Comparison of Measles Antihemolysin Test, Enzyme-Linked Immunosorbent Assay, and Hemagglutination Inhibition Test for Determination of Immune Status

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Sera were collected from 238 high-school students in Prince Edward Island for the determination of immune status before an anticipated measles outbreak. In addition, history of vaccination status and measles infection was obtained. In the subsequent outbreak, 28 students did contract measles. Specificity for hemagglutination inhibition (HI), antihemolysin (AH), and enzyme-linked immunoassay (ELISA) was 100%, compared with the neutralization test. Corresponding sensitivity values for the tests were 66.0% (HI), 99.5% (AH), and 99.0% (ELISA). Predictive values for susceptibility were 26.9% (HI), 77.8% (AH), 75.7% (ELISA), 80% (neutralization), and 41.4% as determined by history of infection or vaccination. The predictive value for immunity as determined by history of previous infection or vaccination was 91.8%, compared with 100% for the four serological tests. No false-positive results were seen with any of these tests. Compared with the neutralization test, the HI test had 69 false-negative results, the AH had 1, and the ELISA test had 2. The AH and ELISA tests provided sensitive and specific alternatives to the commonly used HI test for immune status determination.

Although the reported occurrence of measles in 1983 in Canada and the United States reached the lowest levels ever recorded, the disease continues to be an important cause of morbidity and mortality in underdeveloped countries (2). Kaartinen (6), for example, reported an epidemic in Ethiopia with a mortality rate estimated at 20%, in which 4,000 people were thought to have died. Measles has been targeted for global eradication (5), and to achieve this goal, highly sensitive and specific tests may be required to assess the susceptible population and to monitor vaccine efficacy (8, 13).

The hemagglutination inhibition (HI) test is commonly used to measure measles antibody for the determination of immune status. However, this test has been shown to be less sensitive than the plaque neutralization (NT) test (1) and the enzyme-linked immunosorbent assay (ELISA) test (14). Alternatively, Norrby and Gollmar (10) have described a test which demonstrated a good correlation between titers of neutralizing and hemolysis-inhibiting antibodies.

In this study the results of a new, simple antihemolysin (AH) test, a commercial ELISA test, and an HI test are compared with those of the NT test for measles. Serological test results on sera drawn from high-school students shortly before an outbreak of measles in the institution allowed assessment of the predictive values of the methods used for the determination of immune status to measles.

MATERIALS AND METHODS

Sera from 238 students in a Prince Edward Island high school were collected in 1980 1 month before an anticipated measles outbreak. In the outbreak which ensued, 28 cases of measles were clinically identified. Before the outbreak, sera were collected along with histories of measles or measles vaccination for the 238 students. NT, HI, ELISA, and AH tests subsequently were performed on all sera.

NT test. The virus NT test was conducted with a constant serum dilution of 1:4, with an equal volume (0.025 ml) of four serial 10-fold dilutions of measles virus (Edmonston strain) in 96-well microplates. After an incubation of 2 h at room temperature, a 0.1-ml suspension of Vero cells (in medium 199 with 8% fetal calf serum) was added to the plates. The endpoint of the virus titration was the highest dilution showing syncytial giant cell formation. A parallel virus titration in the presence of a 1:4 dilution of each of the test sera was performed. A 100-fold reduction in virus titer after incubation with serum was considered indicative of the presence of neutralizing antibody.

HI test. The HI test was performed by a modification of the method of Gershon and Krugman (4). Briefly, 4 hemagglutinating units of antigen in a volume of 0.025 ml was added to 0.025 ml of serial twofold dilutions of heat-inactivated and African green monkey erythrocyte-absorbed sera in U-bottom 96-well microplates. Dilutions of virus and sera were made in phosphate-buffered saline (PBS; pH 7.2) containing 0.4% bovine albumin. Each well received 0.025 ml of a 0.5% suspension of African green monkey erythrocytes. Plates were shaken and incubated for 1 h at 36°C. Complete inhibition of agglutination at a $\geq 1:4$ dilution of serum was considered indicative of immunity.

ELISA test. Measles antibody was detected by using Measelisa kits (M. A. Bioproducts, Walkerville, Md.). The tests were performed according to the instructions of the manufacturer, except that the protocol occasionally required modification by increasing the incubation time (from 45 min to 1 h) so that the absorbance of the high positive control serum would fall into the specified optical density range. The low positive control serum optical density sometimes exceeded that specified; when this occurred, absorbance values of the test sera were increased by the amount by which control serum values exceeded the specified value. Equivalent results were repeated. Control plates containing uninfected antigen were not supplied.
The hemolytic activity of measles antigens was assayed by a modification of the technique of Carrigan and Johnson (3). A 1-ml volume of measles hemagglutinin antigen was mixed with 0.20 ml of 50% (vol/vol) African green monkey erythrocytes and incubated at 37°C for 30 min. The absorbance (540 nm) of the supernatant fluid (900 g for 10 min) was read at 0 and 30 min of incubation. The optical density change reflected the hemolysis activity. The minimum hemolysin concentration used was that producing an optical density change of 0.40. For sensitization, 2 ml of antigen diluted 1:2 in PBS was mixed with 0.2 ml of 50% African green monkey erythrocyte suspension and incubated at 37°C for 30 min. The sensitized cells were centrifuged (900 g for 10 min) and suspended in 1.0 ml of PBS with 1.0 ml of goat anti-human immunoglobulin G (IgG; Miles Laboratories, Inc., Rexdale, Ontario, Canada). Agarose (1%) in PBS (agarose II; Sigma Chemical Co., St. Louis, Mo.) was autoclaved for 10 min at 115°C. The sensitized cells, anti-human IgG, and 13 ml of molten agarose were combined at 43°C and poured into petri plates (9 by 9 cm). Wells (diameter, 3 mm; 36 per plate) were punched into the hardened agarose, and 5 μl of sera was added to each well. Control plates were processed similarly, substituting PBS for antigen. Plates were incubated in a humidified incubator for 18 h at 4°C and then transferred to 37°C for 3 h. During this 3-h incubation, the remaining hemolysin lysed the erythrocytes suspended in the solidified agarose. Zones of nonhemolyzed cells were seen around wells containing specific antibody. Limits of the unhemolyzed zones were marked by needle prick and measured with a graduated magnifier (Hyland Diagnostics, Deerfield, Ill.). Diameters greater than 5.0 mm were assessed as indicative of immunity since this exceeded any nonspecific inhibition of hemolysis.

### RESULTS

The results of predicting immune status by testing preexposure sera and by determining measles history or vaccination status for cases and noncases are summarized in Table 1. All 28 cases of clinical measles had sero-converted (by all tests) when postexposure sera were tested. Of the 16 cases with known vaccination or previous history of measles, 10 had documented measles vaccination (3 of whom were vaccinated at 12 months of age and an additional 2 vaccinated between 12 and 13 months of age) and 6 others had evidence of previous infection.

If patients with no history of measles or vaccination or those whose sera were antibody negative were considered susceptible, then predictive values for susceptibility and immunity (12) can be calculated. The predictive values of immunity for all serological tests were 100%, with the value for history or vaccination status being lower at 91.8%. The differences among predictive values of susceptibility were more marked, with that of the HI test being even lower than that of history or vaccination. The HI, AH, and ELISA results are compared with that of the Nt test (Table 2). There were no false-positive reactions seen with any of these tests as compared with Nt results; thus the specificity for all three tests was 100%. The HI test gave 69 false-negative results and a sensitivity of 66.0%. Sensitivities for the AH and ELISA tests were 99.5 and 99.0%, respectively. The one false-negative AH result did not correspond to either of the two found with the ELISA test.

### DISCUSSION

The use of anti-human IgG in the AH test enhanced the nonlytic zones. Norrby and Gollmar (9) found a potentiation

<table>
<thead>
<tr>
<th>Table 1: Prediction of measles immune status by four serological tests and histories of previous infection or vaccination</th>
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<tr>
<td><strong>Method used for prediction</strong></td>
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<tr>
<td></td>
</tr>
<tr>
<td>HI</td>
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<td>History of previous infection or vaccination</td>
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* S, Susceptible; I, immune.

**The predictive value for susceptibility was calculated as number of susceptible individuals who developed measles/total number of susceptible individuals.**

**The predictive value for immunity was calculated as number of immune individuals who did not develop measles/total number of immune individuals.**

**Measles values indicate previous exposure or absence of previous exposure to measles virus by vaccination or infection.**

### Table 2: Comparison of measles immune status* obtained by HI, AH, and ELISA tests with that obtained by the Nt test

<table>
<thead>
<tr>
<th>Test</th>
<th>Nt results</th>
<th>Specificity (%)</th>
<th>Sensitivity (%)</th>
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<tbody>
<tr>
<td>HI</td>
<td>Pos</td>
<td>134</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Neg</td>
<td>69</td>
<td>35</td>
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<tr>
<td>AH</td>
<td>Pos</td>
<td>202</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Neg</td>
<td>1</td>
<td>35</td>
</tr>
<tr>
<td>ELISA</td>
<td>Pos</td>
<td>201</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Neg</td>
<td>2</td>
<td>35</td>
</tr>
</tbody>
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* Pos, Positive; Neg, negative.
of hemolysis-inhibiting antibodies by the inclusion of anti-human sera in their tests. Albrecht et al. (1) used anti-human IgG in his enhanced plaque Nt test. They postulated that anti-human IgG added an extra layer of antibody around the virus and by steric hindrance prevented viral attachment to cells. In the AH test the anti-IgG may act by binding to specific IgG bound to antigen, thus preventing hemolysis of erythrocytes by the measles hemolysin.

With all serological tests, all 28 cases demonstrated seroconversion or a fourfold rise in titers between pre- and postexposure sera. Since this change in antibody titer is usually considerable, even tests of relatively low sensitivity are useful for diagnostic purposes.

Among the 10 cases of clinical measles with a documented vaccination history, 5 of the vaccine failures were in infants of less than 13 months of age. Yeager et al. reported a significantly higher rate of successful immunizations when primary vaccination was delayed until 13 months of age or older, when maternally acquired antibody generally has disappeared (15). As well, many of these individuals were vaccinated in the mid 1960s when inactivated vaccine may have been administered. Inactivated vaccines may have been deficient in their surface antigen composition might not induce antihemolysin antibodies which appear to be important in preventing measles infection (11). It is particularly important to use tests which can measure true neutralizing or AH antibodies since hemagglutination-inhibiting antibodies alone are not indicative of immunity. Marks et al. (7) reviewed various tests methodologies and concluded that the Nt test gave a better overall estimate of immunity after vaccination. The sensitivity of the AH (99.5%) and ELISA (99.0%) tests as compared with the Nt test provides a favorable and more economical alternative.

Two important indices for evaluating the efficacy of diagnostic tests are the positive and negative predictive values (12). The former indicates the likelihood that an individual designated susceptible by a test could contract the disease. The results of the HI test proved to be even less accurate than history or vaccination status in its positive predictive value (26.9% versus 41.4%). The AH, ELISA, and Nt tests were similar in their predictive values of susceptibility (75 to 80%). However, it should be recognized that the predictive values for susceptibility are minimum estimates. These calculations were based on the assumption that all subjects were exposed to a dose of measles infective for truly susceptible individuals. However, individuals designated susceptible by serological tests and who did not develop measles during the outbreak may have remained clinically well because of lack of exposure to the virus. Other factors such as cell-mediated immunity may have contributed to the disease-free status of some. Consequently, the predictive values for susceptibility may well exceed those shown in Table 1.

The predictive value for immunity by all serological tests was 100%. The value for history of infection or vaccination was lower at 91.8%, perhaps reflecting vaccine failures, the inaccurate reporting of histories, or both.

Scott et al. (13) reported on the dubious nature of vaccination histories in establishing immune status. Our findings confirm this observation. As well, the commonly employed HI test for measles was too insensitive to predict the true susceptible population. The Nt test, although the most sensitive and specific, is time consuming and expensive. This drawback may be overcome by the use of commercial ELISA kits. The Measles test was of comparable sensitivity to the Nt test, but initial trials required adjustment of incubation times for control sera to fall into range. This adjustment was not necessary with a subsequent lot ordered from the manufacturer.

The AH and ELISA tests proved to be specific and sensitive alternatives to the HI test for immune status determination when a high positive predictive value is of paramount importance in monitoring populations for true susceptible individuals.

ACKNOWLEDGMENT

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LITERATURE CITED