Erratum: Nao, N., et al. Detection of second case of 2019-nCoV infection in Japan

The authors wish to make the following change to their report.

The reverse primer (NIID_2019-nCOV_N_R2) sequence should be replaced with TGGCAGCTGTGTA**<u>G</u>**GTCAAC. The corrected nucleotide is bold and underlined.

The authors apologize for any inconvenience this may cause.
The report will be updated and the corrected version of the report will be online.

Detection of second case of 2019-nCoV infection in Japan (corrected version)

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Method & Results

1) Nested RT-PCR

Total RNA was extracted from pharyngeal swab 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 reaction was performed using Quick Taq HS Dyemix (TOYOBO, Japan) using two 2019-nCoV 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 1st PCR, nested PCR was performed using 2nd PCR primers and 1μL of 1st PCR product under the same condition as 1st PCR. The primer concentrations were 400nM for all. DDW was used as negative control. The amplicons were visualized by 2% agarose gel electrophoresis (Fig. 1). As the result, both primer sets detected desired size of bands. The rest of PCR products were purified with AMpure XP, and then direct sequencing analysis was performed using seq primers. The analyzed sequences showed 100% match with the sequence of WH-human1 (MN908947).

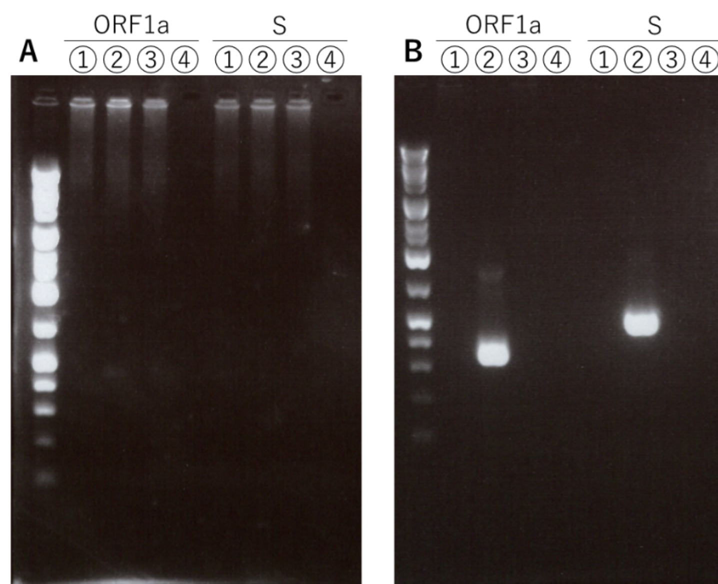

Figure 1. Three specimens were tested this time. Agarose gel electrophoresis of 1st (A) and 2nd (B) PCR reaction. DNA marker (Gene Ladder Wide 1, Nippon gene) was used as a reference for fragment size. Lane 1 and 3 were negative clinical samples, lane 2 was positive clinical sample, and lane 4 was negative control.

Table 1. Primer used for 2019-nCoV.

No.		Name	direction	sequence (5' to 3')	Expected size (bp)
ORF1a set					
1	1st	NIID_WH-1_F501	Sense	TTCGGATGCTCGAACTGCACC	413
2	1st	NIID_WH-1_R913	Antisense	CTTTACCAGCACGTGCTAGAAGG	
3	2nd	NIID_WH-1_F509	Sense	CTCGAACTGCACCTCATGG	346
4	2nd	NIID_WH-1_R854	Antisense	CAGAAGTTGTTATCGACATAGC	
5	Seq	NIID_WH-1_Seq_F519	Sense	ACCTCATGGTCATGTTATGG	
6	Seq	NIID_WH-1_Seq_R840	Antisense	GACATAGCGAGTGTATGCC	
S set					
7	1st	WuhanCoV-spk1-f	Sense	TTGGCAAAATTCAAGACTCACTTT	547
8	1st	WuhanCoV-spk2-r	Antisense	TGTGGTTCATAAAAATTCCTTTGTG	
9	2nd	NIID_WH-1_F24381	Sense	TCAAGACTCACTTTCTTCCAC	493
10	2nd	NIID_WH-1_R24873	Antisense	ATTTGAAACAAAGACACCTTCAC	
11	Seq	NIID_WH-1_Seq_F24383	Sense	AAGACTCACTTTCTTCCACAG	
12	Seq	NIID_WH-1_Seq_R24865	Antisense	CAAAGACACCTTCACGAGG	

2) Real-time RT-PCR

Real-time one step RT-PCR was performed using QuantiTect Probe RT-PCR Kit (Qiagen) on LightCycler96 system (Roche). A 20μL of reaction contains 10 μL of 2×Master Mix, 0.2 μL of RT mix, 3.8 μL of DDW, 1 μL of pre-diluted 20 ×primer and probe mix, and 5μL of extracted RNA. The PCR condition was as follows: 50°C for 30 min; and 95°C for 15 min; and 40 cycles of 95°C for 15 sec and 60°C for 1 min. The synthesized RNA, which contained artificial sequences that could distinguish the laboratory contamination, was used as positive control. DDW was used as negative control. The reaction was performed in duplicate, and both positives within 40 cycles were considered as positive. As the result, the average Cq value of specimen was 36.7, and that of positive control

(500 copies) was 35.0. Negative controls showed no signals. Therefore, the specimen could be considered as positive.

Table 2. Primers and probe sequence for real-time RT-PCR

Primer	Sequence (5' to 3')	Position (MN908947.1)	Concentration
NIID_2019-nCOV_N_F2	AAATTTTGGGGACCAGGAAC	29142-29161	500 nM
NIID_2019-nCOV_N_R2	TGGCAGCTGTGTAGGTCAAC	29299-29280	700 nM
NIID_2019-nCOV_N_P2	FAM-ATGTCGCGCATTGGCATGGA-BHQ	29239-29258	200 nM