

## Performance of a rapid human metapneumovirus antigen test during an outbreak in a long-term care facility

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### SUMMARY

Using a newly developed rapid test, an outbreak of human metapneumovirus (HMPV) infection in a long-term care facility was detected within only 2 days after the onset of symptoms in a putative index case. The outbreak was almost under control within 8 days mainly by zoning patients, with the exception of two cases of HMPV that were diagnosed 16 and 17 days after the onset of the outbreak. According to an immunological diagnosis as well as the rapid test, it was eventually proven that 18 patients had HMPV infections. We suspected that even asymptomatic residents, who had not been completely separated from the facility population, were a source of infection. That suggested that all asymptomatic residents should be tested and that the separation of the infected patients should be absolute, if an outbreak of HMPV infection is suspected in such a facility.

**Key words:** Human metapneumovirus, long-term care facility, rapid HMPV antigen test.

### INTRODUCTION

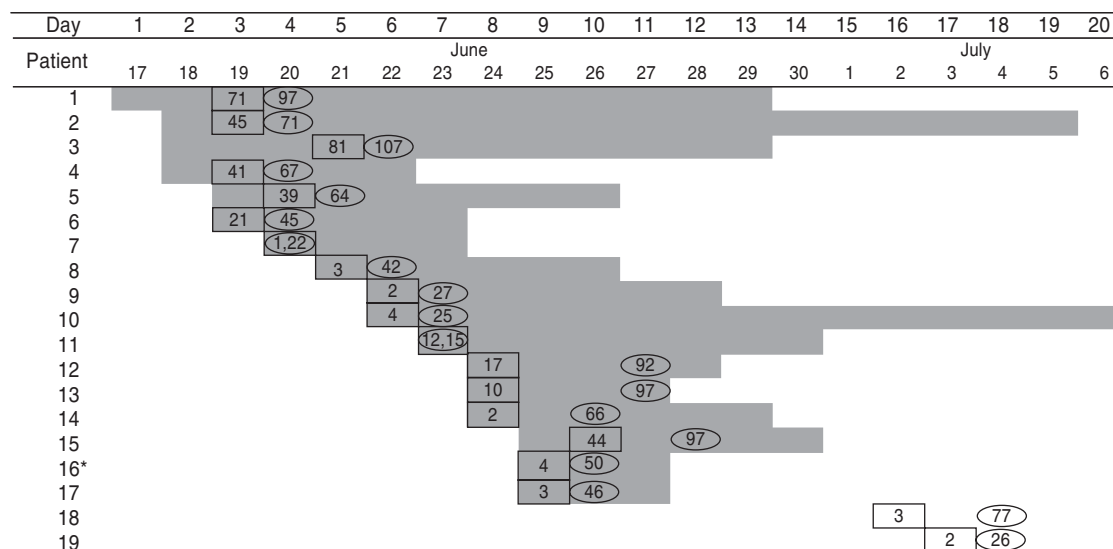
Human metapneumovirus (HMPV) is an important pathogen of the lower respiratory tract that most often affects hospitalized children, immunocompromised patients [1] and elderly occupants of long-term care facilities [2, 3]. Due to the close proximity of living quarters and the reduced levels of personal hygiene and movement of the residents, outbreaks of fatal influenza or norovirus infections have been reported in such facilities [4, 5]. A rapid test has

become a very useful tool in preventing the spread of infections because it results in rapid identification and isolation of the index case. A rapid HMPV antigen test has not been available until recently and the sensitivity and specificity of the assay have been described [6]. The present study focused on evaluating the performance of this new HMPV rapid test during a recent outbreak of HMPV infection in a long-term care facility.

### METHODS

Nasopharyngeal swab specimens were tested using both the Check hMPV assay (Meiji Seika Pharma Co. Ltd, Japan) and a nested RT-PCR [7]. The amplicon fragment (357 bp) was extracted using

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**Fig. 1.** The time-course of the outbreak of human metapneumovirus (HMPV) infection. The grey shading indicates the duration of fever (body temperature  $>37.5^{\circ}\text{C}$ ); rectangles indicate the day of diagnosis by the rapid test (numbers within rectangles represent hours after onset of symptoms); ellipses indicate the day of collection of the acute serum and nasopharyngeal swab (for HMPV RNA extraction) (numbers within ellipses represent hours after the onset of symptoms). \* Patient 16 was accommodated in an intensive respiratory care unit on 28 June.

GeneCleanII (MP Biomedicals, USA). The sequence was determined using a primer specific for the HMPV F gene (5'-CATGCCGACCTCTGCAGGAC-3', 5'-ATGTTGCAYTCYTTGATTG-3') (FASMAC, Kanagawa, Japan). This rapid test was previously known as the SAS hMPV test [6]. An indirect fluorescent antibody test for measuring IgM and IgG antibodies was performed to confirm the HMPV infection as described previously [2, 8]. The serological test was performed once on the serum of all patients. In addition to the detection of viral antigens and nucleic acids, a fourfold increase in serum HMPV IgG titre 2 weeks after onset of symptoms provided evidence of positive immune responses to HMPV. For virus isolation, nasopharyngeal swab samples were suspended in Eagle's minimum essential medium (MEM), and the suspension was inoculated onto LLC-MK2 and Vero E6 cells. The cells were cultured in MEM containing trypsin (1  $\mu\text{g}/\text{ml}$ ). Isolation of HMPV was confirmed via RT-PCR and the rapid test. The present study (number 09040) was approved by the Ethics Committee of Kurume University.

## RESULTS

A total of 18 patients were affected with HMPV over a 20-day period during June–July 2011, in a long-term care facility in Kurume, Japan (Fig. 1). One of 45

residents (case 6) on the first floor and 17 of 41 residents on the second floor were affected in one of several building units in this facility. All residents of each floor were accommodated in one open space. Most patients had a fever lasting 3–6 days. There were 1–3 new patients diagnosed daily during the first 8 days. Using the rapid test, we detected the outbreak in only 2 days after the onset of symptoms in the putative index case (case 1). Infection control measures were instituted to prevent the further spread of infection, which included zoning patients and hand washing. The zone where patients resided was surrounded by a long vinyl curtain in a corner of the ward. Additional patients continued to be diagnosed over the 4- to 5-day HMPV infection incubation period [9]. However, the outbreak was almost under control within only 8 days. Two patients were diagnosed 16 and 17 days after the onset of the outbreak. Case 16 was admitted to the intensive-care unit of another hospital with severe respiratory failure. The attack rates on the first and second floors were 2.2% and 44%, respectively. The total attack rate was 19.8%, which was smaller than that in previous reports [2, 10]. There was no record concerning HMPV infection in staff members.

According to an immunological diagnosis, as well as to the rapid test, it was eventually proven that all symptomatic patients, except for case 11, had been

Table 1. Clinical features of symptomatic patients during a human metapneumovirus (HMPV) outbreak

Patient no.	Age (yr)	Sex	WBC (/mm <sup>3</sup> )	CRP (mg/dl)	Rapid test*	RT-PCR†	Virus‡	IgM titre			IgG titre			
								Acute	2 wk	1 yr§	Acute	2 wk	Ratio	1 yr§
1	32	M	7000	5	+	+	-	20	80	<10	160	2560	16	320
2	28	M	4400	3	+	+	-	<10	10	<10	80	2560	32	80
3	6	F	3700	2	+	+	-	160	320	20	320	10240	32	640
4	17	M	7000	2	+	+	-	40	40	<10	80	10240	128	160
5	17	M	9400	0	+	+	-	1280	160	40	40	5120	128	40
6	42	F	7600	6	-	-	-	80	40	80	10	320	32	640
7	13	M	5200	0	-	-	-	640	80	10	1280	5120	4	320
8	41	M	4600	2	+	+	-	160	320	<10	640	5120	8	160
9	21	M	7400	12	+	+	+	320	160	<10	640	2560	4	160
10	42	F	7700	2	+	+	-	40	160	<10	1280	10240	8	320
11	40	M	5300	2	-	-	-	320	<10	<10	1280	2560	2	80
12	27	M	12000	1	+	+	-	10	80	80	1280	2560	2	160
13	36	F	6800	4	+	+	-	80	80	<10	40	5120	128	80
14	43	M	5400	6	+	+	-	40	80	<10	320	5120	16	160
15	4	F	8400	4	+	+	+	320	160	<10	640	5120	8	80
16	26	M	9000	5	+	-	-	160	320	<10	2560	5120	2	80
17	41	F	6200	1	+	+	-	320	320	<10	40	1280	32	160
18	3	F	8200	0	+	+	+	640	1280	<10	160	1280	8	80
19	10 mo.	F	11100	<0.2	+	+	-	320	160	40	20	160	8	160

WBC, White blood cells; CRP, C-reactive protein.

\* Check hMPV assay (Meiji Seika Pharma Co. Ltd, Japan).

† Genbank accession numbers of the amplicon (F gene) are JX966477–JX966485.

‡ Virus isolation using LLC-MK2 cells.

§ IgM and IgG titres were evaluated 1-year post-outbreak of HMPV.

infected with HMPV (Table 1). Of these, 16 (84%) patients were positive for HMPV according to the rapid test result. These results were confirmed using a nested RT-PCR, with the exception of case 16. HMPV was isolated in the samples from cases 9, 15 and 18. Cytopathic effects of LLC-MK2 cells were seen and rounded and fused cells were clearly observed. The sequences of the amplicon, which codes for a part of the HMPV F protein, were completely identical in all sequences obtained (GeneBank accession numbers JX966477–JX966485), which suggested that the outbreak of HMPV infection would have originated from an index case.

Figure 1 shows the duration of fever and the time lag-between the onset of symptoms and the diagnosis (rapid test, RT-PCR, immunological test). The time lag of the rapid test in nine cases was around 1–4 h. Of these nine cases, only case 7 was negative by the rapid test. The time lag for the remaining cases was between 10 and 81 h. Of these cases, only two were found to be negative by the rapid test. The time lag was not related to the detection rate. Based on the RT-PCR results, the negative results were evenly detected in several cases. The time lag between the onset

of symptoms and the collection of the nasopharyngeal swab for RNA extraction varied from short to long.

## DISCUSSION

In this study, we were able to detect an outbreak of HMPV infection in a long-term care facility at an early phase using a new rapid test. Early detection of HMPV enabled us to prevent widespread infection at the facility and the outbreak had almost ceased within 8 days. We were also able to keep the affected number of patients to within 17 out of a total of 41 residents in a ward on the second floor and to 1 out of 45 in another ward on the first floor.

Influenza outbreaks have been reported in a long-term care facility and fatal cases are common. Many patients in this report had a high fever for at least 3–7 days (Fig. 1). This period seemed to be longer than that seen for influenza infections. However, fatal cases of HMPV infection are relatively rare [2, 11]. Early detection of the HMPV outbreak increased the prospects for a good outcome.

In total, 16 (89%) of the 18 HMPV patients were found to be positive using the rapid test. The rapid

test and RT-PCR results were in accord except for case 16. The time lag between the onset of symptoms and the collection of a nasopharyngeal swab for RT-PCR was 50 h, but the time lag between the onset of symptoms and the rapid test was only 4 h. That suggested that the amount of HMPV might have decreased by the time the nasopharyngeal samples were collected. With respect to the rapid test-positive results for cases 12 and 16, no significant increase in serum IgG titre was detected 2 weeks after the onset of symptoms, because the titre of IgG in acute serum had already increased. This might suggest that cases 12 and 16 were asymptotically infected with HMPV at a much earlier time, although the time lag between the onset of symptoms and the collection of serum samples was relatively early (within 3–4 days). The role of asymptomatic patients in comparatively large HMPV outbreaks has been reported previously [12].

False-negative rapid test results were noted for cases 6 and 7. These patients were also immediately isolated in the same separated area as the rapid test-positive patients because they showed similar clinical symptoms. It is important to note that cases 6 and 7 eventually tested positive for HMPV according to the serological test. This result suggested that the isolation of all symptomatic patients, including patients with false-negative results, was an appropriate control measure to prevent the spread of infection.

We concluded that the rapid HMPV test had high sensitivity, making it very useful for the early detection of an outbreak of HMPV infection in a long-term care facility. Our study also suggested that even asymptomatic patients might be a source of infection. Therefore, we propose rapid diagnosis testing for all asymptomatic residents as well as for symptomatic patients. Testing the asymptomatic residents might be an important measure in preventing the emergence of secondary-infected patients in such a long-term care facility.

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#### DECLARATION OF INTEREST

None.

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