Detection of 2019-novel coronavirus sequence from clinical specimen

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Method & Results

RNA was extracted from 4 nasopharyngeal swab, 3 serum, and 1 whole blood samples (**Table 1**) using QIAamp MinElute Virus Spin Kit (Qiagen, Hilden, Germany). One hundred-μL of RNA solution was eluted from the same volume of each sample. A RNA fragment of 2019-novel coronavirus (2019-nCoV) was amplified with One-step RT-PCR kit (Qiagen) according to manufacture's instruction. Three-μL of RNA solution was used per reaction of 50μL. One-step RT-PCR was performed with specific primers for spike region of 2019-nCoV; forward primer: WuhanCoV-spk1-f 5'-TTGGCAAAATTCAAGACTCACTTT-3' and reverse/RT primer: WuhanCoV-spk2-r 5'-TGTGGTTCATAAAAATTCCTTTGTG-3' amplifying 24370-24916nt in GenBank MN908947 (547bp). RT-PCR was performed in ABI9700 (Applied Biosystems, Foster City, CA) under the following condition: 50°C 30min, 95°C 15min; 40 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min. PCR products were visualized by 2% agarose gel electrophoresis staining ethidium bromide. The results are shown in **Figure 1**. Single band of 547bp was detected in the sample No.2. The amplicon was purified with QIAquick PCR Purification Kit (Qiagen) following manufacture's instruction. Direct sequencing analysis was performed with Big-dye terminator v3.1 cycle sequencing kit and ABI 3130 sequencer (Applied Biosystems and Fasmac, Tokyo, Japan). The 498bp of analyzed sequence excluded PCR primers showed 100% match with the sequence of 2019-nCoV (WH-human1, 24394-24891nt in GenBank MN908947, **Figure 2**).

Table 1 List of samples. *Two swab samples were obtained on day 5.

No.	Sample	Days after hospitalization
1	Nasopharyngeal swab	1
2	Nasopharyngeal swab	2
3	Nasopharyngeal swab*	5
4	Nasopharyngeal swab*	5
5	Whole blood	5
6	Serum	1
7	Serum	4
8	Serum	5

Figure 1 Electrophoresis of PCR products.
Five-μL of PCR product was loaded in each lane. The predicted size of PCR amplicon (547bp) is indicated.

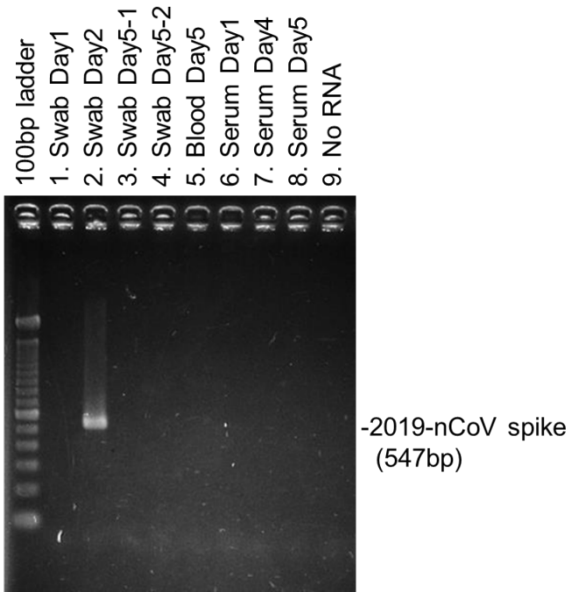

Figure 2 Sequence alignment of the PCR product with 2019-nCoV. The 498bp of analyzed sequence excluded PCR primers (the lower sequence) is aligned with 24394-24891nt in GenBank MN908947 (WH-Human-1-GenBank, the upper sequence).

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WH-Human_1_GenBank 24361 ATAGTGCTATTGGCAAAATTCAAGACTCACTTTCTTCCACAGCAAGTGCACTTGGAAAAC 24420
20-03seq               1 ---------------------------------CTTCCACAGCAAGTGCACTTGGAAAAC 27

WH-Human_1_GenBank 24421 TTCAAGATGTGGTCAACCAAAATGCACAAGCTTTAAACACGCTTGTTAAACAACTTAGCT 24480
20-03seq              28 TTCAAGATGTGGTCAACCAAAATGCACAAGCTTTAAACACGCTTGTTAAACAACTTAGCT 87

WH-Human_1_GenBank 24481 CCAATTTTGGTGCAATTTCAAGTGTTTTAAATGATATCCTTTCACGTCTTGACAAAGTTG 24540
20-03seq              88 CCAATTTTGGTGCAATTTCAAGTGTTTTAAATGATATCCTTTCACGTCTTGACAAAGTTG 147

WH-Human_1_GenBank 24541 AGGCTGAAGTGCAAATTGATAGGTTGATCACAGGCAGACTTCAAAGTTTGCAGACATATG 24600
20-03seq             148 AGGCTGAAGTGCAAATTGATAGGTTGATCACAGGCAGACTTCAAAGTTTGCAGACATATG 207

WH-Human_1_GenBank 24601 TGACTCAACAATTAATTAGAGCTGCAGAAATCAGAGCTTCTGCTAATCTTGCTGCTACTA 24660
20-03seq             208 TGACTCAACAATTAATTAGAGCTGCAGAAATCAGAGCTTCTGCTAATCTTGCTGCTACTA 267

WH-Human_1_GenBank 24661 AAATGTCAGAGTGTGTACTTGGACAATCAAAAAGAGTTGATTTTTGTGGAAAGGGCTATC 24720
20-03seq             268 AAATGTCAGAGTGTGTACTTGGACAATCAAAAAGAGTTGATTTTTGTGGAAAGGGCTATC 327

WH-Human_1_GenBank 24721 ATCTTATGTCCTTCCCTCAGTCAGCACCTCATGGTGTAGTCTTCTTGCATGTGACTTATG 24780
20-03seq             328 ATCTTATGTCCTTCCCTCAGTCAGCACCTCATGGTGTAGTCTTCTTGCATGTGACTTATG 387

WH-Human_1_GenBank 24781 TCCCTGCACAAGAAAAGAACTTCACAACTGCTCCTGCCATTTGTCATGATGGAAAAGCAC 24840
20-03seq             388 TCCCTGCACAAGAAAAGAACTTCACAACTGCTCCTGCCATTTGTCATGATGGAAAAGCAC 447

WH-Human_1_GenBank 24841 ACTTTCCTCGTGAAGGTGTCTTTGTTTCAAATGGCACACACTGGTTTGTAACACAAAGGA 24900
20-03seq             448 ACTTTCCTCGTGAAGGTGTCTTTGTTTCAAATGGCACACACTGGTTTGTAA--------- 498
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