Comparison of the MEASLESTAT M Test Kit with the Sucrose Density Gradient Centrifugation-Hemagglutination Inhibition Method for Detection of Measles Virus-Specific Immunoglobulin M

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A new commercial kit, MEASLESTAT M (Whittaker Bioproducts, Inc.), was compared with the sucrose density gradient centrifugation-hemagglutination inhibition method for the detection of measles virus-specific immunoglobulin M. Overall agreement between the two procedures was 97.1% for 104 single and paired serum samples tested. The sensitivity and specificity of MEASLESTAT M were 98.4 and 95.2%, respectively.

Rapid diagnosis of measles is essential for the timely implementation of control measures to stanch the spread of infection. Serologic confirmation of suspected cases of measles can be accomplished early in the course of illness by the demonstration of measles virus-specific immunoglobulin M (MV-IgM). One of the conventional methods for detecting MV-IgM is a two-step process involving the fractionation of serum proteins by sucrose density gradient centrifugation (SGC) followed by hemagglutination inhibition (HI) testing of IgM-containing fractions for specific anti-measles virus activity. Because SGC is a time-consuming procedure and is unsuitable for large-scale testing, several other technologies have been adapted for the measurement of MV-IgM antibodies. These include radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), and indirect fluorescent-antibody techniques (3, 7-9). Whittaker Bioproducts, Inc., (Walkersville, Md.) has introduced a commercial kit, the MEASLESTAT M test, for the detection of MV-IgM. This assay is an ELISA procedure which includes a serum pretreatment step to remove interfering IgG antibodies prior to the addition of specimens to measles virus antigen-coated microtiter plate wells.

This communication describes a retrospective study comparing MEASLESTAT M with the SGC-HI method, the procedure currently being used in our laboratory, for the detection of MV-IgM.

A total of 130 single and paired serum samples were examined in this study. Except as otherwise noted, they were obtained from our collection of specimens previously tested for antibodies against various infectious agents. Serum specimens from 62 patients had measles virus HI antibody titers of ≥1:10, were positive for MV-IgM and either positive or negative for MV-IgG by SGC-HI (60 single serum samples), or had demonstrated measles seroconversion (2 paired serum samples) (group 1). Thirty-three single serum samples had measles virus HI antibody titers of ≥1:10 and were positive for SGC-HI MV-IgG but negative for SGC-HI MV-IgM (group 2). Eleven single serum samples from patients with rubella, a rash-associated illness frequently confused with measles, were positive for SGC-HI for rubella virus-specific IgM (group 3). Seven acute- and convalescent-phase serum sample pairs from patients with paramyxovirus infections other than measles had demonstrated seroconversion or a fourfold or greater rise in titers of complement fixation antibodies against mumps virus (five pairs) or respiratory syncytial virus (two pairs) (group 4). These samples were kindly provided by Dean D. Erdman, Centers for Disease Control (Atlanta, Ga.). The final panel (group 5) consisted of 17 single serum samples from adult patients (mean age, 65 years) with respiratory illnesses. All group 5 sera tested positive at the time of this study for both MV-IgG (SGC-HI procedure) and rheumatoid factor (Macro-Vue RF Card Test; Becton Dickinson, Cockeysville, Md.). Rheumatoid factor titers ranged from ≤1:80 (12 serum samples) to ≥1:160 (5 serum samples).

Group 3 sera were of recent origin and had been stored in the refrigerator for 2 to 5 days before examination in this investigation. All other specimens had been stored at −70°C for as long as either 2 years (groups 1, 2 and 5) or 5 years (group 4).

For the purpose of this study, the two serum sample pairs in group 1 and the 11 single serum samples in group 3 were assayed for measles virus-specific, class-specific immunoglobulin by SGC-HI. No MV-IgM was detected in group 3 specimens: MV-IgG was present in approximately half of these samples. Both serum sample pairs in group 1, demonstrating measles seroconversion, were positive for SGC-HI MV-IgM. In addition, because the determination of MV-IgM by SGC-HI in sera belonging to groups 1 and 2 had been performed with fresh specimens before storage (except for the two serum sample pairs noted above) and MEASLESTAT M testing was being performed with stored frozen specimens, MV-IgM determinations by SGC-HI were repeated at the time of this study on the seven oldest samples in group 1. All seven serum samples retested MV-IgM positive, thus providing evidence for the persistence of this antibody in sera subjected to long-term storage in the frozen state. For all other sera in groups 1 to 3, retesting for MV-IgM by SGC-HI was undertaken only when there was a discrepancy between SGC-HI and MEASLESTAT M test results. SGC-HI determinations for MV-IgM were not performed with specimens in group 4 because of insufficient serum quantities or with group 5 sera.

Standard procedures were used for the determination of measles and rubella virus HI antibodies (1, 5). Fractionation of serum immunoglobulins by SGC was performed according to the protocol described by Palmer et al. (6). However, for MV-IgM and MV-IgG determinations, a serum pretreatment
TABLE 1. Detection of MV-IgM by MEASLESTAT M in 128 serologically characterized single serum specimens and paired serum samples collected during the acute and convalescent phases of illness

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of single and paired serum specimens tested</th>
<th>Current or recent infection ¹</th>
<th>SGC-HI result for:</th>
<th>No. of specimens with indicated result by MEASLESTAT M</th>
<th>% Agreement between MEASLESTAT M and SGC-HI procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>MV-IgM</td>
<td>MV-IgG</td>
<td>Positive</td>
</tr>
<tr>
<td>1</td>
<td>62</td>
<td>Measles</td>
<td>+</td>
<td>+ or -</td>
<td>61</td>
</tr>
<tr>
<td>2</td>
<td>31</td>
<td>Unknown ²</td>
<td>-</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>Rubella</td>
<td>-</td>
<td>+ or -</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>Mumps, respiratory syncytial virus ³</td>
<td>NT ¹</td>
<td>NT</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>17</td>
<td>Unknown ⁵</td>
<td>NT</td>
<td>+</td>
<td>0</td>
</tr>
</tbody>
</table>

¹ Serologically confirmed infection.
² Specimens in group 2 were from patients suspected of having measles.
³ NT, not tested.
⁴ NA, not applicable.
⁵ Mumps, five cases; respiratory syncytial virus, two cases.

Specimens in group 5 were from patients with symptoms of respiratory illness. All were positive for rheumatoid factor.

Step was added in which a 1:1 serum-phosphate-buffered saline (pH 7.0) mixture was heated inactivated at 56°C for 30 min, adsorbed with a 50% suspension of African green monkey erythrocytes for 1 h, and then centrifuged prior to being layered onto the sucrose gradient. The purity of the IgM- and IgG-containing fractions of each specimen was established by applying them to radial immunodiffusion plates containing monospecific antisera against IgM and IgG. For each specimen that was retested for SGC-HI MV-IgM during this study, class-specific immunoglobulin purity checks were repeated with the fractionated serum.

The MEASLESTAT M test was performed according to the instructions on the package insert. In this procedure, serum specimens are not heat inactivated. All steps took place at room temperature. Briefly, they were as follows. Serum samples were incubated for 30 min with serum pretreatment solution to remove IgG. Each test sample, at a final dilution of 1:80, was then added to two wells of a microtiter plate, alternate wells of which were coated with measles virus antigen and control antigen. Enzyme conjugate, substrate, and stop solution were sequentially added to all the wells. Each addition, except the last, was followed by a 30-min incubation and a brief wash cycle. The reaction was read spectrophotometrically, the intensity of color being directly related to the amount of MV-IgM present. As prescribed by the manufacturer, three calibrators (one high positive, one low positive, and one negative) and one rheumatoid factor control were included in each test run; they were treated in the same manner as the test specimens.

MEASLESTAT M values ≥0.17 and ≤0.12 are interpreted as positive and negative, respectively, for MV-IgM. Values in the range of 0.13 to 0.16 are considered equivocal. The manufacturer recommends retesting equivocal specimens.

All specimens, including both serum samples comprising the acute- and convalescent-phase serum pairs, were tested by MEASLESTAT M. Specimens whose MEASLESTAT M results were in disagreement with SGC-HI results were retested by both methods.

One hundred thirty single and paired serum samples were examined with the MEASLESTAT M test kit. Two specimens in group 2 gave equivocal results. Both samples were of insufficient quantity to permit retesting and were, therefore, not included in the analysis of results. Of the remaining 128 single and paired serum samples, SGC-HI results for MV-IgM were available for specimens in groups 1, 2, and 3. For these 104 single and paired serum samples, there was 97.1% agreement between the MEASLESTAT M and SGC-HI results for MV-IgM (Table 1). Specimens from 61 of 62 patients in group 1, showing MV-IgM by SGC-HI (60 single samples) or both SGC-HI MV-IgM and measles seroconversion (2 paired serum samples), were also positive for MV-IgM in the MEASLESTAT M test (98.4% sensitivity). The one sample in this group giving a negative MEASLESTAT M result was a single serum specimen having a measles virus HI antibody titer of 1:640 and an SGC-HI MV-IgM titer of 1:4. Its MEASLESTAT M value was 0.12, which is the upper limit of a negative interpretation. These results remained unchanged when the specimen was retested by both procedures. Acute- and convalescent-phase sera of both paired serum samples showing measles seroconversion yielded negative and positive MEASLESTAT M results, respectively.

The 31 single serum samples composing group 2 had measles HI antibody titers of ≥1:10 (range, 1:10 to 1:320) and were positive for SGC-HI MV-IgG and negative for SGC-HI MV-IgM. Twenty-nine of these specimens were negative for MV-IgM, and two specimens were positive for MV-IgM by MEASLESTAT M. Both MEASLESTAT M-positive specimens had MEASLESTAT M values of 0.19, which is close to the lower limit of a positive interpretation. The measles virus HI antibody titers for these serum samples were 1:80 and 1:160. On repeat testing for MV-IgM, both specimens were again negative by the SGC-HI method and positive by the ELISA procedure. MEASLESTAT M values on retest were 0.19 and 0.18. All 11 single serum samples in group 3, which, by SGC-HI, were positive for rubella virus-specific IgM and negative for MV-IgM, gave negative results in the MEASLESTAT M test. On the basis of the results for sera in groups 2 and 3, the specificity of the MEASLESTAT M test, relative to SGC-HI, for MV-IgM detection was 95.2%.

In addition, both samples of each of the seven serum sample pairs from patients with serologically confirmed paramyxovirus infections other than measles (group 4) gave negative MEASLESTAT M results. The 17 specimens in group 5, which were positive for both MV-IgG and rheumatoid factor, were also negative in the MEASLESTAT M test.

In this study, three lots of MEASLESTAT M kits were used in a total of 10 test runs (three runs with each of two lots
and four runs with one lot). The between-run coefficient of variation observed with the negative, low-positive, and high-positive calibrators was, respectively, 0.0, 9.4, and 12.4% for the first lot; 0.0, 11.7, and 0.6% for the second lot; and 0.0, 8.7, and 8.9% for the third lot.

There were, in all, three discrepancies between MEASLESTAT M and SGC-HI test results. One specimen was positive for MV-IgM by the SGC-HI procedure but gave a negative MEASLESTAT M result. The reason for this discrepancy was not determined. Although the specimen had a high measles virus HI antibody titer (1:640), it is unlikely that the negative reaction resulted from incomplete removal of MV-IgG during the MEASLESTAT M pretreatment step, followed by subsequent saturation of antigen binding sites by residual IgG antibody. All 16 other single serum samples which were positive for MV-IgM by SGC-HI and had measles virus HI antibody titers of ≥1:640 were also positive for MV-IgM in the MEASLESTAT M test.

Two specimens, both from suspected cases of measles, had measles virus HI antibody titers of 1:160 and 1:80, were negative for MV-IgM by SGC-HI, but yielded low-positive MEASLESTAT M values (0.19 and 0.18). The possibility exists that these are, in fact, true-positive results reflecting the greater sensitivity of ELISA procedures as a class compared with HI methods. It should be noted that false-positive rates of 3 and 10% were observed by Mayo and coworkers (4) when large panels of serum specimens from healthy adults were examined with the MEASLESTAT M kit. However, as indicated by the investigators, no tests were conducted to confirm the absence of MV-IgM in the specimens yielding apparently false-positive results.

The MEASLESTAT M test was not affected by the presence of rheumatoid factor or rubella virus-specific IgM antibodies in serum. In addition, antibodies against measles-related viruses (mumps virus and respiratory syncytial virus) apparently did not interfere with the test. The last observation is made with reservation because, owing to the insufficiency of serum quantities, specimens in group 4 could not be tested for the persistence of respiratory syncytial virus and mumps virus antibodies following their storage at −70°C.

The fact that the near-breakpoint ELISA values for each of the three specimens giving discordant results were reproducible indicates that MEASLESTAT M is a precise assay. It also suggests that routine retesting of specimens to confirm near-threshold values is not necessary. It is, in fact, not recommended by the manufacturer. Further evidence for the reliability of the MEASLESTAT M test was provided by the interassay coefficients of variation for the calibrators. These were 0.0% for the negative calibrator and ranged from 8.7 to 11.7% and from 0.6 to 12.4% for the low-positive and high-positive calibrators, respectively.

Mayo et al. (4) reported a sensitivity of 85.7% and a specificity of 81.3% for the MEASLESTAT M test. The standard of comparison was a significant rise in the titer of measles virus complement fixation antibody or of ELISA IgG in serum sample pairs collected during the acute and convalescent phases of infection. As these investigators noted, a limitation of their study was the reliance on a reference method that measures increases in antibody titer, the detection of which makes the timing of specimen collection critical. Our study enlarges on the work of Mayo et al. by comparing the MEASLESTAT M test with a procedure (SGC-HI) that assays for the same analyte. The findings reported here indicate that the MEASLESTAT M test kit is a satisfactory alternative for the detection of MV-IgM. Furthermore, MEASLESTAT M has several advantages over the SGC-HI procedure for MV-IgM determination. It is relatively easy to perform, all necessary reagents are provided in kit form, and the major piece of equipment required, the spectrophotometer, is far less expensive than is the ultracentrifuge called for in the SGC-HI procedure. An additional benefit is the speed with which results are available: 3.0 h by MEASLESTAT M versus 2 days by SGC-HI testing.

In conclusion, we believe the MEASLESTAT M test is a valuable aid in the rapid diagnosis of measles.

REFERENCES