Supplementary Online Content

Young BE, Ong SWX, Kalimuddin S, et al; for the Singapore 2019 Novel Coronavirus Outbreak Research Team. Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. *JAMA*. doi:10.1001/jama.2020.3204

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This supplementary material has been provided by the authors to give readers additional information about their work.

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eMethods

Diagnostic testing and RNA-dependent RNA polymerase (RdRp) sequencing of Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) from nasopharyngeal samples

Extraction of viral nucleic acid from respiratory specimens was performed using EZ1 virus mini kit v2.0 (Qiagen) according to the manufacturer s instruction. The RNA ZDV HDXWGLQHG LQ ORI \ 9 (EXIIHUDQG XVHG DV WHP SODWH UH all assays.

Three specific real-time RT-PCR methods targeting the N, S, and ORF1ab genes were designed to detect the presence of SARS-CoV-2 in clinical samples. The N gene real-time PCR was modified from and shares the same probe as the SARS-CoV N gene RT-PCR. Changes were made in the forward primer to increase sensitivity towards SARS-CoV-2, and in the reverse primer to differentiate between SARS-CoV-2 and SARS-CoV. The modified primer sequences are: forward primer 5 CTC AGT CCA AGA TGG TAT TTC T; reverse primer 5 AGC ACC ATA GGG AAG TCC. The probe sequence is: 5 FAM-ACC TAG GAA CTG GCC CAG AAG CT-BHQ1, as previously described. Thermal cycling was performed at 50°C for 20 minutes for reverse transcription, 95°C for 15 minutes, 50 cycles of 94°C for 5 seconds, then 55°C for 1 minute.

The sequences for the ORF1ab real-time RT-PCR are: forward primer 5 TCA TTG TTA ATG CCT ATA TTA ACC; reverse primer: 5 CAC TTA ATG TAA GGC TTT GTT AAG; probe: 5 FAM- AAC TGC AGA GTC ACA TGT TGA CA-BHQ1. The sequences for the S gene real-time RT-PCR are: forward primer 5 TATACATGTCTCTGGGACCA; reverse primer 5 ATCCAGCCTCTTATTATGTTAGAC; probe 5 FAM-CTAAGAGGTTTGATAACCCTGTCCTACC-BHQ1. Thermal cycling for both ORF1ab and S gene real-time RT-PCR assays were performed at 50°C for 20 minutes for reverse transcription, 95°C for 15 minutes, 50 cycles of 94°C for 5 seconds, 50°C for 20 seconds, and 72°C for 20 seconds.

) RUDOOD VD OHDFWLRQ FRQWDLQLQJ OO \ WHP SODWH 500nm each of forward and reverse primer, 150nm SUREH DQG O4 XDQW7 HFW5 7 P I[ZLWK SUHGHVLJQHG 4 XDQW7 HFW5 7-PCR kit (Qiagen). All reactions were run on LightCycler 2.0 instrument (Roche). All samples were also tested for endogenous RNAse P as an internal control.

One step RT-PCR for coronaviruses was performed using primers modified from a pan-human coronavirus sequencing assay (forward primer 5 GGTTGGGACTATCCTAARTGTGA; reverse primer 5 GGCATCATCAGATAGAATCATCAT)[1]. This primer set was used to amplify a 440bp region using Superscript

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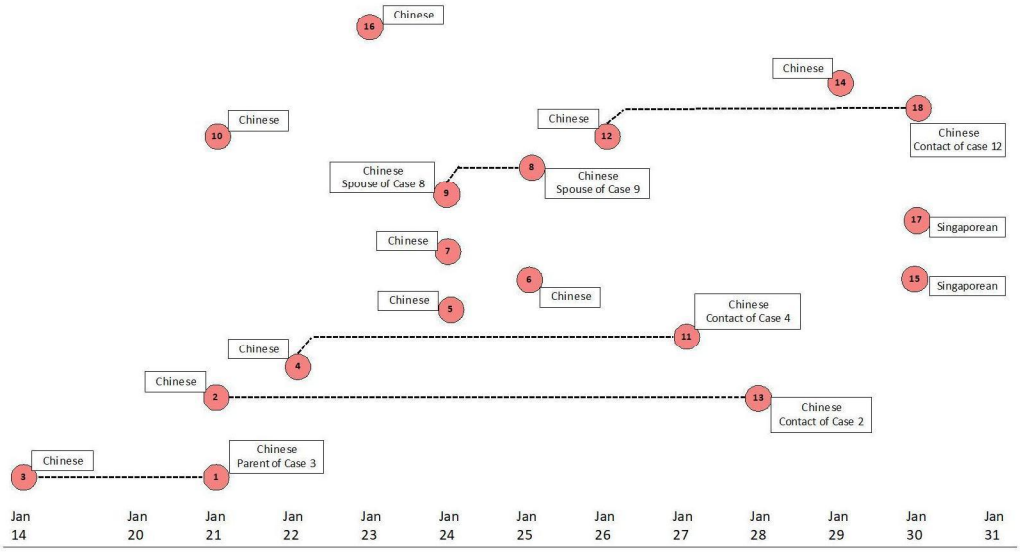
,,,↑3 DMQXP 7DI NLW, QYLWRJHQHQD↓↓↑3 OHDFWRQFRQWLQJ↓↑3 OWP SDW5 1 $ DQG↓↓!!!Q0 of each primer. Reverse transcription was performed at 45°C for 10 minutes, 55°C for 10 minutes, followed by Taq activation at 94°C for 2 minutes. Target amplification was performed in 55 cycles of 94°C for 15 seconds, 48°C for 30 seconds, 68°C for 1 minute, and a final extension at 68°C for 5 minutes. Amplified products were visualized by agarose gel electrophoresis and sequenced using Applied Biosystem 3500xL Genetic Analyzer (Applied Biosystems), using the PCR primers and Bigdye v3.1 (Applied Biosystems).

SARS-CoV-2 PCR from other samples

World Health Organisation interim guidance (updated 17 January 2020) for laboratory testing for Coronavirus disease (COVID-19) in humans was followed for PCR of stool, urine and blood.[1]

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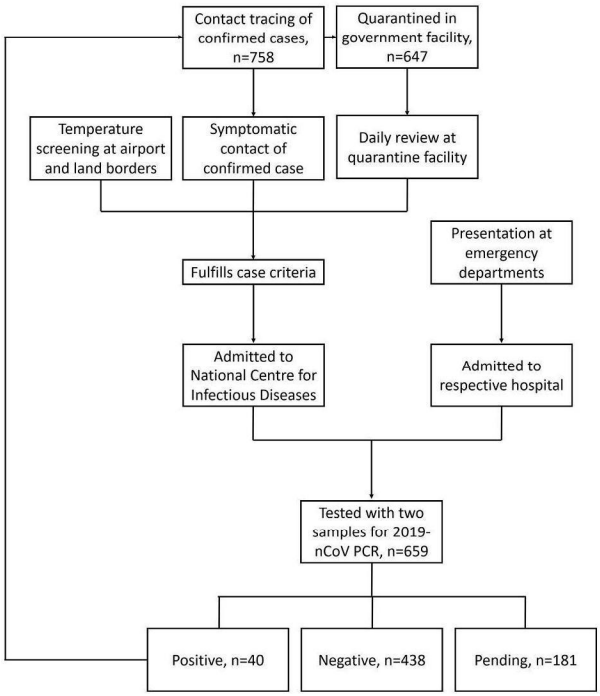
eFigure 1. Case Map of Confirmed 2019-nCoV Cases in Singapore



Dotted lines indicate close contact between cases. Dates refer to date of symptom onset.

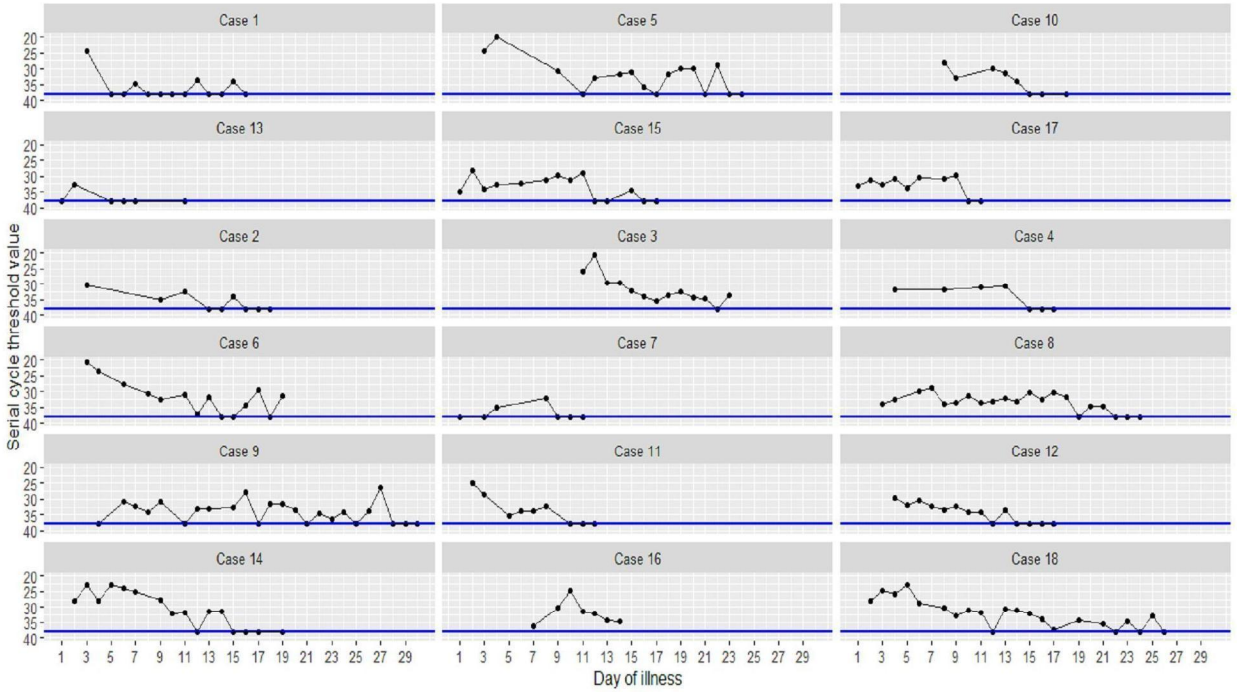
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eFigure 2. Workflow of Screening and Admission for Suspected COVID-19 (as of February 8, 2020)



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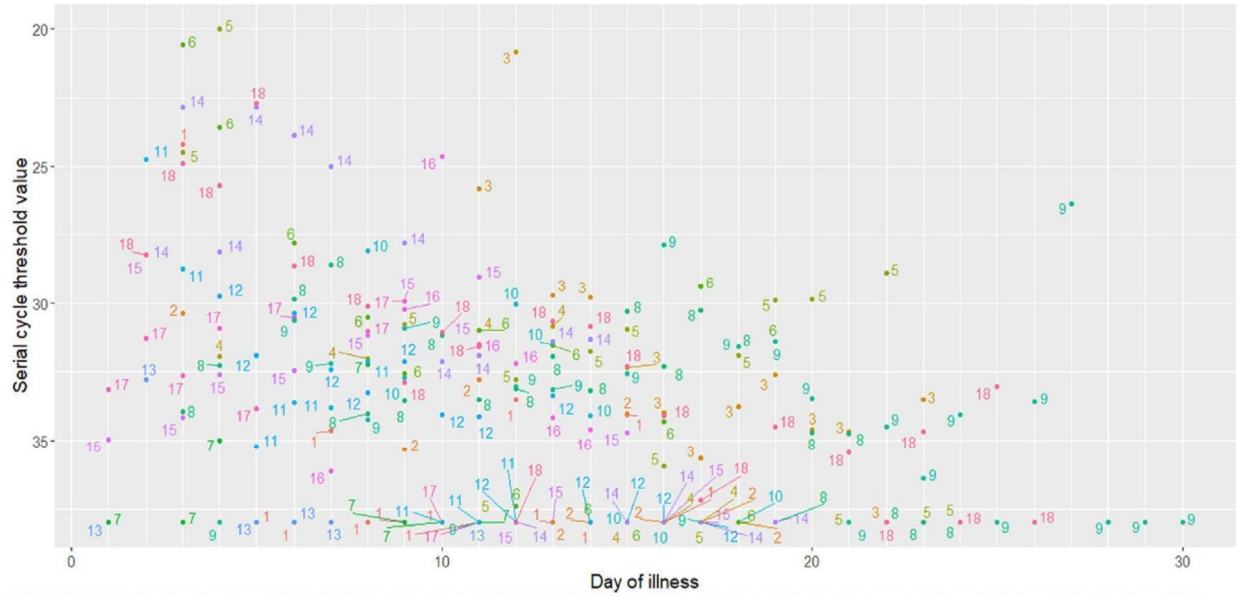
eFigure 3A. Individual Plot of Serial Cycle Threshold (Ct) Values by Day of Illness for Each Patient



Six patients (Case 1, 5, 10, 13, 15 and 17) required supplemental oxygen, while the other 12 patients did not require supplemental oxygen. Cases 1, 5, 10, 15 and 15 also received lopinavir-ritonavir. Negative PCR results (target not detected) are graphed as a Ct value of 38 for ease of viewing and interpretation (blue horizontal blue line). Cycle Threshold Value corresponds with the number of copies of the virus in a biological sample, in an inversely proportional and exponential manner.

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eFigure 3B. Serial Cycle Threshold Values for All Patients by Day of Illness

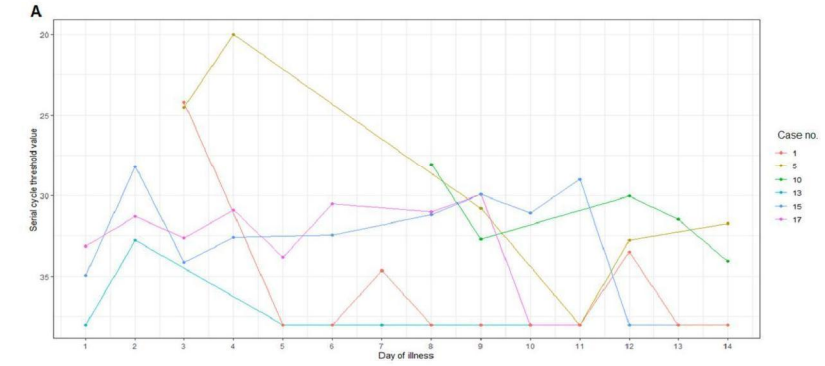


Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
No. measured	4	6	11	11	7	11	8	12	14	10	17	13	13	11	13	13	11	7	7	4	5	5	5	4	2	2	1	1	1	1

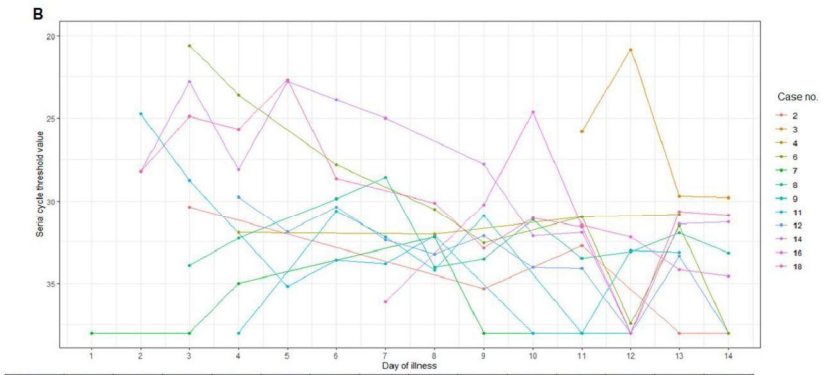
Negative PCR results (target not detected) are graphed as a Ct value of 38 for ease of viewing and interpretation. Each data point represents the lowest Ct value of that 24-hour period for each patient (case number indicated). Different colors correspond to different patients.

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eFigure 4. Serial Cycle Threshold (Ct) Values by Day of Illness up to Day 14, Split by Patients Who (A) Required Supplemental Oxygen and (B) Did Not Require Supplemental Oxygen



Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14
No. measured	3	3	4	3	3	4	2	4	5	3	5	4	3	3

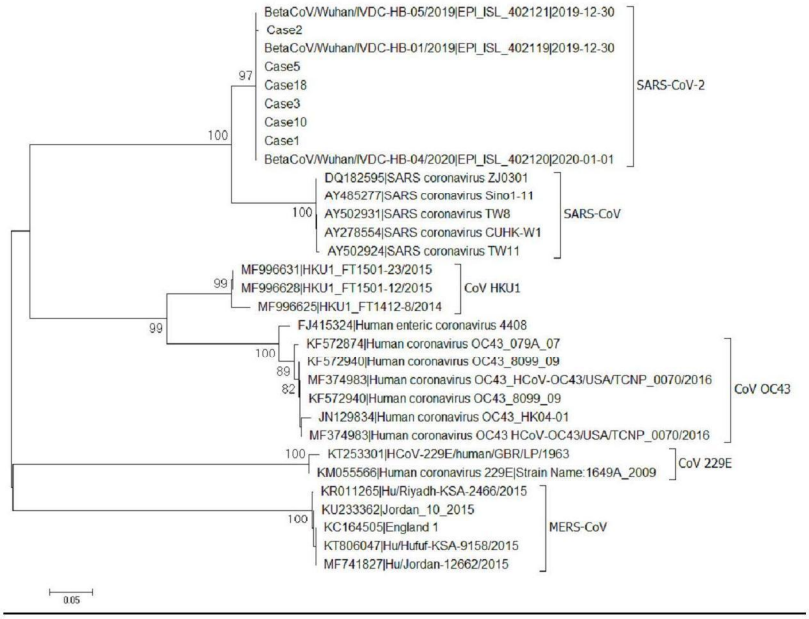


Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14
No. measured	1	3	7	8	4	7	6	8	9	7	12	9	10	8

Negative PCR results (target not detected) are graphed as a Ct value of 38 for ease of viewing and interpretation. Each data point represents the lowest Ct value of that 24-hour period for each patient.

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eFigure 5. Phylogenetic Tree of Six Patients With Available Sequences



Genetic analysis of partial RdRp gene sequence of SARS-CoV-2 strains from six of the cases.. The phylogenetic tree was inferred by Maximum Likelihood, with 1000 bootstrap validation.[2]

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eTable 1. Epidemiologic Features of Patients Infected With 2019-nCoV

		n (%)
Exposure history		
	Travel to Wuhan	18 (100)
	Contact with Huanan seafood market	1 (5.6)
	Contact with healthcare facility in China	3 (17)
	Contact with known case of 2019-nCoV	10 (56)
Source of referral		
	Self-referral to emergency department	10 (56)
	Primary healthcare clinic	1 (5.6)
	Screening at air or land borders	3 (17)
	Contact tracing	4 (22)

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eTable 2. Cycle Threshold Values for Respiratory, Blood, Urine, and Stool Samples by Day of Illness

Case No.	Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	Respiratory			24.2	-	ND	ND	34.7	ND	ND	ND	ND	33.5	ND	ND	34.1	ND	ND	-	ND	ND	ND		
	Blood			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	Urine			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	Stool			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
2	Respiratory			30.4	-	-	-	-	-	35.3	-	32.8	-	ND	ND	34.0	ND	ND						
	Blood			-	ND	-	ND	-	ND	-	-	ND	-	-	-	-	-	-						
	Urine			-	ND	-	-	-		-	-	-	-	-	-	-	-	-						
	Stool			-	-	ND	ND	-	ND	-	ND	-	ND	ND	-	-	-	-						
3	Respiratory											25.8	20.8	29.7	29.8	32.3	34.0	35.6	33.8	32.6	34.6	34.7	ND	33.5
	Blood											-	-	-	-	-	-	-	-	-	-	-	-	-
	Urine											-	-	-	-	-	-	-	-	-	-	-	-	-
	Stool											-	-	-	-	-	-	-	-	-	-	-	-	-
4	Respiratory				31.9	-	-	-	32.0	-	-	31.0	-	30.8	-	ND	ND	ND						
	Blood				-	-	-	-	-	-	-	-	-	-	-	-	-	-						
	Urine				-	-	-	-	-	-	-	-	-	-	-	-	-	-						
	Stool				-	-	-	-	-	-	-	-	-	-	-	-	-	-						
5	Respiratory			24.5	20.0	-	-	-	-	30.8	-	ND	32.8	-	31.8	31.0	36.0	ND	31.9	29.9	29.9	ND	28.9	
	Blood			-	-	-	-	-	-	-	-	-	-	32.7	33.6	-	-	-	-	-	-	-	-	
	Urine			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Stool			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
6	Respiratory			20.6	23.6	-	27.8	-	30.5	32.6	-	31.0	37.4	31.5	ND	ND	34.3	29.4	ND	31.4				
	Blood			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
	Urine			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
	Stool			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
7	Respiratory	ND	-	ND	35.0	-	-	-	32.2	ND	ND	ND												
	Blood	-	-	-	-	ND	-	-	-	-	-	-												
	Urine	-	-	-	-	ND	-	-	-	-	-	-												
	Stool	-	-	-	-	-	-	-	-	-	-	-												

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8	Respiratory			34.0	32.3	-	29.9	28.6	34.0	33.5	31.2	33.5	33.1	31.9	33.2	30.3	32.3	30.3	31.6	ND	34.7	34.8	ND	ND
	Blood			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Urine			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Stool			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	Respiratory				ND	-	30.6	32.2	34.2	30.9	-	ND	33.0	33.2	-	32.6	27.9	ND	31.6	31.4	33.5	ND	34.5	36.4
	Blood				-	-	-	ND	-	ND	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Urine				-	-	-	ND	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Stool				-	-	-	-	ND	ND	-	ND	-	ND	-	ND	-	-	-	-	-	-	-	-
10	Respiratory								28.1	32.7	-	-	30.0	31.5	34.1	-	ND	-	ND					
	Blood								-	-	ND	-	-	-	-	-	-	-	-					
	Urine								-	-	ND	-	-	-	-	-	-	-	-					
	Stool								-	-	-	ND	-	ND	-	-	-	-	-					
11	Respiratory		24.8	28.7	-	35.2	33.6	33.8	32.1	-	ND	ND	ND											
	Blood		-	-	ND	-	ND	-	-	-	-	-	-											
	Urine		-	-	-	-	-	-	-	-	-	-	-											
	Stool		-	-	-	26.0	26.0	-	26.0	-	-	-	-											
12	Respiratory				29.6	31.9	30.4	32.4	33.2	32.1	34.0	34.1	ND	33.4	ND	ND	ND	ND						
	Blood				-	ND	-	-	-	-	-	-	-	-	-	-	-	-						
	Urine				-	ND	-	-	-	-	-	-	-	-	-	-	-	-						
	Stool				-	-	-	-	-	-	-	-	-	-	-	-	-	-						
13	Respiratory	ND	32.8	-	-	ND	ND	ND	-	-	-	ND												
	Blood	-	-	-	ND	-	ND	-	-	-	-	-												
	Urine	-	-	-	-	-	-	-	ND	-	-	-												
	Stool	-	-	-	-	-	-	-	-	36.0	-	-												
14	Respiratory		28.2	22.8	28.1	22.8	23.9	25.0	-	27.8	32.1	31.9	ND	31.4	31.3	ND	ND	ND	-	ND				
	Blood		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
	Urine		-	-	-	-	ND	-	-	-	-	-	-	-	-	-	-	-	-	-				
	Stool		-	-	-	-	-	30.0	29.0	-	-	-	-	-	-	-	-	-	-	-				
15	Respiratory	35.0	28.2	34.2	32.6	-	32.5	-	31.2	29.9	31.1	29.0	ND	ND	-	34.7	ND							
	Blood	-	-	-	-	ND	-	ND	-	-	-	-	-	-	-	-	-							
	Urine	-	-	-	ND	-	-	-	-	-	-	-	-	-	-	-	-							

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	Stool	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-							
16	**Respiratory**							**36.1**	-	**30.2**	**24.6**	**31.5**	**32.2**	**34.2**	**34.6**									
	Blood							-	-	-	-	-	-	-	-									
	Urine							-	-	-	-	-	-	-	-									
	Stool							-	-	-	-	-	-	-	-									
17	**Respiratory**	**33.2**	**31.3**	**32.6**	**30.9**	**33.8**	**30.5**	-	**31.0**	**29.9**	ND	ND												
	Blood	-	-	-	ND	-	ND	-	-	-	-	-												
	Urine	-	-	-	ND	-	-	-	-	-	-	-												
	Stool	-	-	-	-	-	-	-	ND	-	-	-												
18	**Respiratory**		**28.2**	**24.9**	**25.7**	**22.7**	**28.6**	-	**30.1**	**32.9**	**31.1**	**31.6**	ND	**30.7**	**30.9**	**32.3**	**34.1**	**37.2**	-	**34.5**	-	**35.4**		
	Blood		-	-	-	ND	-	ND	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	Urine		-	-	-	ND	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	Stool		-	-	-	-	**30.0**	**29.0**	-	-	-	-	-	-	-	-	-	-	-	-	-	-		

- indicates not done, ND indicates not detected.
Values are indicated to one decimal place.

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eTable 3. Singapore 2019 Novel Coronavirus Outbreak Research Team Members

Name	Highest Degree	Affiliation / Institution
Poh Lian Lim	MD	National Centre for Infectious Diseases, Singapore
Brenda Sze Peng Ang	MPH	National Centre for Infectious Diseases, Singapore
Cheng Chuan Lee	MMed	National Centre for Infectious Diseases, Singapore
Li Min Ling	MBBS	National Centre for Infectious Diseases, Singapore
Lawrence Soon U Lee	PhD	National Centre for Infectious Diseases, Singapore
Chen Seong Wong	MBBS	National Centre for Infectious Diseases, Singapore
Tau Hong Lee	MBBS	National Centre for Infectious Diseases, Singapore
Sapna Pradip Sadarangani	MBBS	National Centre for Infectious Diseases, Singapore
Ray Junhao Lin	MMed	National Centre for Infectious Diseases, Singapore
Po Ying Chia	MBBS	National Centre for Infectious Diseases, Singapore
Mucheli Sharavan Sadasiv	MMed	National Centre for Infectious Diseases, Singapore
Deborah Hee Ling Ng	MBChB	National Centre for Infectious Diseases, Singapore
Chiaw Yee Choy	MBBS	National Centre for Infectious Diseases, Singapore
Tsin Wen Yeo	PhD	National Centre for Infectious Diseases, Singapore
Stephanie Sutjipto	MBBS	National Centre for Infectious Diseases, Singapore
Pei Hua Lee	MBChB	National Centre for Infectious Diseases, Singapore
Jun Yang Tay	MBBS	National Centre for Infectious Diseases, Singapore
Angela Li Ping Chow	PhD	National Centre for Infectious Diseases, Singapore
Wycliffe Wei Enli	MBBS	National Centre for Infectious Diseases, Singapore
Ding Ying	PhD	National Centre for Infectious Diseases, Singapore
Bo Yan Khoo	MBBS	National Centre for Infectious Diseases, Singapore
Woo Chiao Tay	MBBS	National Centre for Infectious Diseases, Singapore
Gabrielle Ng	MBBS	National Centre for Infectious Diseases, Singapore
Yun Yuan Mah	MBBS	National Centre for Infectious Diseases, Singapore
Wilnard Tan	MBBS	National Centre for Infectious Diseases, Singapore
Raymond Kok Choon Fong	MBBS	Changi General Hospital, Singapore
Helen May Lin Oh	MMed	Changi General Hospital, Singapore
Jaime Mei Fong Chien	MMed	Changi General Hospital, Singapore
Humaira Shafi	MBBS	Changi General Hospital, Singapore
Hau Yiang Cheong	MBBS	Changi General Hospital, Singapore
Darren Cheng Han Teo	MBBS	Changi General Hospital, Singapore
Thuan Tong Tan	PhD	Singapore General Hospital, Singapore
Limin Wijaya	MBBS	Singapore General Hospital, Singapore
Indumathi Venkatachalam	MPH	Singapore General Hospital, Singapore
Ying Ying Chua	MMed	Singapore General Hospital, Singapore
Benjamin Pei Zhi Cherng	MBBS	Singapore General Hospital, Singapore
Yvonne Fu Zi Chan	MMed	Singapore General Hospital, Singapore
Lynette Lin Ean Oon	MBBS	Singapore General Hospital, Singapore
Natalie MY Lee	BSc	Singapore General Hospital, Singapore
Jade Xiao Jue Soh	MBBS	Sengkang General Hospital, Singapore
Shuwei Zheng	MBBS	Sengkang General Hospital, Singapore
Pushpalatha Bangalore Lingegowda	MBBS	Sengkang General Hospital, Singapore
Derrick Heng	MPH	Ministry of Health, Singapore
Pream Raj	MPH	Ministry of Health, Singapore
Olivia Oh	PhD	Ministry of Health, Singapore
Constance Low	MPH	Ministry of Health, Singapore
Kelly Foo	MPH	Ministry of Health, Singapore
Khine Nandar	MPH	Ministry of Health, Singapore
Yijun Lin	MPH	Ministry of Health, Singapore
Rachael Pung	Msc	Ministry of Health, Singapore
Guanhao Chan	Bsc	Ministry of Health, Singapore
Xinyi Peh	Bsc	Ministry of Health, Singapore
Charlene Ting Ting Tow	MSc	Ministry of Health, Singapore

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