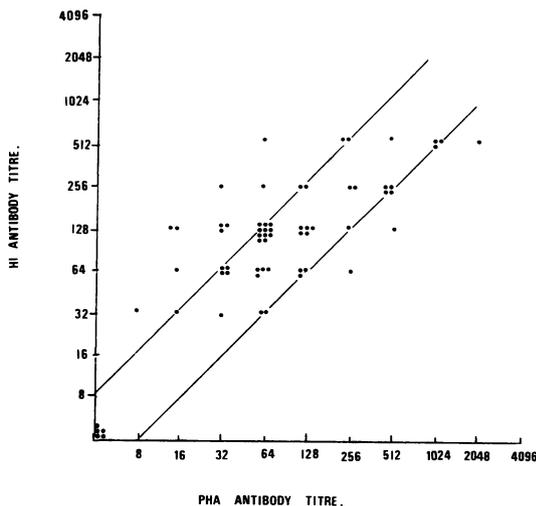


Fig. 2 Comparison of antibody measured by HI and PHA in 64 patients with past infection with rubella.

#### Discussion

Comparison of the HI and PHA tests for detection of rubella-specific antibody revealed similar sensitivity when specimens were obtained from patients having past infections with rubella virus. In addition, sera that had been stored at 4°C for at least six months

had similar titres by both methods. However, levels of rubella-specific antibody detectable in patients with acute or recent infections differed markedly, depending on the method used. The HI response was typical of the normal population (Forbes *et al.*, 1969), although these patients were mentally retarded children with some unusual clinical features, namely, the degree of systemic disturbance and the frequency of purpuric complications. HI antibodies reached maximum levels approximately 10 days after the onset of symptoms, while PHA antibodies were not detected for at least 14 days after the infection and then rose very slowly. A significant rise in PHA antibody titre could be demonstrated between paired sera when the first specimen had been collected as late as four to eight weeks post-infection. Similar results have been reported by Meurman (1978), who also compared HI and PHA with complement fixation and solid-phase radioimmunoassay titres.

The manufacturer of the PHA test recommends that an HI test be used for estimations of antibody titre in specimens obtained from individuals with clinical symptoms of rubella or suspected exposure to rubella virus. However, diagnostic laboratories testing large numbers of specimens are often faced with the difficulty of having insufficient clinical data on which to base an interpretation of a particular rubella titre. In Melbourne, approximately 15-20% of women of child-bearing age do not have rubella-specific antibodies (Lehmann *et al.*, 1969) and are hence considered unprotected. In the absence of reliable clinical data, it is not possible to distinguish between this group and patients who have had a recent infection; in both cases there will be no PHA antibody detectable. In view of the teratogenic potential of rubella virus (Dudgeon, 1967; Forrest and Menser, 1975) the distinction is very important, and all sera negative by PHA would also have to be tested by HI to separate the two groups irrespective of available clinical information. Owing to the urgency of rapid diagnosis of rubella infection in pregnant women, demonstration of seroconversion by PHA is impractical. Because the PHA test detects a population of antibodies which appear later than HI antibodies, the test is of special value in the diagnosis of rubella infection when the patient is first seen several weeks after the onset of symptoms, at a time when HI antibody has reached peak titres and rubella-specific IgM is no longer detectable; a serum negative by PHA but positive by HI is presumptive evidence of a recent infection.

Although realising that the antigens used in the HI and PHA tests were probably not the same, it was thought that the delay in the development of PHA antibody may have been due to the presence of rubella-specific IgM in the serum which, in an

unknown fashion, inhibited the PHA reaction. To test this hypothesis, sera negative by PHA but positive by HI and known to contain rubella-specific IgG and IgM were treated with 2-mercaptoethanol or centrifuged through sucrose gradients to separate the IgG and IgM components. Antibody remained undetectable by PHA in the 2-mercaptoethanol-treated sera and in the IgG and IgM fractions of the serum, whereas antibodies were still detectable by HI in both cases, although at reduced levels in the 2-mercaptoethanol-treated sera. These results suggested that IgM was not involved in a nonspecific blocking reaction, and this was confirmed when two sera, obtained 57 days after the first appearance of the rash, were found to be positive for rubella-specific IgM and positive for total antibody by PHA. When the IgG and IgM fractions from the same two sera were treated with sensitised erythrocytes, only those fractions containing IgG agglutinated, suggesting that the PHA test is IgG specific.

The lack of reaction between sera negative by PHA but positive for IgG and IgM by HI with the rubella-sensitised erythrocytes and anti-human globulin demonstrated the lack of avidity between the antigen on the cells for antibody produced shortly after the appearance of the rash.

In view of the ease of performance of the PHA test and its comparable sensitivity to HI, PHA is a useful addition to the diagnostic laboratory for immune status determinations. The PHA test avoids the necessity for removal of nonspecific inhibitors and nonspecific agglutinins of pigeon erythrocytes from test sera, and does not require several incubation and centrifugation procedures necessary for the performance of the HI test. However, laboratories using the PHA kit routinely also need to be

able to confirm negative PHA results using the HI test or, alternatively, require the facilities provided by a reference laboratory when the diagnosis of a recent rubella infection is in doubt.

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