Rapid Diagnosis of Acute Mumps Infection by a Direct Immunoglobulin M Antibody Capture Enzyme Immunoassay with Labeled Antigen

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A new immunoglobulin M (IgM) antibody capture enzyme immunoassay with peroxidase-labeled mumps antigen (dMACEIA) is described, and its suitability for practical diagnosis of acute mumps infection is evaluated. All 54 patients with proven mumps infection that were tested showed mumps-specific IgM antibodies. On the other hand, no specific IgM antibodies were present in 16 cases of suspected mumps that could not be confirmed by classical complement fixation serology, and IgM mumps virus antibodies could be detected neither in the sera of 100 healthy individuals nor in those of 16 patients positive for rheumatoid factor. In all, 22 children with acute respiratory illness caused by parainfluenza virus and 44 patients with infections due to other viruses showed no IgM response in mumps dMACEIA. The particular characteristic in which complement fixation antibodies against mumps nucleocapsids appear before and disappear earlier than antibodies to the enveloped mumps virus could not be demonstrated in the dMACEIA. In an extensive epidemic of mumps virus infection, the dMACEIA gave a clear diagnosis of mumps infection in 200 out of 371 suspected cases. By day 2 of the illness, 71% of the patients had detectable IgM, and by day 3, all of them had detectable IgM. In 99% of the cases, dMACEIA gave a positive result in the first available serum specimens, most of which were negative for complement fixation antibodies. A positive but only moderate correlation was thus observed between the two serological procedures. IgM antibodies persisted for at least 6 weeks. The dMACEIA, performed in 3 h, offers a reliable, simple, and rapid alternative to routine methods for detection of acute mumps infection.

A rapid and reliable laboratory diagnosis of mumps virus infection is needed since the clinical symptomatology is often atypical, especially in cases of meningitis, orchitis, or pancreatitis without evidence of parotitis. Conventional laboratory methods, such as virus isolation from saliva or spinal fluid, and serological procedures, such as complement fixation (CF) or hemagglutination inhibition, which require paired sera, delay the results. The demonstration of specific immunoglobulin M (IgM) class antibody is considered to be a useful tool for the rapid diagnosis of acute virus infections since, as a rule, this antibody can be detected only for a limited period. The preliminary physical separation of IgM from IgG by either sucrose density gradient centrifugation (27) or exclusion chromatography (12), followed by testing the IgM-containing fractions by a conventional serological method, is generally reliable but laborious. Indirect tests, including the immunofluorescence test (2), a radioimmunoassay (6), or an enzyme-linked immunosorbent assay (ELISA) (9, 16, 18–21, 23, 24) have been used to detect mumps-specific IgM. The problem with these indirect tests is that IgG may inhibit the binding of IgM antibodies, particularly when high IgG antibody titers are present, thus giving false-negative results. Furthermore, rheumatoid factor of the IgM class may cause false-positive results by reacting with antigen-IgG complexes (26). These problems may be overcome by an antibody capture assay in which human IgM is selectively attached to the solid phase by an anti-human IgM antibody. Mumps-specific IgM is then detected by adding either virus hemagglutinin and guinea pig erythrocytes (7, 25) or mumps antigen and its radioisotope-labeled antibody (3). The main disadvantage of the first technique, M-antibody capture hemadsorption, is the difficulty encountered in reading the test, and that of the second technique, M-antibody capture radioimmunoassay, is the use of a radioisotope-labeled reagent. In this report we describe a new direct IgM antibody capture enzyme immunoassay (dMACEIA) with a peroxidase-labeled mumps antigen. A relatively large number of sera collected during an extensive epidemic of mumps that occurred in the eastern area of France during the winter of 1984 allowed us to assess the methodology described.

MATERIALS AND METHODS

Sera. Group 1 consisted of acute- or convalescent-phase sera or both from patients with clinical mumps. Diagnoses were established by the presence of a significant (fourfold or greater) increase in CF antibodies to mumps (40 patients) or by virus isolation from the throat or cerebrospinal fluid (CSF) (14 patients). Group 2 consisted of single sera from 317 patients whose clinical data suggested a mumps infection. Group 3 consisted of 14 single and 16 paired sera from 22 patients with acute respiratory illnesses caused by parainfluenza viruses; diagnoses were established through demonstration of a significant increase in CF antibodies to parainfluenza viruses (6 cases), through a CF antibody titer higher than 64 in a single serum (11 cases), or by virus isolation of parainfluenza virus type 1 (2 cases) or type 3 (3 cases); 9 of these patients were 4 to 9 years old, and 13 were less than 4 years old. Group 4 consisted of 11 sera with rubella-specific IgM antibodies (titers varied between 800 and 12,800 as determined by M-antibody capture hemadsorption), 11 sera with hepatitis A IgM antibodies, 6 sera with herpes simplex

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virus IgM antibodies, and 16 sera with cytomegalovirus IgM antibodies (all determined by dMACEIA with titers between 200 and 2,560). Group 5 consisted of sera from 100 healthy individuals between 40 and 50 years of age. Group 6 consisted of 16 single sera with IgG-ELISA mumps virus antibodies from patients for whom clinical data suggested of a recent mumps infection was lacking. These sera had varying levels of rheumatoid factor.

**dMACEIA antigens.** Allantoic fluid from mumps virus-infected embryos was used as a source of antigen. The Enders strains of mumps virus (kindly provided by G. A. Denoyel, Pasteur Institute, Lyons, France) was propagated by allantoic inoculation in 10-day-old embryos at a dose of 1 hemagglutinin unit per 0.2 ml. After 7 days at 35°C, allantoic fluid and membranes were harvested. Three preparations of allantoic fluid antigen containing the virus-bound (V-) antigen were prepared: (i) crude virus-containing allantoic fluid clarified by low-speed centrifugation and dialyzed overnight against 20 volumes of 0.01 M phosphate-buffered saline (PBS); (ii) pelleted virus obtained by centrifugation of the latter suspension at 100,000 x g for 1.5 h at 4°C in an SW27 rotor and suspension by sonication to ca. 1:30 of the original volume in 0.01 M carbonate buffer (pH 9.5); (iii) virus purified in a discontinuous sucrose gradient as previously described (14). Allantoic fluid from uninfected eggs treated in the same way served as control antigens. Ribonucleoprotein (V-) antigen (S-) antigen was extracted from the membranes of the same eggs from which the pelleted virus was obtained. After washing twice in PBS, a 20% suspension of the membranes in PBS was prepared by homogenization. The homogenate was first clarified by low-speed centrifugation and then centrifuged at 100,000 x g for 1.5 h at 4°C in an SW27 rotor to sediment most of the mumps virus nucleocapsids. These were then suspended by sonication to 1:20 of the original volume in 0.01 M carbonate buffer (pH 9.5). The protein concentrations of the different batches of antigen were determined by the Lowry method.

The peroxidase labeling of the different antigen preparations was carried out by the periodate method in two steps (28). Horseradish peroxidase type IV (4 mg; Sigma Chemical Co., St. Louis, Mo.) was dissolved in 1 ml of distilled water and oxidized by adding 0.2 ml of freshly prepared 0.1 M NaIO4. After stirring for 2 h at room temperature in the dark, the solution was dialyzed overnight at 4°C against 1 mM sodium acetate buffer (pH 4.4). A solution of mumps virus antigen containing 4 mg of protein in 3 ml of 0.01 M carbonate buffer (pH 9.5) was added to 1 ml of the activated enzyme. The mixture was held for 2 h at room temperature in the dark before 0.1 ml of an NaBH4 solution (4 mg/ml) was added. The labeled antigen was kept at 4°C for 5 h, then dialyzed against PBS overnight at 4°C, and finally stored in small volumes at −70°C.

**dMACEIA procedure.** The procedure used was that previously described for detection of cytomegalovirus IgM antibodies (22) but with some modifications. Polystyrene flat-bottom microtiter plates (Kontron Analytique, Trappes, France) were coated with 0.1 ml per well of rabbit anti-IgM immunoglobulin (μ-chain specific; Dakopatts, Copenhagen, Denmark) at a dilution of 1:500 in 0.1 M bicarbonate buffer (pH 9.6) and sealed with tape. After overnight incubation at 4°C in a moist chamber, the plates were washed five times with PBS containing 0.05% Tween 20 (PBS-T) and were shaken dry. On the same day, 0.1 ml of serum specimen at an initial dilution of 1:100 in PBS-T with 0.4% bovine serum albumin was added to each of two wells. The sealed plates were incubated at 37°C for 30 min in a humidified atmosphere and washed five times with PBS-T. A 0.1-ml volume of labeled antigen dilution in PBS-T was then added to each well and incubated for 2 h at 37°C in a humidified atmosphere. The optimal dilution of each kind and batch of antigen was determined by box titration with a known positive serum and a known negative one.

After five washes with PBS-T, substrate solution (0.2 ml per well) was added. The substrate solution was prepared immediately before use by dissolving 10 mg of orthophenylene-diamine in 25 ml of phosphate-citrate buffer (pH 5) followed by the addition of 0.036 ml of 30% H2O2. After a 15-min incubation in the dark at room temperature, the reaction was stopped by adding 0.05 ml of 2 N H2SO4. The absorbance was measured at a wavelength of 486 nm in a vertically measuring photometer (Kontron Analytique). The mean absorbance of eight blank wells each containing 0.2 ml of substrate solution and 0.05 ml of 2 N H2SO4 was automatically subtracted from the absorbance of the test wells. Since comparison of the results obtained with the three preparations of V-antigen showed equal performances for pelleted and purified viruses and insensitive ones with crude virus, we used only pelleted V- or S-antigen (see below). The absorbance values of IgM antibody-positive and -negative sera with control antigen were ca. 10 times lower than those of IgM antibody-negative sera with V-antigen (0.01 and 0.1, respectively). The cutoff level was therefore defined by calculating the absorbance ratio of the specimen serum compared with that of the negative control serum (S/N ratio). An S/N ratio of 2.0, which generally lay between 3 and 6 standard deviations (SD) above the mean value obtained from four negative sera, was arbitrarily considered to indicate a specific reaction. Only nine serum samples collected from patients clinically suspected of having mumps virus infection showed an S/N ratio between 1.6 and 1.9 at a dilution of 1:100 and were considered as negative. The endpoint titer was regarded as the highest serum dilution in which the S/N ratio was equal to or greater than 2.0.

**Indirect ELISA for IgG antibody.** Mumps virus IgG antibodies were determined by a classical indirect ELISA (21) with some differences: the technical conditions were the same as those of the dMACEIA except for the use of pelleted V-antigen without peroxidase labeling; IgG antibody was detected by peroxidase-conjugated rabbit anti-human IgG (γ-chain specific; Dakopatts) diluted at 1:2,000.

**Other methods.** A standard CF test in microplates was used (4). Mumps CF antibodies were determined by using pelleted V- and S-antigen-nondepleted dMACEIA antigens. Parainfluenza CF antigens were purchased from MA Bioproducts (Walkersville, Mass.). Mumps virus isolation from the throat or from spinal fluid and parainfluenza virus isolation from the throat were carried out in an established monkey kidney cell line (MK2 cells). The cytopathic effect was generally noted within 5 to 7 days. The neutralization test with specific reference sera (MA Bioproducts) permitted the identification of the agents. Rheumatoid factor was determined by a commercially available latex test (Hoechst-Behring, Rueil Malmaison, France) and by an enzyme immunoassay, previously described (11), with heat-aggregated IgG. To evaluate the antibody class, sera were fractionated by sucrose density gradient centrifugation (27). The effective separation of IgM and IgG antibodies was demonstrated by the immunodiffusion test of Ouchterlony with rabbit anti-human μ- and γ-chain sera (Dakopatts). IgM and IgG antibodies were found in fractions 1 to 10 and 8 to 20, respectively. Each fraction was diluted 1:50 before its use in the dMACEIA.
RESULTS

Reliability of dMACEIA for detecting acute mumps infection. To assess the specificity of dMACEIA for the IgM antibody class, sera from five patients with clinical mumps and a significant increase in CF antibody titers (group 1) were fractionated by sucrose density gradient centrifugation. The IgM fractions of the five sera were positive in dMACEIA with antibody titers up to 12,800, whereas the IgG fractions were negative. Moreover, the IgM and IgG fractions of five sera from individuals with remote mumps infection showing a mean IgG ELISA titer of 3,200 (group 5) were negative in the dMACEIA. The results obtained with one of the fractionated sera each from groups 1 and 5 are shown in Fig. 1. These data indicate that the specific IgG antibody contained in specific IgM-positive or IgM-negative sera did not give false-positive reactions.

The intraassay variability in the absorbance values of the dMACEIA was determined in three different sera (intermediate positive, low positive, and negative in mumps IgM antibodies) which were diluted 1:100, pipetted into 30 wells, and tested with pelleted V-antigen. The coefficients of variation (SD/mean) were 0.8, 1.2, and 2.0, respectively, in the three sera (Table 1). To investigate the interassay variability of the dMACEIA, the same intermediate positive and negative sera were assayed on 20 different days with the same batch of pelleted V-antigen. The coefficients of variation were 13.8 and 23.2, respectively, in the two sera (Table 1).

Sixty convalescent-phase sera of 54 patients with clinical mumps and a significant increase in CF antibodies to mumps or a virus isolation (group 1) tested by dMACEIA showed positive reactions with IgM-antibody titers ranging from 800 to 102,400. Conversely, 16 patients with a mumps-like illness who had paired serum samples that remained negative or showed low stable titers in the CF test did not show positive reactions in dMACEIA. For comparison, dMACEIA was carried out on sera of 100 healthy individuals (group 5) whose ages varied from 40 to 50 years (Table 2). Although 97% of these had a remote mumps virus infection (IgG-ELISA antibody titer ranging from 800 to 102,400), none showed IgM antibodies.

To explore the possibility of cross-reactivity with parainfluenza and other viruses, sera from 22 patients with acute respiratory illnesses caused by parainfluenza viruses (group 3) and from 44 patients with hepatitis A, rubella, herpes simplex virus, or cytomegalovirus primary infection (group 4) were tested by the dMACEIA. Mumps CF antibodies

![Graph](http://jcm.asm.org/)

**TABLE 1. Intra- and interassay variability of the dMACEIA**

<table>
<thead>
<tr>
<th>Parameter (type of serum)</th>
<th>Absorbance at 486 nm</th>
<th>Coefficient of variation (SD/mean %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Maximum</td>
</tr>
<tr>
<td>Intraassay variability a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate positive</td>
<td>0.822</td>
<td>0.950</td>
</tr>
<tr>
<td>Low positive</td>
<td>0.237</td>
<td>0.280</td>
</tr>
<tr>
<td>Negative</td>
<td>0.108</td>
<td>0.150</td>
</tr>
<tr>
<td>Interassay variability b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate positive</td>
<td>0.860</td>
<td>1.130</td>
</tr>
<tr>
<td>Negative</td>
<td>0.149</td>
<td>0.190</td>
</tr>
</tbody>
</table>

a The sera were tested at a dilution of 1:100 with the same preparation of pelleted V-antigen.

b An intermediate positive serum (titer, 12,800), a low positive serum (titer, 200), and a negative serum were tested 30 times in the same test.

c An intermediate positive serum (titer, 12,800) and a negative serum were tested on 20 different days.
were also assayed in sera from group 3. None of the sera of groups 3 and 4 exhibited mumps IgM antibodies (Table 3). Rheumatoid factor did not give false-positive results in the dMACEIA. In group 6, 16 sera with IgG-ELISA titers against mumps virus of 3,200 to 51,200 and with various levels of rheumatoid factor ranging from 20 to 1,280 and 100 to 12,800 as determined, respectively, by a latex fixation test and ELISA, were negative in the dMACEIA (S/N ratio ± SD, 0.99 ± 0.16). Rheumatoid factor was also determined by ELISA in the 200 patients with acute mumps infection (group 1 and part of group 2) and 20 showed positive reactions with a mean titer ± SD of 225 ± 337. With approximately the same 50% male/female ratio, this group comprised 7 children and 13 adults.

Comparative reactivity of S-antigen and pelleted V-antigen in dMACEIA. Most of the sera sampled on days 1, 2, or 3 after the onset of illness showed IgM antibody against pelleted V- and S-antigens (Fig. 2A). In four cases S/N ratios were lower than 2.0 (1.6 to 1.9) and higher than 2.0 (2.3 to 2.6) against S- and V-antigens, respectively. The reverse was never observed. In particular, all 171 sera from patients whose clinical data were suggestive of a mumps virus infection (group 2), which were IgM negative in the first screening with V-antigen were also negative with S-antigen.

**TABLE 2.** Distribution of mumps IgG and IgM antibody titers in sera from 100 healthy individuals.a  

<table>
<thead>
<tr>
<th>Antibody class</th>
<th>No. of sera based on antibody titer:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>IgGb</td>
<td></td>
</tr>
<tr>
<td>IgMb</td>
<td></td>
</tr>
</tbody>
</table>

a Aged 40 to 50 years.  
b Assayed by ELISA with pelleted V-antigen.  
c Assayed by dMACEIA with pelleted V-antigen.  
d Mean S/N ratio ± SD, 0.85 ± 0.24.

Of eight sera sampled more than 28 days after the onset of mumps, four showed a negative IgM result against S-antigen and one showed a negative result against V-antigen (Fig. 2B). Late sera always showed a higher S/N ratio for V-antigen as compared with that for S-antigen, respective values being 4.7 and 1.8. Based on these data, we chose to use V-antigen only to diagnose acute mumps infection by IgM antibody assays.

**Diagnosis of current mumps infection by dMACEIA.** The development of the IgM response was studied in the sera of 371 patients whose clinical symptoms could be attributed to

**FIG. 2.** Correlation between the use of pelleted enveloped virus (V-antigen) and nucleocapsids (S-antigen) in dMACEIA in sera sampled during the first 3 days (A) or 28 days (B) after the onset of mumps virus infection. A ratio of the specimen serum absorbance value to that of the negative control serum (S/N ratio) equal or greater than 2.0 correlates with the presence of IgM antibodies.

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**TABLE 3.** IgM antibodies and increases or high levels in CF mumps virus antibodies in patients with parainfluenza virus infection, hepatitis A, rubella, herpes simplex virus, and cytomegalovirus primary infections

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of patients tested</th>
<th>No. of patients with increased or high titer in mumps CF antibodiesa</th>
<th>No. of patients with IgM antibodiesb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parainfluenza</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF titer increase</td>
<td>6</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>CF titer higher than 64</td>
<td>11</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Isolation of parainfluenza type 1</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Isolation of parainfluenza type 3</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other virusesa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis A</td>
<td>11</td>
<td>NDc</td>
<td>0</td>
</tr>
<tr>
<td>Rubella</td>
<td>11</td>
<td>NDc</td>
<td>0</td>
</tr>
<tr>
<td>Herpes simplex virus</td>
<td>6</td>
<td>NDc</td>
<td>0</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>16</td>
<td>NDc</td>
<td>0</td>
</tr>
</tbody>
</table>

a Fourfold increase or titer higher than 64.  
b Assayed by dMACEIA with pelleted V-antigen; mean S/N ratio ± SD, 0.99 ± 0.25.  
c Sera of these patients showed specific IgM antibodies (titers between 200 and 25,600) determined by IgM antibody capture hemagglutination assay or dMACEIA.  
d ND, Not done.
mumps virus infection (groups 1 and 2). In 200 patients (54%), IgM antibodies were found. These appeared on day 1 or 2 after the onset of the disease. Indeed, all except two sera sampled on day 1 and all sera collected on days 2 or 3 contained IgM antibodies (Fig. 3). Also, 84 of 86 acute-phase sera in which CF antibodies were still absent exhibited IgM antibodies (Fig. 4). A moderate but statistically significant positive correlation ($r = 0.17; P < 0.01$) between dMACEIA and CF is shown in Fig. 4. In effect, antibodies revealed by the first test appeared earlier than those revealed by the second (Fig. 3). The mean IgM titer was 117 times that of CF (4,802 and 41, respectively). When CF antibodies determined with S-antigen and pelleted V-antigen were first detected on days 2 and 3 after the onset of the symptoms, dMACEIA with pelleted V-antigen already showed mean titers of 663 and 1,280 on days 2 and 3, respectively. Study of the temporal course of the antibody titers also revealed that the peak was reached on weeks 1 and 3 for IgM and CF antibodies, respectively. Mumps IgM persisted for at least 41 days in one patient but disappeared by day 64 in another patient.

CSF from 16 meningitis patients who showed mumps IgM antibodies in their sera with titers ranging from 20 to 12,800 were also tested by dMACEIA and IgG-ELISA. Although 11 of these CSF samples yielded mumps virus on culture and 5 did not, none showed specific IgM or IgG antibodies.

**DISCUSSION**

The aim of this study was to develop a new assay for demonstration of mumps IgM antibodies, to analyze it, and to evaluate its suitability for practical serological diagnosis of mumps infection as compared with that of the commonly used techniques.

The dMACEIA is easy to perform. Antigen preparation is relatively simple. Since sensitized microtiter plates can be stored at $-80^\circ$C for several weeks, the test can be carried out in 3 h and needs only a small amount of serum. We also demonstrated that dMACEIA is specific for the IgM class of antibody to mumps (Table 1). All sera from patients with clinical mumps and a significant increase in CF antibodies or from which the virus was isolated showed specific IgM (group 1). Conversely, patients with an illness suggestive of mumps whose paired sera remained antibody negative or
displayed a low stationary titer of CF antibodies did not show a positive reaction in dMACEIA. Sera were also negative in 100 healthy individuals aged 40 to 50 years (group 5). These results would be expected since only the IgM of the sera from these patients attached to the wells coated with anti-human IgM. Other studies with the same highly specific procedure for capturing IgM antibody were used in detecting IgM antibodies to hepatitis A virus (8), rubella virus (15), and cytomegalovirus (22). For diagnosis of mumps infection, we considered dMACEIA preferable to M-antibody capture radioimmun assay (RIA) and M-antibody capture hemadsorption (7, 25), which require radio-labeled antibody or sometimes present difficulties in interpretation of the test results.

The dMACEIA was reproducible since the intraassay variability of absorbance values in the same test showed coefficients of variation ranging from 0.8 to 2.0. The interassay variability of the test system assessed on 20 different days exhibited higher coefficients of variation (13.8 and 23.2) but was still considered acceptable. We chose a cutoff level between negative and positive scoring, defined as 3 to 6 SD above the mean absorbance of the negative reference serum, keeping in mind the possibility that such a level might give false-negative results.

To find out which kind of mumps antigen was preferable for the diagnosis of acute mumps, we compared results obtained with pelleted enveloped virus (V-antigen) and nucleocapsids (S-antigen). It is well known that the S-antibody frequently appears in the course of infection before the V-antibody, but the latter persists longer (13). This was also observed in our results for CF antibodies but could not be confirmed for IgM by using pelleted V- and S-antigens in the dMACEIA (Fig. 2). Thus, the diagnosis of acute mumps could not be made earlier by demonstrating S-IgM. On the contrary some acute-phase sera showed IgM against V-antigen but not against S-antigen. A possible explanation for this may be the difficulty of obtaining high specific activity with membrane-extracted nucleocapsids as compared with pelleted virus particles from allantoic fluid. Mumps virus has an antigenic relationship with other viruses of the paramyxovirus group (5) as evidenced by serological cross-reactions (17). In accordance with certain other procedures for detecting mumps IgM (18, 24) but contrary to the M-antibody capture hemadsorption (25), our data showed that none of the patients with acute respiratory illnesses caused by parainfluenza viruses (group 3) had IgM antibodies to mumps virus (Table 3). We could not determine the reason for this discrepancy. Cross-reactions were also not observed in 44 sera containing IgM antibodies against hepatitis A virus, rubella virus, herpes simplex virus, or cytomegalovirus (Table 3).

Rheumatoid factor often gives false-positive results in indirect assays where the antibodies of different classes are not separated before testing (26). It is known that the immunocapture of IgM reduces or eliminates this problem (10), as we observed in our results. The use of the latter procedure is highly desirable since virus-infected individuals frequently exhibit rheumatoid factor in their serum (1), as was the case in our study, in which 10% of mumps-infected patients showed rheumatoid factor.

The dMACEIA is a sensitive method for the early diagnosis of acute mumps infection. Mumps IgM antibodies appeared soon after the onset of clinical symptoms. By day 2 of the illness, 71% of the patients had detectable IgM in their serum, and by day 3, all of the patients had detectable IgM (Fig. 3). The dMACEIA thus gave a clear diagnosis of mumps infection in 200 out of 371 patients whose clinical symptoms were suggestive of mumps infection and did so with the first available serum specimen in 99% of these cases. Despite a statistically positive correlation between the results obtained with CF and dMACEIA, the latter assay was more sensitive. This was indicated, as previously shown (23), by the ability to demonstrate relatively high dMACEIA absorbance values in the early-phase sera which were negative or had only a low level of CF antibodies (Fig. 3). Peak titers of IgM were reached in week 1 after the onset of the illness. IgM persisted in a patient 41 days after the onset of infection but was absent at day 64 in another patient. Adequate convalescent-phase sera were not available in our study to determine how long these antibodies actually persist in the course of infection. The possibility that prolonged IgM responses may occur (2, 25) emphasizes the importance of interpreting laboratory results in the light of clinical and epidemiological details.

Contrary to what has been observed in neurological diseases due to herpes simplex virus or cytomegalovirus, mumps IgM and IgG antibodies could not be found in the CSF of 16 patients with proven mumps meningitis. Of these patients, 11 had CSF which contained mumps virus, but 5 did not. The reason for the absence of specific IgM and IgG antibodies did not seem to be a technical one attributable to the occurrence of immune complexes. Our data indicate that detection of IgM in serum may be preferable to virus isolation from CSF, in view of the greater sensitivity of the former.

The dMACEIA is a simple, rapid, and reliable test for mumps-specific IgM, and it is particularly valuable for the rapid diagnosis of atypical mumps infection and for confirming clinically suspected cases of the disease. The dMACEIA is also useful when sera are taken too late for diagnosis based on a significant increase in CF titer or when the only serum collected is from the convalescent phase.

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LITERATURE CITED


