Comparison of a Lateral-Flow Immunochromatography Assay with Real-Time Reverse Transcription-PCR for Detection of Human Metapneumovirus[▽]

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Received 22 September 2007/Returned for modification 30 November 2007/Accepted 20 December 2007

A lateral-flow immunochromatography (IC) assay for the detection of human metapneumovirus (hMPV) has been developed by using two mouse monoclonal antibodies to the nucleocapsid protein of hMPV. The purpose of this study was to compare the virus detection rate in nasopharyngeal secretions by the IC assay with that by real-time reverse transcription-PCR (RT-PCR). We collected nasopharyngeal swab samples from 247 children with respiratory symptoms in Sapporo, Japan, during the period from April to July 2007. Sixty-eight of the 247 children were positive for hMPV by real-time RT-PCR. When the real-time RT-PCR was used as the reference standard, the IC assay results were positive for 48 of the 68 real-time RT-PCR-positive children (70.6% sensitivity) and 8 of the 179 real-time RT-PCR-negative children (95.5% specificity). Although the sensitivity of the IC assay is lower than that of real-time RT-PCR, the IC assay is a rapid and useful test for the diagnosis of hMPV infections in children.

Human metapneumovirus (hMPV), first isolated in The Netherlands in 2001, is a member of the genus *Metapneumovirus* of the subfamily *Pneumovirinae* of the family *Paramyxoviridae*. This subfamily also includes the genus *Pneumovirus*, which includes respiratory syncytial virus. hMPV causes upper respiratory tract infection and influenza-like illness but is also associated with lower respiratory tract infections, such as bronchitis, bronchiolitis, and pneumonia (2, 4, 11, 13).

There are four different kinds of methods for the diagnosis of hMPV infections: serological study, virus isolation by culture, RNA detection by reverse transcription-PCR (RT-PCR), and antigen detection (2, 3, 4, 5, 6, 11). Serological study is important for the retrospective differentiation between primary infection and reinfection with hMPV (4). Isolation of viruses in cell cultures is considered the “gold standard” for detection. However, hMPV is difficult to detect by cell culture due to its slow growth and mild cytopathic effects without apparent syncytium formation (4, 11). In a previous study, hMPV was not detected by culture from two-thirds of RT-PCR-positive children. Therefore, RT-PCR is concluded to be the most sensitive and specific procedure for hMPV detection at present (2, 4, 11, 13). However, RT-PCR can be performed only in special laboratories, and it takes more than 6 h to obtain results. An immunofluorescent-antibody (IFA) test with

a mouse monoclonal antibody (MAb) to hMPV has been reported to enable the detection of hMPV antigens of hMPV-infected cells in nasopharyngeal secretions (5). However, the IFA test requires a highly trained technologist to interpret the staining results and a fluorescence microscope. An immunochromatography (IC) assay is generally inferior to the PCR method with regard to sensitivity and specificity. However, the assay is easy to perform, can be completed in approximately 15 min, and does not require an expert technologist or special instruments. IC assays are widely used for the management of patients with infections caused by respiratory viruses, influenza viruses, respiratory syncytial virus, and adenovirus, especially when other viral assays are unavailable (6). However, at present there are no available IC assays for the detection of hMPV that can be used at the bedside or in an outpatient clinic without special techniques. The development of a new rapid assay for the detection of hMPV is needed in order to prevent nosocomial infections; the prolonged isolation of patients, especially in intensive care units and nurseries; and the unnecessary use of antibiotics. For the rapid diagnosis of hMPV infection, we have applied a previously reported IC assay (8) to the detection of hMPV antigens in nasopharyngeal secretions. The purpose of this study was to compare the virus detection rate in nasopharyngeal secretions by the IC assay with that by real-time RT-PCR.

MATERIALS AND METHODS

Patients and sample collection. Two hundred forty-seven nasopharyngeal swab samples were collected from 100 hospitalized patients and 147 outpatients (age range, 1 month to 12 years) with acute respiratory infections who were

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[▽] Published ahead of print on 3 January 2008.

treated at three private clinics or six hospitals by eligible practicing pediatricians in Sapporo, Japan, during the period from April to July, an epidemic season, 2007. The male-to-female ratio for the 247 patients was 0.9 to 1. The mean age of the 247 children was 1 year and 10 months (age range, 2 months to 8 years and 11 months). All samples were collected after informed consent was obtained from the children's parents.

Each nasopharyngeal swab sample was placed in 600 μ l of extraction buffer and was immediately examined for the presence of the hMPV nucleocapsid (N) antigen by the IC assay. The remainder of each sample was stored at -20°C , transferred to our laboratory (Mitsubishi Chemical Medience Corporation), and examined for the presence of the RNA sequence of hMPV by a real-time RT-PCR based on the fusion glycoprotein (F) gene within 14 days after sampling. In order to determine the time frame for the stability of virus RNA in the extraction buffer, two hMPV samples with different virus concentrations (6.7×10^4 copies/ μ l and 2.0×10^5 copies/ μ l) were examined for their virus titers after storage in extraction buffer at various temperatures (room temperature, 4°C , -20°C , and -80°C) for various periods (1, 7, 10, and 14 days). Analysis of the stability of the hMPV RNA revealed that the samples could be stored for 14 days at any of the temperatures tested other than room temperature without a decrease in the virus concentrations.

Generation and characterization of MAbs against hMPV N protein. A panel of MAbs reactive to hMPV antigens was obtained by immunizing BALB/c mice with LLC-MK2 cells infected with hMPV strain JPS02-76 (subgroup B1; GenBank accession number AY530089) (8). We developed two MAbs to the N protein of hMPV, designated MAbs 3D1 and 5B10, which were characterized by IFA and immunoprecipitation assays with *Trichoplusia ni* (strain Tn5) insect cells infected with a recombinant baculovirus-expressing hMPV N protein (8). Both MAbs were reactive to two groups of hMPV by an IFA assay with two groups of hMPV-infected cells (8).

Lateral-flow IC assay. The IC assay reported previously (8) uses a paper membrane with a gold colloid-conjugated MAb (MAb 5B10) in a liquid phase and an MAb (MAb 3D1) in a solid phase to detect the N protein of hMPV. The sample extract migrates along the membrane, and the N protein of hMPV reacts with the signal antibody (MAb 5B10). Then the hMPV-signal antibody complex reacts with MAb 3D1 and forms a test line that develops within 15 min. The signal antibody also reacts with goat anti-mouse immunoglobulin G (heavy and light chains; Shibayagi Co., Ltd., Ishihara, Japan) and forms a control line. Four drops (approximately 100 μ l) of the sample extract is added to each test device. A sensitivity similar to that obtained with hMPV strain JPS02-76 was obtained with hMPV strain JPS03-180 (subgroup A1; GenBank accession number AY530092) by the IC assay (8). A positive test result is indicated by the presence of the test line and a control line on a white background. A negative test result is indicated by the presence of only the control line.

RNA extraction and cDNA synthesis. Total RNA was extracted from 50 μ l of the specimen extract by using a Sumitest R kit (Medical & Biological Laboratories Co., Ltd., Nagoya, Japan), according to the manufacturer's protocol. Five microliters of each RNA sample was incubated in a solution containing 100 pmol of a primer (F primer [5'-GCTTCAGTCAATCAACAG-3']; GenBank accession number NC_004148; positions 3626 to 3644) specific for the hMPV F gene, 20 nmol of deoxynucleoside triphosphates, and 6 U of Moloney murine leukemia virus reverse transcriptase (Invitrogen, Carlsbad, CA) in a final volume of 20 μ l at 37°C for 60 min to synthesize the cDNA. The specific primer was also used as a forward primer for the real-time PCR assay.

Real-time PCR. cDNA was amplified by a real-time PCR procedure with a LightCycler FastStart DNA Master SYBR green I kit in a LightCycler instrument (Roche Diagnostics K.K., Tokyo, Japan). Each reaction mixture had a total volume of 20 μ l and included 5 μ l of cDNA, 2 μ l of LC buffer, 2 μ l of 25 mM MgCl_2 , and 20 pmol of hMPV F primers. The forward primer sequence was 5'-GCTTCAGTCAATCAACAG-3' (subgroup A1; GenBank accession number NC_004148; positions 3626 to 3644), and the reverse primer sequence was 5'-CCTGCAGATGTTGGCATGT-3' (subgroup A1; GenBank accession number NC_004148; positions 3767 to 3749) (4, 7). The cycling conditions included an initial denaturation step of 10 min at 95°C , followed by 40 cycles of 15 s at 94°C , 10 s at 63°C , and 30 s at 72°C . At the end of each cycle, the fluorescent signal was measured at a wavelength of 530 nm by using a LightCycler fluorimeter. Tenfold serial dilutions of plasmid DNA, which contained one copy of the hMPV strain JPY88-12 (subgroup A2; GenBank accession number AY622381) F gene (1,620 bp) or the hMPV strain JPS03-194 (subgroup B1; GenBank accession number AY530094) F gene (1620 bp), were amplified by the LightCycler PCR. When the threshold cycles were plotted against the $\log_{10}$ of the copy number of the plasmid DNA, linearity was obtained over the range from 1×10^2 to 1×10^7 copies/reaction mixture. When the samples contained 1×10^7 copies/reaction mixture, the predicted 142-bp fragment was identified by electrophore-

sis through a 3.0% agarose gel in two of three reactions. The real-time RT-PCR assay could detect four hMPV subgroups.

Statistical analyses. Quantitative variables were compared by using Welch's *t* test, and qualitative variables were compared by using the chi-square test with Fisher's exact test. For all statistical comparisons, a *P* value of <0.05 was considered to be significant.

RESULTS

Whereas 56 (22.7%) of the 247 samples were positive for hMPV by the IC assay, 68 (27.6%) of the 247 samples were positive by real-time RT-PCR. Eight samples were positive by the IC assay but negative by real-time RT-PCR. They could not be confirmed to be positive for hMPV by another RT-PCR assay with primers specific for a part of the hMPV N gene (data not shown). Four of the eight samples had reactions with very weak intensities by the IC assay. Therefore, the results for the eight IC assay-positive and real-time RT-PCR-negative samples must be regarded as false positive. In contrast, there were 20 IC assay-negative and real-time RT-PCR-positive samples. The possibility of contamination of the virus during the procedures was ruled out by another RT-PCR assay and by real-time RT-PCR-positive results obtained in independent real-time PCR runs. Therefore, the results for the 20 IC assay-negative and real-time RT-PCR-positive samples were considered to be true positive. Therefore, when real-time RT-PCR was used as the reference standard, the IC assay results were positive for 48 of the 68 real-time RT-PCR-positive samples (70.6% sensitivity) and 8 of the 179 real-time RT-PCR-negative samples (95.5% specificity). The agreement of the results of the IC and real-time RT-PCR assays was 88.7%.

The clinical findings, IC assay results, and virus loads for the real-time RT-PCR-positive children are shown in Table 1. The 68 hMPV-positive nasopharyngeal swab samples tested by real-time RT-PCR were collected from 22 hospitalized patients and 46 outpatients. The male-to-female ratio for the 68 children was 0.8 to 1. The mean age of the 68 hMPV-positive children was 2 years and 3 months. Exacerbation of bronchial asthma was observed in 9 (13.2%) of the 68 children. The duration of fever ranged from 1 to 9 days, with a mean duration of 4.9 days and a standard deviation (SD) of 2.0 days. All of the 68 children had cough. Fifty-one (75%) and 14 (21%) of the 68 children suffered from wheezing and dyspnea, respectively. The durations of cough, wheezing, and dyspnea ranged from 2 to 17 days, 2 to 11 days, and 1 to 7 days, respectively. None of the patients had any underlying disease except bronchial asthma.

The numbers of hMPV copies/ml in the 68 real-time RT-PCR-positive samples ranged from 2.24×10^3 to 6.57×10^9 , with a mean of 5.25×10^8 and an SD of 1.12×10^9 . The lowest number of hMPV copies/ml among the IC assay-positive samples was 3.78×10^6 . The mean hMPV copy number for samples that were positive by both the IC assay and real-time RT-PCR was 7.41×10^8 copies/ml, whereas the mean was 5.80×10^6 copies/ml for samples positive by real-time RT-PCR only ($P = 0.00022$).

When onset was defined as the first day of fever, the mean period of sampling from the onset of disease was 3.0 days (range, 0 to 7 days). The onset in two children without fever was defined as the first day of cough. The mean numbers of hMPV copies/ml at 0, 1, 2, 3, 4, 5, and more than >5 days after

TABLE 1. Clinical findings, IC assay results, and virus loads by real-time RT-PCR assay for 68 children with hMPV infection

Case	Age	Sex ^a	Sampling day	Diagnosis	Duration of fever (day)	Wheezing	Dyspnea	IC assay result	No. of copies/ml ^b
1	7 mo	F	0	Wheezy bronchitis, pneumonia	3	+	+	Positive	3.78E + 06
2	3 mo	M	0	Bronchitis	2	+			6.22E + 07
3	4 yr 6 mo	M	0	Pneumonia	1	+			2.72E + 05
4	1 yr 10 mo	M	0	Bronchitis	5			Positive	9.72E + 06
5	2 yr 10 mo	M	1	Bronchitis	3			Positive	8.89E + 07
6	1 yr 4 mo	F	1	Pneumonia	5			Positive	1.29E + 08
7	3 yr 8 mo	F	1	Pneumonia	3				4.95E + 03
8	11 mo	F	1	Wheezy bronchitis	4	+		Positive	4.60E + 08
9	3 yr 11 mo	F	1	Bronchitis	4	+			5.99E + 04
10	1 yr 9 mo	M	1	Bronchitis	2			Positive	6.41E + 08
11	4 yr 4 mo	F	1	Pneumonia	8	+		Positive	3.50E + 08
12	7 mo	M	1	Wheezy bronchitis	9	+	+	Positive	5.02E + 08
13	1 yr 11 mo	F	2	Wheezy bronchitis, pneumonia	7	+		Positive	4.16E + 07
14	1 yr 6 mo	F	2	Pneumonia	4				1.06E + 04
15	4 mo	M	2	Bronchitis	5	+	+	Positive	5.67E + 08
16	9 mo	M	2	Wheezy bronchitis	2	+		Positive	2.51E + 08
17	2 yr 5 mo	M	2	Bronchitis	5	+		Positive	1.95E + 08
18	1 yr 7 mo	M	2	Wheezy bronchitis	3	+		Positive	2.09E + 08
19	2 yr 7 mo	M	2	Wheezy bronchitis	3	+	+	Positive	2.79E + 08
20	6 mo	M	2	Wheezy bronchitis	2	+			7.21E + 06
21	3 yr 1 mo	F	2	Bronchitis	3	+		Positive	1.36E + 09
22	1 yr 11 mo	F	2	Wheezy bronchitis	6	+		Positive	1.75E + 09
23	9 mo	F	2	Wheezy bronchitis	4	+		Positive	1.76E + 09
24	5 mo	M	2	Wheezy bronchitis	6	+		Positive	1.10E + 09
25	3 yr 7 mo	F	2	Bronchitis	3	+	+	Positive	1.79E + 09
26	1 yr 2 mo	M	3	Wheezy bronchitis, pneumonia	5	+		Positive	9.63E + 08
27	4 yr 5 mo	F	3	Wheezy bronchitis, pneumonia	5				1.27E + 04
28	3 yr 5 mo	M	3	Wheezy bronchitis, pneumonia	4	+	+	Positive	2.12E + 07
29	2 yr 8 mo	F	3	Wheezy bronchitis, pneumonia	4	+		Positive	4.57E + 08
30	8 mo	M	3	Wheezy bronchitis	4	+	+	Positive	2.59E + 08
31	1 yr 6 mo	M	3	Pneumonia	6	+		Positive	6.57E + 09
32	2 yr 1 mo	M	3	Bronchitis	4	+			9.09E + 05
33	2 yr	M	3	Bronchitis	4	+		Positive	2.60E + 08
34	3 yr	F	3	Pneumonia	7	+	+	Positive	3.80E + 09
35	4 yr 2 mo	F	3	Bronchitis	5			Positive	1.90E + 08
36	3 yr 3 mo	M	3	Bronchitis	6	+		Positive	2.30E + 08
37	1 yr 7 mo	F	3	Pneumonia	5	+	+		1.75E + 04
38	3 yr 4 mo	F	3	Pneumonia	4				3.96E + 04
39	3 yr 4 mo	F	3	Bronchitis	5	+			2.23E + 06
40	1 yr 2 mo	F	3	Wheezy bronchitis	8	+		Positive	4.45E + 08
41	3 mo	F	3	Wheezy bronchitis	0	+	+	Positive	3.56E + 09
42	3 yr 2 mo	F	3	Bronchitis	5			Positive	1.01E + 09
43	1 yr	M	3	Wheezy bronchitis	5	+	+	Positive	6.17E + 07
44	5 mo	M	3	Wheezy bronchitis	0	+			5.42E + 06
45	1 yr 1 mo	F	3	Wheezy bronchitis	2	+		Positive	4.52E + 08
46	4 yr 6 mo	F	3	Bronchitis	4			Positive	6.16E + 07
47	2 yr	M	3	Wheezy bronchitis	4	+		Positive	2.92E + 07
48	10 mo	F	4	Pneumonia	6			Positive	2.65E + 08
49	7 mo	M	4	Wheezy bronchitis	5	+	+		9.40E + 05
50	2 yr 10 mo	F	4	Wheezy bronchitis	7	+		Positive	6.87E + 07
51	3 yr 5 mo	F	4	Pneumonia	7			Positive	6.00E + 08
52	6 yr	M	4	Pneumonia	5			Positive	4.72E + 07
53	8 yr 11 mo	F	4	Bronchitis	7	+		Positive	1.57E + 08
54	1 yr 8 mo	F	4	Wheezy bronchitis	5	+		Positive	1.47E + 07
55	2 yr 1 mo	F	4	Wheezy bronchitis	7	+		Positive	2.43E + 08
56	4 yr 9 mo	M	4	Bronchitis	6	+		Positive	3.87E + 09
57	5 mo	M	4	Pneumonia	6	+	+	Positive	4.20E + 07
58	2 yr 7 mo	F	4	Wheezy bronchitis	5	+		Positive	1.77E + 08
59	1 yr	F	5	Bronchitis	8				1.85E + 04
60	1 yr 7 mo	M	5	Bronchitis	5	+	+		5.52E + 04
61	2 yr 4 mo	F	5	Wheezy bronchitis	7	+		Positive	6.53E + 07
62	2 yr	F	5	Wheezy bronchitis	7	+			3.59E + 07
63	2 yr 2 mo	F	5	Wheezy bronchitis	6	+		Positive	8.11E + 07
64	8 mo	M	5	Wheezy bronchitis, pneumonia	6	+		Positive	9.27E + 07
65	2 yr 8 mo	F	6	Pneumonia	4	+			4.30E + 04
66	5 yr	F	7	Pneumonia	8				3.23E + 05
67	4 yr 1 mo	F	7	Wheezy bronchitis	9	+			2.24E + 03
68	3 yr 10 mo	M	7	Pneumonia	9				3.60E + 05

^a F, female; M, male.^b The mean numbers of copies/ml $\pm$ SDs were 1.90E + 07 $\pm$ 2.91E + 07 for cases 1 to 4 (sampling day 0), 2.71E + 08 $\pm$ 2.49E + 08 for cases 5 to 12 (sampling day 1), 7.16E + 08 $\pm$ 7.24E + 08 for cases 13 to 25 (sampling day 2), 8.35E + 08 $\pm$ 1.66E + 09 for cases 26 to 47 (sampling day 3), 4.99E + 08 $\pm$ 1.13E + 09 for cases 48 to 58 (sampling day 4), 4.58E + 07 $\pm$ 4.03E + 07 for cases 59 to 64 (sampling day 5), and 1.82E + 05 $\pm$ 1.86E + 05 for cases 65 to 68 (sampling days 6 and 7, i.e., >5).

TABLE 2. Relationships of virus loads with gender, age, and duration of fever

Characteristic	Mean $\pm$ SD no. of copies/ml
Gender	
Male.....	5.45E + 08 $\pm$ 1.34E + 09
Female.....	5.09E + 08 $\pm$ 9.16E + 08
Age (yr)	
<1.....	5.58E + 08 $\pm$ 9.31E + 08
1-2.....	7.05E + 08 $\pm$ 1.63E + 09
2-3.....	1.41E + 08 $\pm$ 1.32E + 08
3-4.....	7.34E + 08 $\pm$ 1.14E + 09
>4.....	4.67E + 08 $\pm$ 1.20E + 09
Duration of fever (no. of days)	
<3.....	6.23E + 08 $\pm$ 1.21E + 09
3.....	5.33E + 08 $\pm$ 7.29E + 08
4.....	2.54E + 08 $\pm$ 4.84E + 08
5.....	2.11E + 08 $\pm$ 3.36E + 08
6.....	1.56E + 09 $\pm$ 2.26E + 09
7.....	6.26E + 08 $\pm$ 1.29E + 09
>7.....	1.85E + 08 $\pm$ 2.35E + 08

onset were 1.90×10^7 , 2.71×10^8 , 7.16×10^8 , 8.35×10^8 , 4.99×10^8 , 4.58×10^7 , and 1.82×10^5 by real-time RT-PCR, respectively. The virus number at 1, 2, and 3 days was statistically higher than that at 0 days and more than 5 days. Although the virus number at 4 days was high, the difference was not significant. The sensitivity of the IC assay with the 68 real-time RT-PCR-positive samples at 1 to 4 days was statistically higher than that at 0 and more than 5 days ($P = 0.00699$). There was statistically no association of the virus loads with gender, age, or the duration of fever (Table 2). The hMPV load in the nasopharyngeal swab samples was not associated with age; gender; or the severity of disease, such as the period of fever, the presence of dyspnea, and hospitalization.

DISCUSSION

Virus isolation in cell culture is the gold standard for the detection of respiratory viruses and remains indispensable as a source for analyzing genetic and antigenic changes in virus populations. However, some investigators now consider that RT-PCR should be the gold standard for the detection of respiratory viruses. Recent studies suggest that RT-PCR is more sensitive than viral culture for the detection of respiratory viruses in clinical samples (6, 9, 14). Since hMPV grows poorly in cell culture, it is difficult to isolate hMPV from clinical samples (3, 4, 11). Therefore, the RT-PCR assay shows the greatest sensitivity and specificity and is actually considered the gold standard for the detection of hMPV. We previously reported on the usefulness of a lateral-flow IC assay for the detection of the hMPV N antigens using two MAb against the N protein of hMPV, which reacted to the N proteins of the two lineages of hMPV (8). Furthermore, the IC test is cheaper than real-time RT-PCR (relative expense per test of the IC test versus that of real-time RT-PCR, 1:10) from a commercial point of view. A large-scale study was then performed to assess whether the assay detects hMPV in clinical samples. The sensitivity and the specificity were 70.6% and 95.5%, respectively. The predicted amino acid sequences of the N protein are

highly conserved between the two groups of hMPV (1, 7, 11, 12). Furthermore, since the N protein is highly expressed in the cytoplasm of hMPV-infected cells and exists in viral particles as a structural protein, this protein is likely to be the preferred antigenic target for the detection of hMPV. Nasopharyngeal swab samples contain many viral particles, antigenic material from the hMPV-infected cells, and other cellular and inflammatory debris. The IC assay detects the N protein expressed in the cytoplasm of hMPV-infected epithelial cells rather than the N protein in viral particles. Therefore, it is not necessary for there to be a direct correlation between the threshold by the IC assay and the copy number of hMPV present in clinical samples. However, among the 68 RT-PCR-positive samples, the 20 IC-negative samples apparently had lower viral loads than the samples positive by both assays, suggesting a lower limit of sensitivity for the IC assay. The RNA sequences of hMPV were detected in 68 (27.59%) of the 247 samples by real-time RT-PCR. The mean number of 5.25×10^8 hMPV copies/ml among all hMPV-positive samples in the present study was slightly higher than that found by Kuypers et al. (10).

Since two nasopharyngeal swab samples obtained from a child at the same time by different samplings may have differences in quality and quantity, we used one nasopharyngeal swab sample for both the IC and the real-time PCR assays. Although nasopharyngeal swab samples contain large amounts of RNase, surfactants contained in the extraction buffer of the samples inhibit the effect of the RNase and induce the stability of viral RNA for a long period before the RT-PCR assay. The sensitivity of the IC assay was influenced by the interval between the onset of symptoms and the time of sample collection. The sensitivity of the IC assay with the 68 real-time RT-PCR-positive samples obtained 1 to 4 days after the onset of fever was statistically higher than the sensitivities with samples obtained earlier than 24 h and later than 5 days after the onset of fever, indicating that secretions with the highest viral titers may occur in hMPV-infected children at 1 to 4 days after the onset of fever. It was likely that children shed fewer viral particles within 24 h after onset and from 5 days after onset than during the period from 1 to 4 days after onset and that the viral load within 24 h after onset and from 5 days after onset did not reach the threshold of detection for the IC assay. The viral loads were not influenced by age, gender, or the severity of disease. Since the IC assay, however, was performed with samples collected at different times, further investigations are necessary to determine any associations between viral load and age and between viral load and the severity of disease. In general, the sensitivities of rapid tests for influenza virus is variable (median, 70 to 75%) and lower than the sensitivity of cell culture, while their specificities are high (median, 90 to 95%) (15). Although we could not perform virus isolation by culture in the present study, the IC assay seemed to be more sensitive than virus isolation by culture (4). Furthermore, although the IC assay had a sensitivity of 70.6% for all samples, the sensitivity for all samples during a period from 1 to 4 days after onset increased to 79.6% (43/54). Ideally, a nasopharyngeal swab for the IC assay should be taken 1 to 4 days after the onset of fever. The IC assay can rapidly provide useful information for diagnosis and for the establishment of a treatment plan for patients with suspected hMPV infections.

ACKNOWLEDGMENT

We thank Stewart Chisholm for proofreading the manuscript.

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