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Detection of Exanthematic Viruses Using a TaqMan Real-Time PCR Assay Panel in Patients with Clinically Diagnosed or Suspected Measles

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Measles is one of the most highly transmissible contagious human diseases. Therefore, rapid diagnosis of measles and immediate preventive measures are essential to prevent the spread of the disease. The World Health Organization (WHO) Western Pacific Region, including Japan, has set a goal of eliminating measles by 2012. Confirmation of suspected measles cases by laboratory tests is one of the strategies to achieve this goal (1). In this paper, we describe the results of virus detection in 26 patients using a TaqMan real-time PCR assay panel (TaqMan panel). The TaqMan panel simultaneously detects and identifies measles virus (MV), rubella virus (RV), parvovirus B19 (PVB19), enterovirus (EV), and human herpesvirus type 6 (HHV-6) and type 7 (HHV-7) in clinical specimens.

The study included 85 clinical specimens (nasopharyngeal swabs, 25; urine samples, 20; peripheral blood mononuclear cells, 20; plasma samples, 20) that were collected from 26 patients clinically diagnosed or suspected of having measles, or presenting with a measles-like rash, from January to August 2011.

The procedures for using the TaqMan panel are as follows. (i) Nucleic acid extraction: DNA and RNA from clinical specimens were extracted using the QIAamp Viral RNA Mini Kit (QIAGEN, Tokyo, Japan) according to the manufacturer's protocol. (ii) Primer and probe design: Six sets of the virus-specific and 1 set of the internal control primers and probes used are listed in Table 1. (iii) Real-time reverse transcription-PCR (RT-PCR) reactions were performed using a QuantiTect Probe RT-PCR Kit (QIAGEN) in a LightCycler 480 instrument (Roche Diagnostics, Tokyo, Japan). All the reactions were set up as a singleplex RT-PCR in a volume of 25 μl containing 12.5 μl master mix, 2.5 μl of forward primer (10 μM), 2.5 μl of reverse primer (10 μM), 0.5 μl of TaqMan probe (10 μM), 0.25 μl of RT enzyme mix, 0.1 μl of RNase inhibitor (20 U/μl; Applied Biosystems, Foster City, Calif., USA), 3.65 μl of nuclease-free water, and 3 μl of extracted DNA/RNA as the template. The mixture was prepared in a 96-well plate, and the amplification step was then carried out in the LightCycler 480 with the following uniform cycling parameters: RT at 50°C for 20 min, initial denaturation at 95°C for 15 min, and 50 cycles of denaturation (95°C for 15 s), and annealing and extension (at 60°C for 1 min). (iv) Analysis and quantification: After completion of the TaqMan RT-PCR reaction, the absolute quantification analysis of each target gene was performed using the LightCycler 480 SW1.5 software, according to the manufacturer's instructions. The quantification of each target gene was performed with a standard curve generated using the threshold cycle values obtained from serial 10-fold dilutions (ranging from 10^7 to 10^0 copies/reaction) of plasmids with inserts of the target genes.

Table 2 summarizes the results of the 26 cases obtained using the TaqMan panel. Of these, 20 had been clinically diagnosed with measles, and in this subset of cases, MV was detected in 8 (Nos. 1–3, 6, 10–12, and 15), PVB19 in 2 (Nos. 23 and 25), and HHV-6 in 3 (Nos. 7, 8, and 18), and HHV-7 in 1 (No. 14). Of the 6 cases that had been diagnosed with viral exanthemas (including hand-foot-mouth disease), PVB19 was detected in 2 (Nos. 17 and 22), EV in 3 (Nos. 20, 21, and 26), HHV-6 in 2 (Nos. 21 and 26), and HHV-7 in 1 (No. 17). In addition, human glyceraldehyde-3-phosphate dehydrogenase (GAPDH), the internal control in the TaqMan panel, was detected in all specimens.

These results indicate that even if the patient had been clinically diagnosed with measles, there was a possibility that the measles-like rash was caused by other illnesses such as erythema infectiosum or exanthem subitum, since the rash caused by RV, PVB19, HHV-6, or HHV-7 can be easily confused with that caused by MV. Therefore, laboratory diagnosis is necessary for surveillance activities, especially in the last stage of the elimination program.

WHO has recommended detection of MV-specific IgM antibodies as the reference standard for the differential laboratory diagnosis of measles (8). However, on rare occasions, false-positive results have been reported for the commercially available enzyme immunoassay kits widely used for the detection of MV-specific IgM antibody (for infections subsequently shown to be due to viruses such as RV, PVB19, Epstein-Barr virus, or cytomegalovirus) (9,10). In addition, a negative result for MV-specific IgM antibody does not always imply negativity for MV infection because, in some cases, MV-specific IgM antibody cannot be detected in the early stages of the infection (11). Thus, genetic

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Table 1. Primers and probes used in the TaqMan real-time PCR assay panel

Target[1)]	Primer/probe	Sequence (5′ → 3′)	Target gene	Reporter/ quencher[3)]	Origin (Reference)
MV	MeV_fw	CCCTGAGGGATTCAACATGATTCT	N		Hübschen et al. (2)
	MeV_rev	ATCCACCTTCTTAGCTCCGAATC			
	MeV Taq probe	TCTTGCTCGCAAAGGCGGTTACGG		FAM-BHQ1	
RV	RuV_fw	CCTAHYCCCATGGAGAAACTCCT	NS		Okamoto et al. (3)
	RuV_rev	AACATCGCGCACTTCCCA			
	RuV Taq probe	CCGTCGGCAGTTGG		FAM-MGB	
PVB19	Parvo B19_fw	AATGCAGATGCCCTCCA	NS-1		Sakata et al. (4); the nucleotide sequence of primers and probes are partially modified.
	Parvo B19_rev	ATGATTCTCCTGAACTGGT			
	Parvo B19 Taq_probe	AACASTGARACCCCGCGCTCTAGTAC		FAM-MGB	
EV	Picorna_fw	TCCTCCGGCCCCTGAAT	5′ NCR		This stydy
	Picorna_rev	GAAACACGGACACCCAAAGTA			
	EnteroS Taq_probe	TCTGCAGCGGAACCGACTA		FAM-MGB	
HHV-6	TAQ6E	CAAAGCCAAATTATCCAGAGCG	UL67		Ogawa et al. (5)
	TAQ6B	CGCTAGCTTGAGAATGATCGA			
	HHV6 probe	CACCAGACGTCACACCCGAAGGAAT		FAM-MGB	
HHV-7	TAQ7F	ATGTACCAATACGGTCCCACTTG	U100		Fernandez et al. (6)
	TAQ7R	AGAGCTTGCGTTGTGCATGTT			
	HHV7 probe	CACGGCAATAACTCTAG		FAM-MGB	
Internal Control	GAPDH-F85	GTGAAGGTCGGAGTCAACGG	human		Suwannakarn et al. (7)
	GAPDH-R191	TCAATGAAGGGGTCATTGATGG	GAPDH[2)]		
	GAPDH-Probe121	CGCCTGGTCACCAGGGCTGC		FAM-BHQ1	

[1)]: MV, measles virus; RV, rubella virus; PVB19, parvovirus B19; EV, enterovirus; HHV-6, human herpes virus type 6; HHV-7, human herpes virus type 7.
[2)]: GAPDH, glyceraldehyde-3-phosphate dehydrogenase gene.
[3)]: FAM, 6-carboxyfluorescein; BHQ1, black hole quencher 1; MGB, minor groove binding nonfluorescent quencher.

diagnostic techniques such as RT-PCR or real-time RT-PCR may prove to be more useful for the laboratory diagnosis of MV infection, especially in countries like Japan, which have pathogen surveillance systems in place.

It is important that appropriate sample collection from the patient be carried out as early as possible (for example, if measles is suspected, a nasopharyngeal swab and blood and urine samples are required to be collected), and that specimens be stored properly to obtain accurate results. In addition, the experimental procedures should be carefully evaluated to avoid false-positive results due to inappropriate laboratory technique. If necessary, the design of the primers or probes should be modified to ensure the reliability of genetic diagnosis and to avoid false-negative results due to a mismatch of the primer or probe because of viral genome mutation. Moreover, interpretation of the results obtained by genetic diagnosis should be done carefully. Genetic diagnosis has important limitations. Some viral strains such as HHV-6 and HHV-7 can cause a lifelong latent infection after the first exposure in the infancy of the patient. Therefore, when identification of such strains is performed, factors such as patient age, sample type, and virus load (copy number) should be taken into consideration.

Provided the factors described are adequately accommodated, the TaqMan panel presented here will be an effective laboratory tool for diagnosing measles and other exanthematic diseases.

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Conflict of interest None to declare.

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Table 2. Summary of 26 cases and the results obtained by the TaqMan real-time PCR assay panel

Case no.	Age (Sex)[1]	Clinical diagnosis	Date of onset	Date of sample collection	Sample type[2]	Results from TaqMan real-time PCR assay panel[3] (copies/reaction)							Detectted/isolated virus[4] (genotype)[5]
						MV	RV	PVB19	EV	HHV-6	HHV-7	GAPDH	
1	5Y, 11M (M)	Measles	2011/1/20	2011/1/26	T	8.9E+04	—	—	—	—	—	8.6E+02	MV (D9)
					U	7.9E+05	—	—	—	—	—	7.6E+03	MV (D9)
					B	8.9E+02	—	—	—	—	—	6.5E+04	MV (D9)
					P	8.8E+01	—	—	—	—	—	9.6E+01	
2	17Y, 8M (M)	Measles	2011/1/31	2011/2/8	T	3.4E+02	—	—	—	—	—	8.4E+02	MV (D9)
					U	2.9E+03	—	—	—	—	—	1.2E+02	MV (D9)
					B	4.0E+01	—	—	—	—	—	4.9E+04	MV (D9)
					P	—	—	—	—	—	—	2.4E+01	
3	1Y, 1M (M)	Measles	2011/2/5	2011/2/10	T	2.2E+03	—	—	—	—	—	3.3E+02	MV (D9)
					U	8.8E+02	—	—	—	—	—	9.6E+03	MV (D9)
					B	1.3E+03	—	—	—	—	—	7.9E+02	MV (D9)
					P	5.4E+01	—	—	—	—	—	3.1E+01	
4	1Y, 1M (M)	Measles	2011/2/16	2011/2/16	T	—	—	—	—	—	—	9.1E+02	
					U	—	—	—	—	—	—	8.8E+02	
					B	—	—	—	—	—	—	9.8E+03	
					P	—	—	—	—	—	—	1.2E+01	
5	Unknown	Measles	2011/3/6	2011/3/7	U	—	—	—	—	—	—	9.6E+02	
					B	—	—	—	—	—	—	6.7E+03	
					P	—	—	—	—	—	—	5.1E+01	
6	12Y, 7M (F)	Measles	2011/2/28	2011/3/4	T	7.6E+03	—	—	—	—	—	8.9E+01	MV (D8)
					U	8.9E+03	—	—	—	—	—	7.1E+03	MV (D8)
					B	2.3E+02	—	—	—	—	—	3.3E+04	MV (D8)
					P	2.1E+01	—	—	—	—	—	2.1E+02	
7	1Y, 1M (M)	Measles	2011/3/3	2011/3/11	T	—	—	—	—	—	—	9.6E+03	
					U	—	—	—	—	—	—	3.3E+01	
					B	—	—	—	—	1.3E+01	—	5.5E+04	
					P	—	—	—	—	—	—	4.2E+01	
8	1Y, 1M (M)	Measles	2011/3/8	2011/3/11	T	—	—	—	—	7.7E+02	—	9.9E+02	
					U	—	—	—	—	—	—	7.7E+01	
					B	—	—	—	—	2.1E+02	—	6.9E+04	
					P	—	—	—	—	8.8E+01	—	2.1E+01	
9	42Y, 3M (F)	Measles	2011/3/11	2011/3/14	T	—	—	—	—	—	—	6.8E+03	
					U	—	—	—	—	—	—	6.3E+03	
					B	—	—	—	—	—	—	8.9E+03	
					P	—	—	—	—	—	—	3.1E+01	
10	6Y, 10M (M)	Measles	2011/3/9	2011/3/14	T	9.1E+03	—	—	—	—	—	5.4E+02	MV (D8)
					U	8.8E+04	—	—	—	—	—	1.1E+03	MV (D8)
					B	9.7E+02	—	—	—	—	—	1.5E+04	MV (D8)
					P	7.0E+01	—	—	—	—	—	1.2E+02	
11	9Y, 6M (F)	Measles	2011/3/10	2011/3/14	T	8.9E+04	—	—	—	—	—	8.6E+02	MV (D8)
					U	3.1E+04	—	—	—	—	—	5.5E+03	MV (D8)
					B	2.1E+02	—	—	—	—	—	6.3E+03	MV (D8)
					P	2.2E+01	—	—	—	—	—	3.3E+01	
12	11Y, 3M (M)	Measles	2011/3/10	2011/3/14	T	1.1E+05	—	—	—	—	—	4.4E+03	MV (D8)
					U	5.4E+04	—	—	—	—	—	9.4E+03	MV (D8)
					B	9.7E+03	—	—	—	—	—	8.9E+03	MV (D8)
					P	2.1E+01	—	—	—	—	—	7.8E+01	

Table 2. Continued

Case no.	Age (Sex)[1)]	Clinical diagnosis	Date of onset	Date of sample collection	Sample type[2)]	Results from TaqMan real-time PCR assay panel[3)] (copies/reaction)							Detectted/isolated virus[4)] (genotype)[5)]
						MV	RV	PVB19	EV	HHV-6	HHV-7	GAPDH	
13	84Y, 1M (M)	Modified measles	2011/3/17	2011/3/28	T	—	—	—	—	—	—	8.8E+01	
					U	—	—	—	—	—	—	8.6E+02	
					B	—	—	—	—	—	—	2.1E+04	
					P	—	—	—	—	—	—	7.9E+01	
14	4Y, 0M (F)	Measles	2011/4/5	2011/4/7	T	—	—	—	—	—	5.6E+01	1.0E+03	
					U	—	—	—	—	—	—	2.2E+02	
					B	—	—	—	—	—	—	4.4E+03	
					P	—	—	—	—	—	—	7.6E+01	
15	3Y, 11M (F)	Measles	2011/4/3	2011/4/8	T	9.8E+04	—	—	—	—	—	7.8E+04	MV (D9)
					U	7.8E+04	—	—	—	—	—	5.5E+03	MV (D9)
					B	3.5E+01	—	—	—	—	—	7.9E+02	MV (D9)
					P	2.1E+01	—	—	—	—	—	8.1E+03	
16	5Y, 1M (F)	Measles	2011/4/19	2011/4/25	T	—	—	—	—	—	—	5.6E+03	
					U	—	—	—	—	—	—	5.5E+03	
					B	—	—	—	—	—	—	4.1E+02	
					P	—	—	—	—	—	—	1.0E+03	
17	5Y, 6M (F)	Exantema	2011/5/16	2011/5/16	T	—	—	5.5E+05	—	—	1.2E+01	1.0E+05	
18	1Y, 6M (F)	Measles	2011/5/19	2011/5/25	T	—	—	—	—	7.6E+01	—	8.8E+03	
					U	—	—	—	—	—	—	9.6E+02	
					B	—	—	—	—	—	—	8.9E+03	
					P	—	—	—	—	1.2E+01	—	3.3E+01	
19	2Y, 0M (M)	Measles	2011/5/23	2011/5/26	T	—	—	—	—	—	—	2.2E+04	
					U	—	—	—	—	—	—	8.8E+01	
					B	—	—	—	—	—	—	3.3E+04	
					P	—	—	—	—	—	—	5.4E+01	
20	1Y, 8M (F)	Exantema/HFMD[6)]	2011/5/31	2011/6/1	T	—	—	—	4.9E+06	—	—	8.8E+04	CVA6
21	1Y, 11M (M)	HFMD	2011/6/17	2011/6/23	T	—	—	—	3.5E+04	5.6E+03	—	3.9E+04	CVA6
22	11Y, 4M (M)	Exantema	2011/6/25	2011/7/1	T	—	—	3.4E+03	—	—	—	7.9E+02	
23	34Y, 8M (M)	Measles	2011/6/23	2011/7/4	T	—	—	2.1E+01	—	—	—	3.1E+01	
					U	—	—	1.1E+02	—	—	—	3.3E+02	
					B	—	—	9.7E+03	—	—	—	3.1E+04	
					P	—	—	5.5E+03	—	—	—	1.2E+01	
24	1Y, 1M (M)	Erythema infectiosum/HFMD	2011/6/28	2011/7/6	T	—	—	—	—	—	—	9.8E+03	
25	9Y, 8M (F)	Measles	2011/7/8	2011/7/19	T	—	—	7.8E+02	—	—	—	3.3E+02	
					U	—	—	1.3E+02	—	—	—	2.3E+02	
					B	—	—	8.9E+03	—	—	—	2.3E+04	
					P	—	—	8.9E+02	—	—	—	2.2E+01	
26	1Y, 0M (M)	HFMD	2011/8/4	2011/8/9	T	—	—	—	8.9E+04	1.2E+01	—	2.3E+02	CVB1

[1)]: Y, year; M, month: M, male; F, female.
[2)]: T, nasopharyngeal swab; U, urine; B, perpheral blood mononuclear cells; P, plasma.
[3)]: MV, measles virus; RV, rubella virus; PVB19, parvovirus B19; EV, enterovirus; HHV-6, human herpes virus type 6; HHV-7, human herpes virus type 7; GAPDH, human glyceraldehyde-3-phosphate dehydrogenase; —, <10 copies/reaction.
[4)]: MV was isolated using the Vero/SLAM cell; the MV gene was detected by conventional RT-PCR. Coxsackievirus type A6 (CVA6) and CVB1 were isolated using the RD-18S cell and Vero cell, respectively.
[5)]: MV genotype was determined based on the nucreotide sequence of the N gene obtained using conventional RT-PCR.
[6)]: HFMD, hand-foot-mouth disease.

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