THE LANCET

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Chan J F-W, Yuan S, Kok K-H, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* 2020; published online Jan 24. http://dx.doi.org/10.1016/S0140-6736(20)30154-9.

Supplementary Methods

Whole genome sequencing

Specimen preparation

To deplete host cells, nasopharyngeal and sputum specimens were centrifuged at 16,000 × g for 2 min, and supernatant was used for subsequent RNA extraction (1). An internal RNA control (*Escherichia coli* bacteriophage MS2 [ATCC® 15597B1]) was added to the supernatant. RNA was extracted from 140 μL of supernatant or no-template control (viral transport medium) using QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) as we described previously (2). DNase treatment (TURBO DNA-free Kit, Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) was used in removing residual host DNA.

Sequence-independent single-primer amplification (SISPA)

SISPA was performed as described previously with modifications (1,3). Briefly, DNase-treated RNA was reverse transcribed to single strand cDNA using primer A (5'-GTTTCCCACTGGAGGATA-N9-3'). Second strand cDNA synthesis was performed using Klenow Fragment (3'→5' exo-) (New England BioLabs, Ipswich, Massachusetts). PCR using primer B (5'-GTTTCCCACTGGAGGATA-3') was used in generating the amplified cDNA libraries.

Nanopore sequencing library preparation was performed according to manufacturer's instructions for Ligation Sequencing Kit (SQK-LSK109, Oxford Nanopore Technologies). Briefly, amplified PCR products were purified by 1 × AMPure XP bead (Beckman Coulter, California, CA). Equal molar of each amplified PCR products were then subjected to DNA repair, end preparation, and native barcode ligation (EXP-NBD104, Oxford Nanopore Technologies).

Barcoded samples were pooled and were ligated to sequencing adaptor. Sequencing was performed with Oxford Nanopore MinION device using R9.4.1 flow cell for 12-48 hours.

Bioinformatics analysis

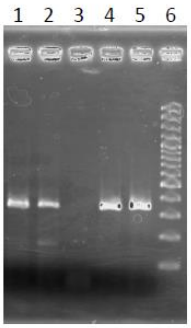
After sequencing, Guppy v3.4.4 was used in converting the raw signal data into FASTQ format, demultiplexing, removal of nanopore and SISPA adaptor sequences. Only reads with a minimum Q score of 7 were included for subsequent analysis. The sequencing run was quality-checked using MinIONQC (4). Human reads were depleted by mapping to reference human genome hg38, and unmapped reads were extracted using SAMTools (5). BCFtools Mpileup was used in creating a variant file (6). BCFtools call (6), vcfutils.pl (5), and Seqtk seq (7) were used in generating the FASTA consensus sequence. Finally, the coverage data was obtained using SAMtools (5).

Supplementary figure legends:

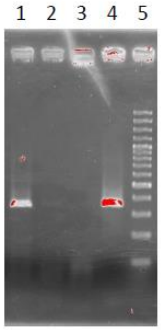
Figure S1. Gel electrophoresis after reverse transcription-polymerase chain reaction using primers targeting RNA-dependent RNA polymerase of SARS-related coronaviruses.

Figure S2. Melting curve of SYBR green real-time polymerase chain reaction targeting Spike of the novel coronavirus showing a unique clean peak and a melting temperature of ~78 ^{o}C.

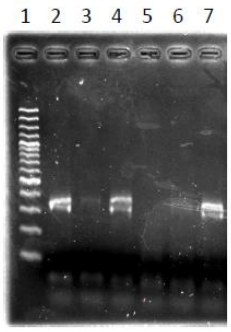
S1



Lane 1: P2 NPS
Lane 2: P1 NPS
Lane 3: Negative control
Lane 4: High positive control
Lane 5: Low positive control
Lane 6: GeneRuler 100 bp Plus DNA Ladder



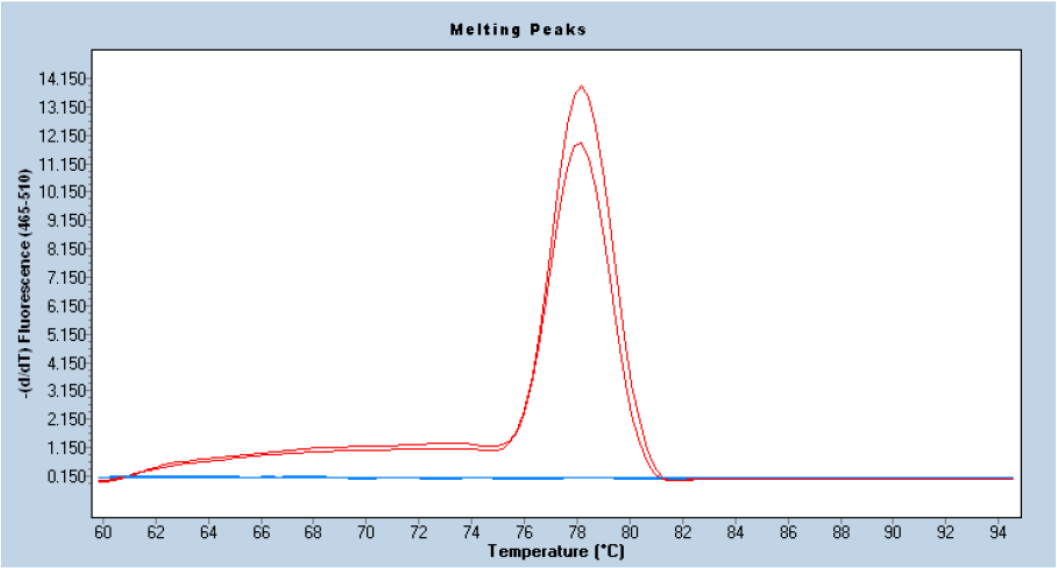
Lane 1: P4 NPS (1st sample)
Lane 2: P4 NPS (2nd sample)
Lane 3: Negative control
Lane 4: Positive control
Lane 5: GeneRuler 100 bp Plus DNA Ladder



Lane 1: GeneRuler 100 bp Plus DNA Ladder
Lane 2: P7 NPS
Lane 3: P7 Throat swab
Lane 4: P7 Sputum
Lane 5: Negative control
Lane 6: P4 NPS (3rd sample)
Lane 7: P5 Sputum

Target size: 344 bp RdRp

S2



Tm of P1 and P2: about 78°C

Supplementary Table S1. Names and accession numbers of the coronavirus strains in the present study.

	Name	Accession number		
P1		RdRp	Spike	Whole genome
NPS	HKU-SZ-001	MN938385	MN938387	-
P2				
NPS*	HKU-SZ-002a	-	-	MN938384
Serum	HKU-SZ-002b	-	MN938388	-
P4				
NPS	HKU-SZ-004	MN938386	MN938389	-
P5				
TS	HKU-SZ-005	-	MN938390	-
Sputum*	HKU-SZ-005b	-	-	MN975262
P7				
NPS	HKU-SZ-007a	MN975263	MN975266	-
TS	HKU-SZ-007b	MN975264	MN975267	-
Sputum	HKU-SZ-007c	MN975265	MN975268	-

* RdRp and Spike sequences can be inferred from whole genome sequence.

References

(1) Lewandowski K, Xu Y, Pullan ST, Lumley SF, Foster D, Sanderson N, et al. Metagenomic Nanopore Sequencing of Influenza Virus Direct from Clinical Respiratory Samples. J Clin Microbiol 2019;58

(2) To KKW, Chan WM, Li KSM, Lam CSF, Chen Z, Tse H, et al. High prevalence of four novel astrovirus genotype species identified from rodents in China. J Gen Virol 2017;10.1099/jgv.0.000766

(3) Greninger AL, Naccache SN, Federman S, Yu G, Mbala P, Bres V, et al. Rapid metagenomic identification of viral pathogens in clinical samples by real-time nanopore sequencing analysis. Genome Med 2015;7:99

(4) Lanfear R, Schalamun M, Kainer D, Wang W, Schwessinger B. MinIONQC: fast and simple quality control for MinION sequencing data. Bioinformatics 2019;35:523-5

(5) Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009;25:2078-9

(6) Narasimhan V, Danecek P, Scally A, Xue Y, Tyler-Smith C, Durbin R. BCFtools/RoH: a hidden Markov model approach for detecting autozygosity from next-generation sequencing data. Bioinformatics 2016;32:1749-51

(7) GitHub. https://github.com/lh3/seqtk (accessed on Jan 10, 2020).