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Polymerase Chain Reaction for Detection of Measles Virus in Clinical Samples

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A rapid and sensitive one-step reverse transcription polymerase chain reaction assay was developed to detect measles virus (MV) in nasal aspirates from patients with suspected MV infection. Oligonucleotide primers and probe were targeted to highly conserved regions of the matrix gene. Assay conditions were optimized to allow detection of as little as 1 PFU of an MV stock whose titer was known. Extraction of RNA from 38 nasal aspirates and then reverse transcription and MV matrix gene amplification yielded a polymerase chain reaction product of the predicted size in 14 of 14 MV culture-positive patients. Matrix gene amplification provides a rapid, sensitive, and specific supplementary assay to the currently available modalities for MV detection.

Despite the availability of an effective vaccine, sporadic and epidemic measles virus (MV) infection continues to occur in the United States. The clinical diagnosis of classical MV infection in the setting of a high prevalence of disease in the community poses no problem to the experienced clinician. With increasing frequency, however, patients are presenting with incomplete clinical syndromes and often lack the characteristic rash (10, 13). In addition, sporadic outbreaks of MV have increased the importance of making an accurate and rapid diagnosis to allow implementation of public health measures to contain the spread of disease. In these situations, clinicians must rely on the laboratory for assistance in the diagnosis of MV infection. Serology, culture, and antigen detection are the currently available diagnostic modalities. While isolation of the virus in monkey kidney cells provides unequivocal evidence of MV infection, the procedure requires expedited handling of clinical samples obtained early in the course of the disease and results are not available for 2 to 3 weeks. The indirect fluorescent antibody test (IFA) provides a more rapid alternative which allows MV antigen detection in nasal aspirates and urine (14, 23). The technique, however, requires experienced personnel to interpret the assay, which is subject to interobserver variability. Assays for the detection of MV-specific immunoglobulin M (IgM) and rising titers of MV-specific IgG provide additional alternatives for documentation of MV infection. Serologic methods, however, require that a convalescent-phase serum sample (IgG) be obtained and cannot be used in patients with defects in antigen-specific antibody production (e.g., human immunodeficiency virus-infected patients). Gene amplification followed by nucleic acid hybridization provides a sensitive and specific alternative method for the diagnosis of viral infections (7, 19). We used oligonucleotide primers and a probe which recognize highly conserved regions of the MV matrix gene for use in a gene amplification assay. We report here the results of this assay for the detection of the MV genome in nasal secretions from patients and controls.

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MATERIALS AND METHODS

Virus and cell culture. Moraten vaccine strain virus (Attenuvax; Merck Sharp and Dohme, West Point, Pa.) was reconstituted in 0.7 ml of sterile water and was diluted with 2.8 ml of Dulbecco's modified Eagle medium (Mediatech, Herndon, Va.) plus 2% heat-inactivated fetal calf serum (Gemini Bioproducts, Calabasas, Calif.). Virus was allowed to adsorb to Vero cells (80% confluent in a 25-cm^2 flask) for 4 h at 35°C in 5% CO_2. The medium was removed and replaced with Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum–5 mM glutamine–100 U of penicillin per ml–100 μg of streptomycin per ml. Virus was harvested at 3 days when typical multinucleated giant cells covered approximately 80% of the monolayer. For experiments to determine the sensitivity of the reverse transcription (RT) polymerase chain reaction (PCR) assay, we used Edmonston MV of known titer (10^6 PFU/ml), which was kindly donated by Stephen Udem, New Jersey Medical School, Newark.

Preparation of viral RNA. Infected Vero cells from a 25-cm^2 flask were washed once with phosphate-buffered saline (PBS; pH 7.2) and lysed directly by previously published protocols for acid guanidinium isothiocyanate extraction of RNA (4). Briefly, cells were lysed in 500 μl of a chaotropic solution (4 M guanidinium isothiocyanate [GIBCO BRL Gaithersburg, Md.], 25 mM sodium citrate [pH 7.0], 0.5% sarcosyl, 0.1 M 2-β-mercaptoethanol) and the RNA was extracted by the sequential addition of 50 μl of 2 M sodium acetate (pH 4.0), 500 μl of phenol, and 100 μl of chloroform-isoamyl alcohol (24:1). The suspension was vortexed for 10 s, cooled on ice for 15 min, and centrifuged at 10,000 × *g* for 20 min. The nucleic acid was precipitated from the aqueous phase with an equal volume of isopropanol at −20°C for 1 h; this was followed by centrifugation at 10,000 × *g* for 20 min. The pellet was redissolved in 150 μl of the chaotropic solution and was precipitated again in isopropanol at −20°C for 1 h. After centrifugation for 10 min, the RNA pellet was washed with 70% ethanol and was

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TABLE 1. Nucleotide sequence and map position of oligonucleotide primer pairs and probe

Oligonucleotide	Gene	Map position	Sequence (5′→3′)
Primer			
MV01	Matrix	13–35[a]	ATTGCCTCCCAAGTTCCACAATG
MV02	Matrix	247–268	CCTAAGGGCAGGGACCCAAATG
MV03	Matrix	499–520	GTGTTGTTTATATGAGCATCAC
MV04	Matrix	727–749	CATTTTGCAATAATCGGCAGAGT
MV05	Matrix	891–913	AATCGATTAAGGTCTTCATTGAT
MV06	Nucleocapsid	1–23[b]	ATGGCCACACTTTTAAGGAGCTT
MV07	Nucleocapsid	460–482	TCAGGGTCTTGCACTTCAATATC
Probe	Matrix	809–838[a]	CATTAGAAGCACAGGCAAAATGAGCAAGAC

[a] Matrix gene position as described previously (2).
[b] Nucleocapsid gene position as described previously (17).

dissolved in 0.1% diethylpyrocarbonate-treated sterile water. The RNA concentration was determined by measuring the A_{260}. An absorption of 1.0 unit at an optical density of 260 nm was assumed to be 40 μg of RNA per ml.

Plasmids. Purified plasmid DNA containing a full-length MV cDNA cloned into the Bluescript vector [peMV(−)] (1) (kind gift of Stephen A. Udem) was used for quantitative assessment of MV amplification.

Oligonucleotide primers. Oligonucleotide primers were the kind gift of John J. Sninsky and David Mack (Roche Molecular Systems, Alameda, Calif.), who designed three sets of primer pairs from highly conserved regions of the MV genome identified by computer-assisted analysis of nucleic acid sequence alignments of MV with two other morbilliviruses (canine distemper virus and rinderpest virus) (see Fig. 1). The conserved regions among these viruses were targeted to increase the probability that all wild-type isolates would share the genomic sequence recognized by the primers. We tested the relative sensitivity of the different primer pairs by amplification of peMV(−) in a 20-μl reaction mixture containing 1× PCR buffer (10 mM Tris [pH 8.0], 50 mM KCl, 2.5 mM $MgCl_2$), 0.5 μM (each) oligonucleotide primers, 200 μM (each) deoxynucleoside triphosphates (dUTP, dATP, dCTP, dGTP), and 2 U of *Taq* DNA polymerase (Perkin-Elmer, Norwalk, Conn.). The mixture was overlaid with 20 μl of mineral oil, and gene amplification was carried out in a thermal cycling device (MJ Research, Cambridge, Mass.). The cycling conditions were 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min for 40 cycles, with a final extension step at 72°C for 5 min.

An additional primer (MV04) was designed in the antisense orientation to be used with the MV03/MV05 product as a nested primer to provide additional confirmation of the sequence of the amplified product (Table 1).

Control amplifications for the extracted nucleic acid in the clinical samples were performed with oligonucleotide primers from the HLA-DQα gene (18), yielding a 242-bp PCR product.

Clinical samples, IFA, and virus culture. Nasal aspirates in sterile PBS were collected from 38 children evaluated for MV and other respiratory virus infections as described previously (23). The majority of samples were obtained during the MV outbreak in Chicago in 1989. Aliquots (0.25 ml) of antibiotic-treated specimens were inoculated onto the full range of cell lines routinely used for virus isolation, including the RMK and CV-1 cell lines. Monolayers were observed for a cytopathic effect two times per week for a period of 4 weeks. Confirmation of virus isolates was performed by IFA as reported previously (23). Aliquots of aspirates were stored at −70°C for up to 3 years before analysis by PCR. Coded samples were submitted for PCR testing, and the patient's diagnosis was unknown to the investigators who performed the assays (H.S. and J.C.B.).

RT-PCR for nasal aspirates. RNA was extracted from 300 μl of the nasal aspirates by the acid guanidinium isothiocyanate method in the presence of 25 μg of carrier yeast (*Saccharomyces cerevisiae*) tRNA. After the addition of 500 μl of guanidinium isothiocyanate solution, the extraction was carried out as described above. The resulting pellet was resuspended in 20 μl of diethylpyrocarbonate-treated water, and 5 to 8 μl was used for amplification by a one-step RT PCR protocol modified from Godec et al. (8). In the optimized PCR protocol, RNA samples were overlaid with 20 μl of light mineral oil in microcentrifuge tubes (0.5-ml Micro Test Tubes "EZ"; Bio-Rad Laboratories, Richmond, Calif.), heated at 90°C for 10 min, and quick chilled on ice. The RT PCR mixture (chilled on ice) was added to each tube to yield a 25-μl reaction volume containing 1× PCR buffer (as above), 0.5 μM (each) primers MV03 and MV05, 200 μM (each) deoxynucleoside triphosphates (dUTP, dATP, dGTP, dCTP), 1 to 2 U of *Taq* DNA polymerase, and 25 U of Moloney murine leukemia virus reverse transcriptase (GIBCO BRL). The tubes were then placed directly into the thermal cycler (prewarmed to 42°C), and the following cycling profile was initiated: 42°C for 5 min, 94°C for 3 min (94°C for 1 min, 60°C for 1 min, 72°C for 1 min) (the steps in parentheses were repeated for 39 cycles), and 72°C for 5 min. The PCR product was held at room temperature until gel analysis.

Amplification of the HLA-DQα gene was accomplished by the protocol described above, with the following modifications. The total reaction volume was 10 μl with 2 μl of nucleic acid template, 0.5 μM HLA-DQα primers, and 10 U of Moloney murine leukemia virus reverse transcriptase. The reverse transcriptase was included only to control for any inhibitory effect on the PCR.

We used the following control templates for amplifications: for MV03/MV05 amplifications, 17 to 34 ng of RNA extracted from MV-infected Vero cells; for HLA-DQα amplifications, 0.6 μg of human genomic DNA (positive control) and 5 μg of yeast tRNA (negative control).

Analysis of PCR product. We analyzed 10 μl of PCR product on 2% agarose gels (electrophoresis-grade agarose; Fisher Scientific, Fair Lawn, N.J.) stained with 20 μg of ethidium bromide per 30 ml of gel and compared the molecular sizes with a 123-bp ladder (GIBCO BRL). For Southern transfer, gels were denatured for 20 min in 1.5 M NaCl–0.5 M NaOH and were transferred to a nylon membrane (Mag-

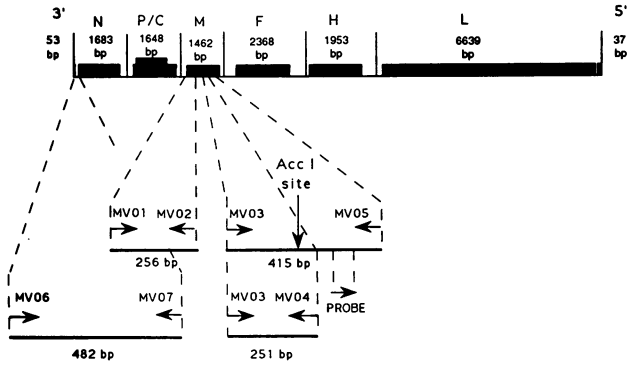

FIG. 1. Schematic representation of the MV genome with locations of the oligonucleotide primers for PCR, nonoverlapping internal probe, and *Acc*I restriction site in the PCR product. The black bars represent the open reading frame for each gene (6). Key for MV genes: N, nucleocapsid gene; P/C, phosphoprotein-nonstructural protein gene; M, matrix gene; F, fusion gene; H, hemagglutinin gene; L, polymerase gene. Arrows for each primer indicate the direction of priming from cDNA. Numbers are molecular sizes (in base pairs).

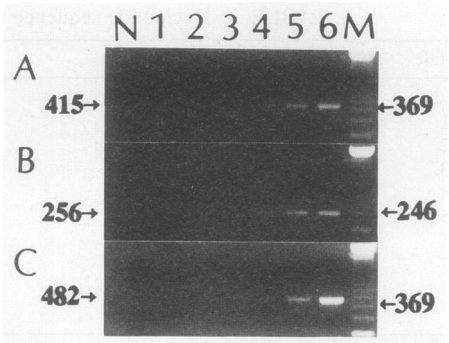

FIG. 2. Analysis of the PCR product from amplifications of serially diluted full-length MV plasmid [peMV(−)] with three different primer pairs. A 10-μl aliquot of each PCR was resolved on a 2% agarose gel containing 0.6 μg of ethidium bromide per ml. (A) Amplification with MV03/MV05; a 415-bp product is faintly visible in lane 3. (B) Amplification with MV01/MV02. (C) Amplification with MV06/MV07. Lanes: M, molecular mass marker (123-bp ladder); N, no template; lanes 1 to 5, PCR product from amplifications with the following increasing concentrations of peMV(−): 0.5 fg (lane 1), 5 fg (lane 2), 50 fg (lane 3), 500 fg (lane 4), 5 pg (lane 5), and 50 pg (lane 6).

nagraph; Micron Separation, Inc., Westboro, Mass.) under vacuum (model 785 Vacuum Blotter; Bio-Rad) at 5 in. (ca. 12.7 cm) Hg for 30 min in 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). DNA was cross-linked to the membrane by exposure to 120 mJ of UV radiation (UV Crosslinker; Fisher Scientific) per cm^2. Membranes were prehybridized for 15 min at 68°C in 1 ml of QuikHyb (Stratagene, La Jolla, Calif.) and were hybridized overnight at 68°C with labeled probe in 50 μl of salmon sperm DNA (10 mg/ml). Oligonucleotide probes were labeled by a standard kinase method (20) with [γ-^{32}P]ATP (3,000 Ci/mmol) to a specific activity of 1.4×10^8 cpm/μg. Membranes were washed twice in 2× SSC–0.1% sodium dodecyl sulfate (SDS) at room temperature for 15 min and once in 0.1× SSC–0.1% SDS at 55°C for 30 min. Hybridized probe was detected by autoradiography with an intensifying screen for 0.5 to 4 h at −70°C.

To further confirm the specificity of the PCR product, 1 μl of PCR product was reamplified (under the conditions described above) for nine cycles, with the MV04 hemi-nested primer used in conjunction with MV03, and the PCR product was analyzed by electrophoresis in a 4% gel (3% NuSieve agarose, 1% standard agarose).

Additional confirmation of the specificity of the PCR product was derived from restriction enzyme digestion of 10 μl of PCR product with 4 U of *Acc*I (GIBCO BRL) incubated overnight at 37°C. Digestion of the amplified product was predicted to yield two fragments (191 and 224 bp), which were resolved by analysis on a 4% agarose gel.

RESULTS

Selection of primers. The structure of the MV genome and the location of the primer pairs are shown in Fig. 1. We tested the sensitivity of the three primer pairs at different concentrations of template [peMV(−)] (Fig. 2). The results indicated superior amplification with the MV03/MV05 primers. The remaining primer pairs were not examined further. We determined the optimal concentration of $MgCl_2$ (2.5 mM) and the optimal annealing temperature (60°C) for this primer pair (data not shown). All subsequent assays were performed with the MV03/MV05 primer pair under optimal conditions, which yielded a single band of 415 bp. Sequence alignments with five wild-type isolates collected in the United States from 1983 to 1989 revealed 100% sequence homology with MV04 and a single-base-pair mismatch for one of five isolates for MV03 and four of five isolates for MV05. No mismatches were located at the 3′ end of the primer, and all mismatches resulted in T:G pairing (11, 16a).

Sensitivity of PCR for the MV genome. The sensitivity of our PCR assay for detection of the MV genome is shown in Fig. 3. As few as 3,000 virus equivalents as cDNA (50 fg of plasmid DNA) could be detected by direct examination of 50% of the PCR product from a 20-μl reaction resolved on a 2% agarose gel stained with ethidium bromide. Southern hybridization increased the sensitivity of detection down to 30 virus particle equivalents as cDNA (0.5 fg). Amplification of as little as 1 PFU of MV stock of known titer yielded a clear ethidium bromide-stained band of the predicted molecular mass (data not shown).

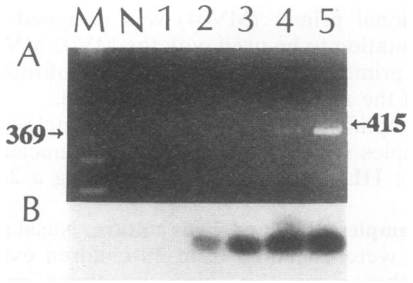

FIG. 3. Assessment of the lower limit of detection of MV plasmid amplified with the MV03/MV05 primer pair: (A) A 10-μl aliquot of each PCR resolved on a 2% agarose gel containing 0.6 μg of ethidium bromide per ml. (B) Southern blot of the gel in panel A hybridized with a ^{32}P-labeled oligonucleotide probe. Lanes: M, molecular mass marker (123-bp ladder); N, no template; lanes 1 to 5, PCR product from amplifications with the following increasing concentrations of peMV(−): 50 ag (lane 1), 500 ag (lane 2), 5 fg (lane 3), 50 fg (lane 4), and 500 fg (lane 5).

TABLE 2. Results of diagnostic assays for MV infection

RT-PCR result	Sample no.	Age (yr)	Culture results[a]		IFA	HLA PCR
			RMK	CV-1		
PCR positive (n = 18)	1	4	10	−	+	+
	3	2.3	17	22	+	+
	5	4	14	13	+	+
	9	7	15	31	+	+
	14	1.3	15	−	+	+
	15	0.9	12	15	+	+
	16	0.7	23	23	+	+
	18	10	16	−	+	+
	22	3	21	−	+	+
	28	5	12	−	+	−
	30	1	16	−	+	+
	7	0.7	17	17	−	+
	11	0.5	17	−	−	+
	12	1.2	14	19	−	+
	19	2.9	−	−	+	+
	24	4	−	−	+	+
	31	1	−	−	+	+
	13	1.3	NA[b]	NA	+	+
PCR negative (n = 20)	25	14	−	−	+	−
	34	0.8	−	−	+	+
	35	4	−	−	+	+
	2	0.2	−	−	NA	+
	4	0.2	−	−	NA	+
	6	0.1	−	−	NA	+
	8	0.9	−	−	NA	+
	10	0.5	−	−	NA	+
	20	0.4	−	−	NA	+
	26	0.1	−	−	NA	+
	32	0.4	−	−	NA	+
	33	0.1	−	−	NA	+
	36	0.7	−	−	NA	+
			Culture result			
	17	3	Influenza virus type B		NA	+
	21	0.6	Influenza virus type A		NA	+
	23	0.3	RS virus[c]		NA	+
	27	2.4	Parainfluenza II		NA	+
	29	0.8	Parainfluenza III		NA	+
	37	0.7	RS virus		NA	−
	38	0.6	Enterovirus		NA	+

[a] Numbers indicate the elapsed time (in days) from inoculation of the sample to a cytopathic effect; minuses indicate negative results. RMK, rhesus monkey kidney cells; CV-1, African green monkey cell line.
[b] NA, not available.
[c] RS virus, respiratory syncytial virus.

Analysis of clinical samples. We examined nasal aspirates from 38 infants and children (mean age, 2.14 ± 2.89 years; range, 0.1 to 14 years) enrolled in a study to compare methods of MV diagnosis. For all patients, a viral culture was performed on a 0.25-ml aliquot of a nasal secretion. For a subset of patients, IFA was also performed for MV antigen detection and MV serology. The results of these assays and our PCR studies are summarized in Table 2. A single PCR product of the predicted size (415 bp) was amplified from 300 μl of nasal aspirate from the 14 patients from whom MV was cultured. The derivation of the PCR product from the MV genome was confirmed by *Acc*I digestion of the PCR product and hemi-nested amplification with the MV03/MV04 primer pairs, which yielded the anticipated fragments (191 and 224 bp for the *Acc*I digestion and 251 bp for the hemi-nested PCR) when resolved on a 4% agarose gel (data not shown).

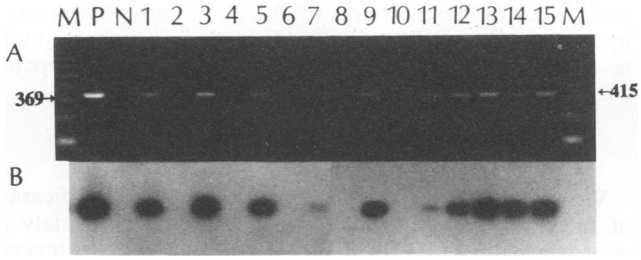

FIG. 4. One-step RT-PCR amplification of MV in nasal aspirates from infected and control patients. A representative analysis of 15 unselected samples is shown. Lanes: M, molecular mass marker (123-bp ladder); P, positive control (17 ng of RNA extracted from MV-infected Vero cells); N, negative control (5 μg of yeast tRNA; lanes 1 to 15, 10-μl aliquots of PCR product from amplification of RNA extracted from 300 μl of coded nasal aspirates. The lane numbers are identical to the sample numbers used in Table 2. (A) A 2% agarose gel containing 0.6 μg of ethidium bromide per ml. (B) Southern blot of the gel in panel A hybridized with ^{32}P-labeled oligonucleotide probe.

Southern hybridization with an MV-specific oligonucleotide probe further confirmed the identity of the PCR product (Fig. 4).

For four patients (patients 19, 24, 31, and 13) for whom virus culture results were either lost (patient 13) or negative (three patients), the MV genome was detected by PCR analysis of their nasal secretions that were also positive by IFA. Three patients (patients 19, 24, and 31) had positive IgM serology for MV. No serology was available for patient 13. All four patients had fever and Koplik spots and an exposure history consistent with MV infection.

Three patients (patients 25, 34, and 35) were positive for MV by IFA but were negative for MV by virus culture and PCR. No amplifiable nucleic acid could be detected in the nasal secretion of one of the patients (patient 25). The presence of an inhibitor to PCR DNA amplification was ruled out by spiking 0.6 μg of human genomic DNA into the reaction with the HLA-DQα primers, which yielded the anticipated 242-bp product. Repeat extraction of an aliquot of nasal secretion from this patient again failed to yield amplifiable nucleic acid. Serology was negative for IgM and positive for IgG on the second day of her illness, which suggests that the patient did not have acute MV infection.

For the two remaining IFA-positive, culture-negative patients, no MV genome was detected in their nasal secretions, even though HLA-DQα sequences were detected. In one patient with a negative culture (patient 35), there was a delay of 4 days before the aspirate was processed. IFA of this sample revealed only one positively staining cell. Culture of a sample from this patient obtained 1 day previously and processed appropriately was positive, and IFA revealed two positively staining cells. PCR assay of this sample yielded the diagnostic 415-bp amplification product (data not shown). Therefore, PCR results agreed with the culture results for this patient.

Patient 34 had been immunized with the measles-mumps-rubella vaccine 2 months prior to the onset of his illness, which was characterized by fever associated with a diffuse rash but no evidence of conjunctivitis or Koplik spots. The possibility that the IFA results were falsely positive for this patient and patient 25 appears likely.

The remaining 17 patients had no evidence of MV infection by culture, IFA, or PCR. A different virus was cultured

from seven of these patients (Table 2), but no cross-reactivity was noted by PCR with the MV-specific primers. Amplification with the HLA-DQα primers yielded the anticipated 242-bp product in 16 of 17 patients.

DISCUSSION

We reported here the sensitive and specific amplification of the MV genome from nasal aspirates from acutely ill pediatric patients. A PCR assay that permits the reverse transcription of extracted RNA and the amplification of the cDNA in a single tube was devised. The assay was sufficiently sensitive to permit direct visualization of the resolved PCR product on a 2% agarose gel without a need for nested amplification or Southern hybridization except to further confirm the identity of the amplified sequence.

The PCR assay compared favorably with culture and IFA. The rapid availability of a result (1 versus 10 to 23 days) and the enhanced sensitivity (samples from three patients were culture negative but PCR, IFA, and MV IgM positive) were distinct advantages of the PCR assay over culture for MV. The ease of interpretation of PCR results and the ability to perform the test on archival samples were additional advantages. The potential problems of false-positive IFA results are highlighted by the conflicting data for patients 25 and 34. In choosing the optimal assay for any given clinical setting, one must weigh the possible disadvantages of the PCR (potential for contamination resulting in false-positive results and the labor-intensive nature of the procedure) against the advantages (rapid availability of easily interpreted results).

The oligonucleotide primers and probe used in our study were specifically designed to amplify conserved regions of the genome shared by other members of the *Morbillivirus* genus. While clinical isolates of MV all share serologic cross-reactivities, genomic sequence variation has been documented in wild-type isolates (5, 16, 24). Our primers would be expected to amplify the RNA of currently circulating MV isolates on the basis of analysis of matrix gene sequences from clinical isolates from the United States and the published sequences of four wild-type isolates from Great Britain (5, 22). All four isolates shared 100% homology with the MV03 and MV05 primers and three of four isolates had a single-base-pair mismatch with the MV04 primer. The MV03/MV05 primer pair successfully amplified a sequence of the predicted length from canine distemper virus RNA, further confirming the broad range of this primer pair (data not shown).

The amplifications of HLA-DQα sequences used to verify the presence of amplifiable nucleic acid were negative from three patients. In one case (patient 28), the MV amplification was positive, which suggested the preferential stability of the encapsidated MV genome compared with that of cellular nucleic acid during storage and freeze-thaw cycles. Use of primers that hybridize to different exons of a host cell gene (e.g., β-actin primers from exons 4 and 5 [3]) and that therefore yield PCR products of different lengths from RNA or DNA templates would be suitable controls for this RT PCR assay.

Other reports have described PCR assays for detection of the MV genome in specialized clinical settings. Godec et al. (8) and Schmid et al. (21) designed primers from different MV genes to amplify MV sequences from frozen and formalin-fixed brain tissues from patients with subacute sclerosing panencephalitis. Jackson et al. (9) used PCR primers from the nucleocapsid gene to amplify MV RNA from lung tissue from an immunocompromised patient who died of giant cell pneumonia. In none of those studies was an effort made to choose primers capable of hybridizing to a potentially wide range of different MV isolates. In a fourth study, degenerate primers were designed to amplify a conserved region in the nucleocapsid genes of MV, canine distemper virus, parainfluenza virus type 3, and Sendai virus (15). These studies, in conjunction with the present one, document the wide range of primers available for detection of MV in different clinical and research applications.

In designing our assay, we made efforts to eliminate, where possible, opportunities for PCR contamination by both the exogenous template and the PCR product. The reverse transcription and gene amplification steps were carried out in a single reaction tube, with the RNA template protected by an overlay of mineral oil. Incorporation of dUTP into the reaction mixture would have permitted use of uracil-*N*-glycosidase, according to published protocols (12), if contamination of reactions by previously synthesized product had occurred.

In summary, we reported the sensitive and specific detection of MV matrix gene sequences in nasal aspirates from infected patients. This assay should provide a useful addition to the currently available modalities for the detection of MV infection.

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