Detection of WN-Human1 sequence from clinical specimen.

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Method & Results

Total RNA was extracted from four nasopharyngeal swabs using QIAamp viral RNA mini kit (Qiagen) following manufacture's instruction. First strand cDNA was synthesized using Super Script IV Reverse Transcriptase (Thermo) with random primer (Thermo) and oligodT primer (Thermo). PCR reactions were performed using Quick Taq HS Dymix (TOYOBO, Japan) and pancoronaviral or specific primers (Table 1). The PCR condition was as follows: 94°C for 1 min; 40 cycles of 94°C for 30 sec, 56°C for 30 sec, and 68°C for 1 min. After PCR, amplicons were visualized by agarose gel electrophoresis staining ethidium bromide. The results are shown in Table 2. Two amplicons were detected in primer set 1 of specimen No.3 and primer set 4 of specimen No.4. The amplicons were gel-purified using Wizard SV Gel and PCR Clean-Up System (Promega) following manufacture's instruction. The sequencing analysis was performed with Bigdye tterminator v3.1 cycle sequencing kit and Ampure XP purification. Direct sequencing analysis of the amplicons were unsuccessful maybe due to low intensity of band. Therefore, to increase the amount of template, the amplicons were re-amplified by 2nd PCR with the same enzyme and primer sets with some modification of PCR condition as follows: 94°C for 2 min; 40 cycles of 94°C for 30 sec, 52°C for 30 sec, and 68°C for 45 sec. The sequencing analysis using re-amplified template was performed and the sequence of amplicon of primer set 1 could be decoded. The 376bp of analyzed sequence showed 100% match with the sequence of WH-human1 (MN908947). HCoV229E (VR740) and MERS-CoV (EMC) could be used as positive control for pan coronavirus primer set, but there was no positive control for WN-human1 specific primers. Water only was used as negative control.

Table 1. Primer used for WH1 detection.

	Name	direction	sequence (5' to 3')	Expected size (bp)
	IN-6	sense	GGTTGGGACTATCCTAAGTGTGA	440
	IN-7	antisense	CCATCATCAGATAGAATCATCATA	
	IN7hemi	antisense	ATCAGATAGAATCATCATAGAGA	435
No.	Name	direction	sequence (5' to 3')	Expected size (bp)
1	NIID_WH-1_F501	sense	TTCGGATGCTCGAACTGCACC	413
	NIID_WH-1_R913	antisense	CTTTACCAGCACGTGCTAGAAGG	
2	NIID_WH-1_F2396	sense	TAGGTGAAACATTTGTCACGCACTC	347
	NIID_WH-1_R2742	antisense	TGGTGCACCGCCTTTGAGTGTG	
3	NIID_WH-1_F5822	sense	GCATAGACGGTGCTTTACTTACAAAGTC	568
	NIID_WH-1_R6389	antisense	ATTCCCTGCGCGTCCTCTGAC	
4	NIID_WH-1_F9436	sense	ATTGTAGCTATCGTAGTAACATGCC	548
	NIID_WH-1_R9983	antisense	AGATGACAACAAGCAGCTTCTCTG	
5	NIID_WH-1_F11625	sense	AGTTTATTGTTTCTTAGGCTATTTTTGTAC	361
	NIID_WH-1_R11985	antisense	AGCTAAGAGAATGTCATTGTGTAA C	
6	NIID_WH-1_F23061	sense	ATATGGTTTCCAACCCACTAATGGTG	685
	NIID_WH-1_R23650	antisense	ATTGACTAGCTACACTACGTGCC	
7	NIID_WH-1_F24855		AGGTGTCTTTGTTTCAAATGGCACACA	485
	NIID_WH-1_R25339		AGCAGGATCCACAAGAACAACAG	
8	NIID_WH-1_F28659		TGGTGCTAACAAAGACGGCA	307
	NIID_WH-1_R28965		GTCAAGCAGCAGCAAAGCAA	
9	NIID_WH-1_F29062		CCTCGGCAAAAACGTACTGC	386
	NIID_WH-1_R29447		TGTCTCTGCGGTAAGGCTTG	

Table 2. 1st PCR results

	Specimen number						
Primer sets	1	2	3	4	NC	MERS-CoV	HCoV229E
IN-6 & IN-7	-	-	-	-	-	+	+
IN-7 heminested	-	-	-	-	-	+	Not done
1	-	-	+	-	-		
2	-	-	-	-	-		
3	-	-	-	-	-		
4	-	-	-	+	-		
5	-	-	-	-	-		
6	-	-	-	-	-		
7	-	-	-	-	-		
8	-	-	-	-	-		
9	-	-	-	-	-		