Investigation of Optimal Specimen Type and Sampling Time for Detection of Measles Virus RNA during a Measles Epidemic

MICHAELA A. RIDDELL,* DORIS CHIBO, HEATH A. KELLY, MICHAEL G. CATTON, AND CHRISTOPHER J. BIRCH

Victorian Infectious Diseases Reference Laboratory, North Melbourne, Victoria, Australia

Received 13 July 2000/Returned for modification 13 September 2000/Accepted 23 October 2000

At various times postonset of rash, 74 patients positive for measles virus-specific immunoglobulin M provided samples for detection of measles virus RNA by a reverse transcriptase PCR. Of lymphocytes, urine, throat swab, and serum specimens, throat swab specimens were optimal for detection of measles virus RNA during the first 2 weeks after the rash.

Detection of measles virus RNA by reverse transcriptase (RT) PCR (RT-PCR) from various clinical specimens, including throat swab (TS), nasopharyngeal aspirate, urine, cerebrospinal fluid, and blood specimens, has been described by several groups (7, 8, 10). However, to our knowledge, no investigation of optimal specimen type or sampling time for maximization of the chance of measles virus RNA recovery has been reported. Identification of the optimal specimen type and sampling time for the detection of measles virus RNA will enhance the rapid confirmation of true measles virus infections and the identification of measles virus genotypes for molecular epidemiological studies.

A measles outbreak in the state of Victoria in Australia in 1999 (6) provided the opportunity to collect clinical material from naturally infected individuals. In addition to clinical information, plain blood for serum, blood containing EDTA for recovery of peripheral blood leukocytes (PBLs), urine, and TS samples were collected as part of the enhanced measles surveillance system (5). TS samples were collected with a sterile dry swab from the back of the throat. The swab tip was placed in 3 ml of sterile viral transport medium and was transported to the laboratory at 4°C. On the day of collection these samples were stored at 4°C and analyzed by RT-PCR within 1 week. Serum was tested for the presence of measles virus-specific immunoglobulin M (IgM) and IgG antibodies by a commercial enzyme immunoassay (Dade Behring Enzygnost, Marburg, Germany). All sera were tested for the presence of IgM and IgG antibodies to human parvovirus B19 (Biotrin, Dublin, Ireland) and rubella virus (Beckman Access, Chaska, Minn.). All sera were positive for measles virus-specific IgM and negative for parvovirus B19- and rubella virus-specific IgM. Total RNA was extracted directly from 100 μl of the clinical specimen by a guanidinium isothiocyanate technique (2). Following reverse transcription of RNA with random primers and avian myeloblastosis virus RT, specific primers targeted to the nucleoprotein gene of the measles virus Edmonston strain were used in a heminested RT-PCR to amplify a 528-bp measles virus-specific fragment (1). Statistical analysis was performed with Stata statistical software (release 6.0; Stata Corporation, College Station, Tex.). A 95% confidence interval (CI) for the proportion of positive samples for each specimen type was calculated by using the binomial distribution. Tests for association were performed by chi-square or Fisher’s exact test, as appropriate. Multiple-comparison testing was used to investigate differences in proportions between specimen types.

A total of 249 samples collected from 74 patients with confirmed measles were available for RT-PCR analysis. Not all patients provided all four specimen types. Thirty-six patients provided at least one sample each of PBLs, urine, TS, and serum. The TS sample was the only positive sample in six of these patients, whereas in two patients each the urine or PBL sample was the only positive sample. Table 1 shows the proportion (with 95% CI) of measles virus RNA-positive samples for each specimen type according to days postonset of the rash (day of rash onset = day 0). Overall, at least one RT-PCR-positive sample was obtained from 48 of the 74 (65%) measles virus-specific IgM-positive patients. Measles virus RNA was recovered from a high proportion of TS, urine, and PBL samples compared to the proportion of serum samples from which it was recovered in the first 3 days after rash onset. Only the difference between TS and serum samples approached significance (P = 0.054). Between days 4 and 7, measles virus RNA was detected in TS and urine samples significantly more often than in serum samples (P = 0.002 and 0.032, respectively), but there was no significant difference in RT-PCR detection rates between PBL, urine, and TS samples.

During the second week after onset of rash, TS and PBL samples yielded a higher proportion of measles virus RNA-positive samples than urine samples, although no difference between specimen type reached significance. Fourteen days or more after the onset of a rash, the rate of RNA detection for all specimen types was significantly lower than that of the first 14 days (14 versus 43% [P < 0.001]) but was not significantly different between PBL, urine, and TS samples. When comparing specimen types for all samples collected during the outbreak, only TS samples remained significantly more likely than serum samples to be RT-PCR positive (P = 0.005).

Serum samples were significantly more likely to be RNA positive before the appearance of an IgG response. Prior to the appearance of IgG, 23 of 56 (41%) serum samples were positive for measles virus RNA. However, only 2 of 47 (4%) serum
samples were positive once specific IgG was present \((P < 0.001)\). A 1992 study has suggested that measles virus viremia may be prolonged in patients with more severe disease (3). Twenty-eight of the 74 patients with confirmed cases (38%) were hospitalized, and our results suggest that disease severity, using hospitalization as a surrogate marker for severe disease, was not associated with an increased rate of detection of viral RNA by RT-PCR \((P = 0.8)\).

Diagnosis of acute measles virus infection based on clinical presentation may have a positive predictive value as low as 5% during nonepidemic periods (5), and in low-prevalence countries, laboratory confirmation by IgM serology is important. RT-PCR provides a rapid and sensitive method for the detection of measles virus RNA from a variety of clinical specimens (4, 7–10) and plays an important supporting role in measles diagnosis. In our hands, the measles virus RT-PCR is 1,000-fold more sensitive than virus isolation in B<sub>92a</sub> cells (data not shown). We have found RT-PCR for measles virus to be useful for clarification and confirmation of measles virus infection in a variety of circumstances, including atypical or complicated clinical presentations, cases in which IgM reactivity to multiple viruses is present, and cases in which serum for antibody testing is not available. Combined with sequencing, RT-PCR facilitates identification of measles virus genotypes (1) and differentiation between vaccine-associated and wild-type measles virus infection (4).

We conclude that a TS sample is the preferred specimen for the detection of measles virus RNA by RT-PCR. Collection of multiple specimens does not enhance the recovery rate sufficiently to justify this as routine practice. In cases in which serum collected before development of a measles virus IgG antibody response is the only specimen available, RT-PCR may be worth attempting. While the sample size in this study was too small for most relative differences in recovery rate between specimens to reach significance, it is unlikely that a sufficiently large set of high-quality specimens could be collected in countries with high measles vaccination rates. The current study is therefore likely to provide the best indication of optimal specimen types for the recovery of measles virus RNA.

We thank Stephen Lambert Department for Human Services, Melbourne, Victoria, Australia, for clinical data, Debbie Gercovich for collection of samples, Jennie Leydon for serology testing, and Graham Byrnes for statistical advice.

**REFERENCES**


**TABLE 1. Analysis of measles virus RT-PCR positivity with clinical samples according to days postonset of rash (70 patients) or other clinical symptoms (4 patients)**

<table>
<thead>
<tr>
<th>Days postonset of rash</th>
<th>Serum</th>
<th>PBLs</th>
<th>Urine</th>
<th>TS</th>
<th>All specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of samples&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No. (%; 95% CI) positive</td>
<td>No. of samples&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No. (%; 95% CI) positive</td>
<td>No. of samples&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>&lt;0–3</td>
<td>64</td>
<td>22 (34; 23–47)</td>
<td>10</td>
<td>7 (70; 35–93)</td>
<td>15</td>
</tr>
<tr>
<td>4–7</td>
<td>21</td>
<td>2 (10; 1–30)</td>
<td>12</td>
<td>5 (42; 15–72)</td>
<td>15</td>
</tr>
<tr>
<td>8–13</td>
<td>7</td>
<td>0</td>
<td>5</td>
<td>3 (60; 15–95)</td>
<td>6</td>
</tr>
<tr>
<td>14–20</td>
<td>4</td>
<td>0</td>
<td>6</td>
<td>2 (33; 4–78)</td>
<td>7</td>
</tr>
<tr>
<td>&gt;21</td>
<td>2</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>No. rash&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5</td>
<td>1 (20; 0.5–72)</td>
<td>3</td>
<td>1 (33; 0.8–91)</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>103</td>
<td>25 (24; 16–34)</td>
<td>42</td>
<td>18 (43; 28–59)</td>
<td>53</td>
</tr>
</tbody>
</table>

<sup>a</sup> Twenty-four patients submitted more than one serum sample, four patients submitted more than one urine and TS sample each, one patient submitted more than one serum, urine, and TS sample each.

<sup>b</sup> Specimens collected up to 19 days postonset of symptoms. Specimens which were PCR positive were collected up to 7 days postonset of symptoms.