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Detection of Measles Virus RNA in Urine Specimens from Vaccine Recipients

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Analysis of urine specimens by using reverse transcriptase-PCR was evaluated as a rapid assay to identify individuals infected with measles virus. For the study, daily urine samples were obtained from either 15-month-old children or young adults following measles immunization. Overall, measles virus RNA was detected in 10 of 12 children during the 2-week sampling period. In some cases, measles virus RNA was detected as early as 1 day or as late as 14 days after vaccination. Measles virus RNA was also detected in the urine samples from all four of the young adults between 1 and 13 days after vaccination. This assay will enable continued studies of the shedding and transmission of measles virus and, it is hoped, will provide a rapid means to identify measles infection, especially in mild or asymptomatic cases.

Despite the existence of an effective vaccine, measles virus continues to cause sporadic outbreaks and epidemics of disease in the United States and throughout the world. Most recent outbreaks have involved either children who were too young to be vaccinated or older children and teenagers (5 to 19 years), most of whom had been previously vaccinated (3, 8). Because of the sporadic nature of outbreaks in populations with high rates of vaccination, the altered presentation of clinical signs that occurs in "mild measles" infections (1, 11, 20), and the presence of other exanthem-causing infections, effective public health measures to control measles outbreaks are more dependent on laboratory confirmation of infection than on diagnosis based on clinical presentation. Currently available diagnostic techniques, which include virus isolation, viral antigen detection, and serologic antibody studies, are very sensitive and specific. However, these techniques are labor intensive, require specimen collection by medically trained personnel, and would be inappropriate for screening large numbers of individuals.

The detection of measles virus RNA in urine by using reverse transcriptase-PCR (RT-PCR) would be a potentially rapid means of detecting measles infections with a clinical specimen which is more readily and conveniently accessible than serum or nasopharyngeal aspirates. Collection of urine specimens could be done in the absence of medical professionals, and on-site specimen-processing requirements are minimal. Measles virus can be isolated from urine specimens from infected individuals for as long as 10 days after the onset of the rash (16, 28), and measles antigen has been detected by immunofluorescence in urine samples from asymptomatic case contacts (5).

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Since urine specimens from naturally infected individuals were unavailable at the time this study was conducted, the RT-PCR assay was evaluated by using specimens obtained from recently vaccinated individuals. In all cases, RNA was extracted from urinary sediment by the guanidinium acid-phenol method (9) and resuspended in 25 μl of RNase-free water.

For the measles virus-specific RT-PCR, a nested set of primers that hybridized to the nucleoprotein (N) gene was used (MV41, CAT TAC ATC AGG ATC CGG; and MV42, GTA TTG GTC CGC CTC ATC). The internal primers (MV43, digoxigenin [DIG] -GA GCC ATC AGA GGA ATC A; and MV44, DIG-CA TGT TGG TAC CTC TTG A) were 5′ labeled with DIG. The target sequences for these primers are located between bases 57 and 389 of the coding region of the N gene, and these sequences are conserved among the N genes of all wild-type measles viruses examined thus far (24). DIG-5′-labeled primers that amplified beta-actin mRNA (BA4 and BA1) were used as controls for RNA extraction.

Before the RT reaction, the RNA was heated to 95°C for 90 s and then placed on ice. The RT reaction mixture contained 50 mM Tris-HCl (pH 8.3), 8 mM $MgCl_2$, 30 mM KCl, 5 mM dithiothreitol, 1 mM each deoxynucleoside triphosphate (dNTP), 10 μM each forward and reverse primer (MV41 and MV42 or BA1 and BA4), 24 U of avian myeloblastosis virus RT, and 40 U of human placental RNase inhibitor. The reaction mixture was incubated at 42°C for 45 min and then at 95°C for 5 min.

For PCR, 5 μl of the cDNA sample was added to a 45-μl PCR mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 200 μM each dNTP, 5 μM each primer (MV43 and MV44 or BA1 and BA4) and 5 U of *Taq* DNA polymerase. PCR conditions were as follows: 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min. After 39 cycles, 20 μl of each sample was analyzed by electrophoresis on a 1.5% agarose gel. DNA was visualized by ethidium bromide staining and UV illumination. Immunochemiluminescence detection of PCR products that were not visible after ethidium bromide staining was performed as described previously (10).

In all RT-PCR assays, samples containing water were used as contamination controls. Positive control RNA was extracted from Vero cells that had been infected with measles virus, and negative control RNA was extracted from uninfected Vero cells or from urine specimens donated by laboratory personnel who had not recently been vaccinated. For measurement of the

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