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Correspondence

First isolation of SARS-CoV-2 from clinical samples in India

Sir,

The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has, as on March 31, 2020, spread to over 207 countries around the world[1,2], with a total of 896,475 confirmed cases and 45,525 deaths[2]. The number of reported SARS-CoV-2 cases in India is also on an increase with 1,636 cases and 38 deaths[2]. In the current pandemic situation, the isolation of SARS-CoV-2 is important for developing and evaluating diagnostic reagents, for antiviral studies and for screening of vaccine candidates. Earlier studies showed that SARS-CoV-2 could not replicate in several cell lines, which are routinely used for isolation of respiratory viruses[3]. Human and animal cell lines that were found to support SARS-CoV-1 replication during the first outbreak of SARS in China, 2002[4], are currently being studied. The virus was first isolated in the human airway epithelial cells from clinical specimens as part of early attempts to identify the aetiologic agent of infection[5]. We describe here the successful isolation and characterization of SARS-CoV-2 from clinical samples in India using Vero CCL-81 cells by observing cytopathic effects (CPEs) and cycle threshold (Ct) values in real-time reverse transcription-polymerase chain reaction (RT-PCR), electron microscopy and next-generation sequencing (NGS).

The first three SARS-CoV-2 cases were reported from Kerala during January 27-31, 2020. Later during March 2020, cases were also reported from a group of Italian tourists (n=15) and their contacts in New Delhi, India. Simultaneously, cases were reported in Agra, Uttar Pradesh, which was the outcome of close contact of an infected Delhi-based individual who returned from Italy. The designated COVID-19 testing laboratories of Virus Research Diagnostic Laboratory network (All India Institute of Medical Sciences, New Delhi; Sawai Man Singh Medical College, Jaipur; and King George's Medical University, Lucknow) referred the specimens (throat swab/nasal swab, oropharyngeal swab/sputum) to the Indian Council of Medical Research-National Institute of Virology (ICMR-NIV), Pune, after screening for envelope (*E*) gene by real-time RT-PCR was done[6]. A total of 12 SARS-CoV-2 positive specimens having a Ct <30 for the *E* gene were included in the study. Of these, eight samples were from positive cases of Italian tourists and their contacts in New Delhi. The rest of the specimens were from four positive cases at Agra, Uttar Pradesh, and the close contact cases of an infected Delhi-based individual who returned from Italy.

The clinical specimens of the 12 cases were used for infecting Vero CCL-81 which was maintained in Eagle's minimum essential medium (MEM; Gibco, UK) supplemented with 10 per cent foetal bovine serum (FBS) (HiMedia, Mumbai), penicillin (100 U/ml) and streptomycin (100 mg/ml). Likewise, 100 µl was inoculated onto 24-well cell culture monolayers of Vero CCL-81, before growth medium was decanted. The cells were incubated for one hour at 37°C to allow virus adsorption, with rocking every 10 min for uniform virus distribution. After the incubation, the inoculum specimen was removed and the cells were washed with 1X phosphate-buffered saline (PBS). The MEM supplemented with two per cent FBS was added to each well. The cultures were incubated further in five per cent CO_2 incubator at 37°C and observed daily for CPEs under an inverted microscope (Nikon, Eclipse Ti, Japan). Cellular morphological changes were recorded using a camera (Nikon, Japan). From each well of cell culture plate, on the third post-infection day (PID-3) of passage-1 (P-1), 50 µl of supernatant was taken and tested for SARS-CoV-2 using real-time RT-PCR for *E* and RNA-dependent RNA polymerase (*RdRp*) (2) genes

Supplementary material available from *http://www.ijmr.org.in/article.asp?issn=0971-5916;year=2020;volume=151;issue=2;spage=244;epage=250;aulast=Sarkale*

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as described earlier[7,8]. Similar testing was repeated on the cell supernatant of passage-2 (P-2) at PID-4 for observing viral copy number. Cultures that showed CPE on PID-4 were centrifuged at 4815 × *g* for 10 min at 4°C; the supernatants were processed immediately or stored at −86°C. Further, those that showed CPE were grown in T-25 cm^2 flasks at P-2 and titration was done after serial dilution. Tissue culture infective dose 50 per cent ($TCID_{50}$) values were calculated by the Reed and Muench method[9]. CPEs were observed in 9 of 12 cultures in the P-1. The $TCID_{50}$ values ranged from $10^{5.5}$ to $10^{6.4}$/ml for the different clinical specimens passaged in Vero CCL-81 at P-2. The cells were examined microscopically for cellular morphological changes following inoculation.

Vero CCL-81 cells infected with SARS-CoV-2 strain NIV-2020-770 and uninfected cells (CC) were transferred onto microcavity slides and fixed with acetone. Serum samples (1:25 dilution) from the confirmed COVID-19 cases (POD nCOV-S11, nCOV-S13 and nCOV-S7) and negative serum samples were added followed by incubation at 37°C for 1.5 h[10]. Antibody reactivity was visualized using anti-human immunoglobulin fluorescein-isothiocynate. In immunofluorescence assay of COVID-19 positive patients, three serum samples exhibited specific reactivity against SARS-CoV-2 virus isolate (Fig. 1).

Vero CCL-81 cells that were inoculated with the samples showed evidence of cell rounding and detachment from 9 of 12 clinical samples in P-1 at PID-4. Syncytial cells formed large cell masses that increased in size and number as the infection progressed. Enhanced CPE was noted in P-2 at PID-2. The cells were detached from the tissue culture plate surfaces by PID-3. Similar cellular morphological changes were observed after passaging of the above nine samples up to P-2. No cellular changes were observed in the cell control during both passages. Figure 2 depicts the day-wise changes during the passage of a representative clinical isolate (NIV-2020-770). Virus replication was confirmed using real-time RT-PCR with RNA extracted from the cell culture medium on PID-3. The Ct values ranged from 9.79 to 15.41 (in Vero CCL-81 cells) for the isolates at P-2, which were lower than the Ct values of 16-25.1 in the clinical samples (Table I). The number of virus copies in the isolates at P-1 in Vero CCL-81 cells ranged from 5.18×10^7 to 8.12×10^8 copy/ml and increased 1-26 fold to a range of 1.69×10^8 to 6.77×10^9 in the cell culture supernatants at P-2 (Table I).

On PID-4, enhanced CPE was observed. The P-1 material was reinoculated in a new batch of cells, and it showed progressive enhancement of CPE as observed day-wise. Further, an aliquot of cell culture supernatant was harvested from infected Vero CCL-81 showing CPE and the supernatant used for negative staining as described elsewhere[11,12]. Distinct CoV particles with an average size of 95±10 nm having a distinct envelope fringe could be detected in the fields scanned (Fig. 3), as observed earlier[13].

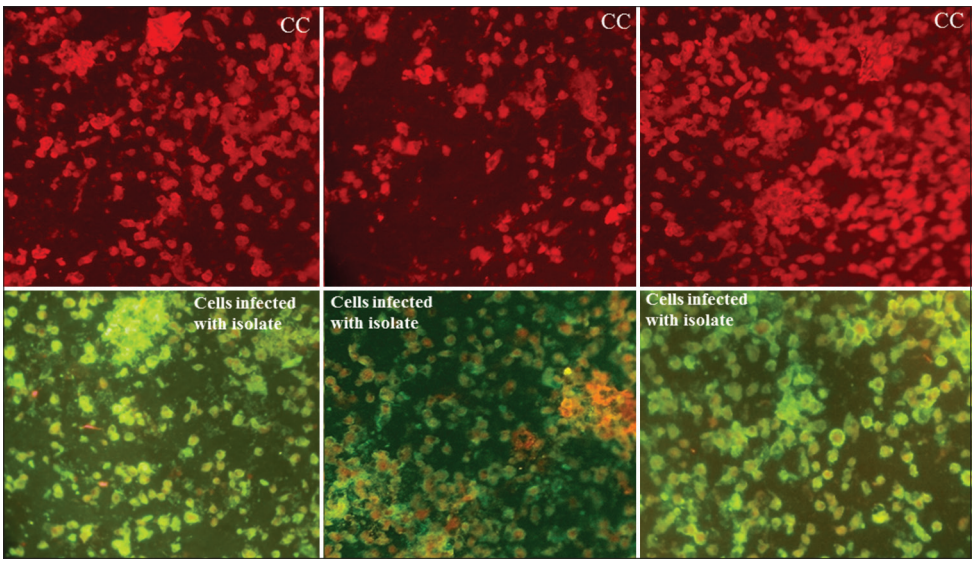

Fig. 1. Immunofluorescence images (red panel) showing uninfected Vero CCL-81 cells probed by positive patient serum samples after post infection day of 13th (left), 11th (middle) and seventh (right) and with SARS-CoV-2 strain NIV-2020-770 infected Vero CCL-81 cells probed by positive patients serum (green panel) showing the reactivity of virus and antibody.

Table I. Cycle threshold (Ct) of SARS-CoV-2 positive clinical specimens and respective viral copy number in isolates in different passages for two different cell culture types using real-time reverse transcription-polymerase chain reaction (RT-PCR). *E* gene was targeted in all

Serial number	Sample ID	Isolate ID	Ct (copy number) of viral RNA in real-time RT-PCR		
			Original (clinical) samples by qRTPCR (Ct)	Vero CCL-81 passage-1 Ct (copy number)	Vero CCL-81 passage-2 Ct (copy number)
1	nCoV-763	NIV-2020-763	18.07	10.56 (4.08×10^9)	11.14 (2.77×10^9)
2	nCoV-770	NIV-2020-770	18	15.15 (1.96×10^8)	11.62 (2.02×10^9)
3	nCoV-772	NIV-2020-772	20.2	14.00 (4.18×10^8)	10.93 (3.19×10^9)
4	nCoV-773	NIV-2020-773	25.1	17.15 (5.18×10^7)	15.41 (1.69×10^8)
5	nCoV-781	NIV-2020-781	22.1	14.91 (2.27×10^8)	10.0 (5.91×10^9)
6	nCoV-C132	NIV-2020-C132	16	13.68 (5.12×10^8)	10.73 (3.64×10^9)
7	nCoV-777	NIV-2020-777	23.3	13.31 (6.57×10^8)	9.99 (5.92×10^9)
8	nCoV-C31	NIV-2020-C31	25	12.99 (8.12×10^8)	9.79 (6.77×10^9)
9	nCoV-C32	NIV-2020-C32	16	13.21 (7.01×10^8)	10.25 (5.05×10^9)

Serial numbers 1-7: Italian tourists who arrived in Delhi, India and an Indian contact of the cohort; Serial numbers 8-9: Close contacts in Agra, Uttar Pradesh, of an infected Delhi-based person who returned from Italy. qRT-PCR, quantitative RT-PCR

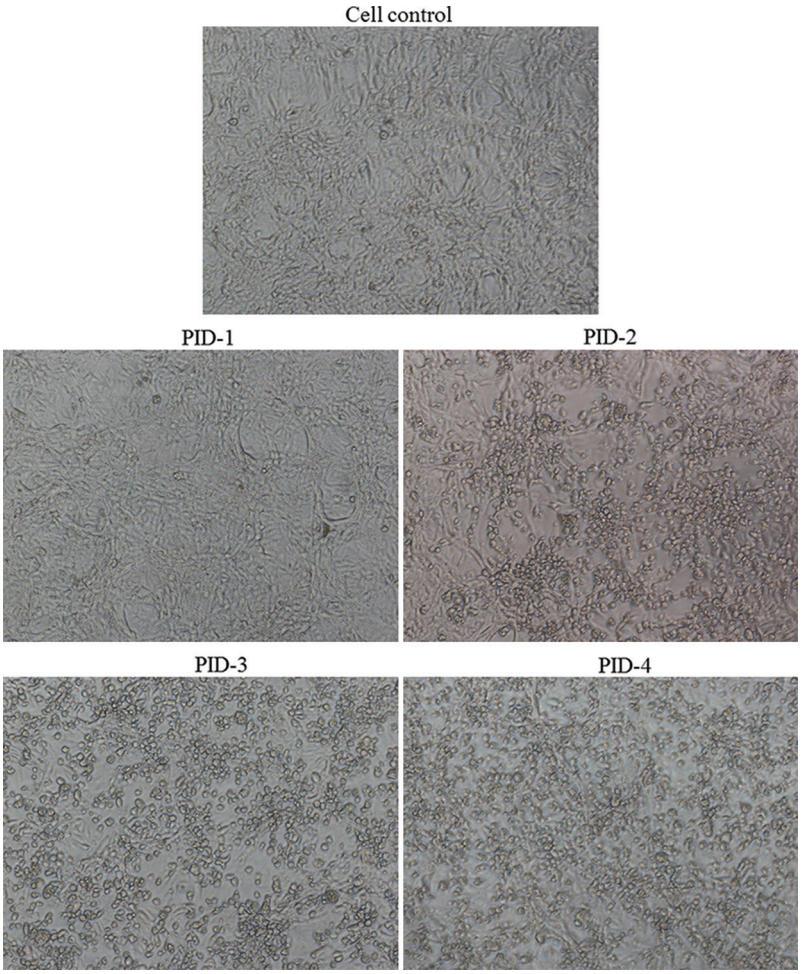

Fig. 2. Cytopathic effect of the SARS-CoV-2 isolate (NIV-2020-770) demonstrated in Vero CCL-81 cells on different post-infection days (PID).

Table II. Per cent of the reads mapped, total reads and the per cent of genome coverage recovered for the clinical samples and the isolates

Sample type	Sample/isolate details	Total reads	Per cent of reads mapped	Per cent of genome recovered	Position of nucleotide in genome[17]	
					8782	28144
Isolate	NIV-2020-763	10,054,258	94.8	100	C	T
	NIV-2020-770	4,384,130	99.0	100	C	T
	NIV-2020-772	3,482,648	98.4	99.9	C	T
	NIV-2020-773	5,952,758	94.2	99.9	C	T
	NIV-2020-777	3,949,748	98.7	100	C	T
	NIV-2020-781	2,226,464	91.6	99.9	C	T
	NIV-2020-C32	4,159,878	99.0	100	C	T
Clinical sample	nCoV-763	8,721,610	84.9	99.9	T	T
	nCoV-770	5,197,614	93.1	99.9	T	T
	nCoV-772	4,222,912	81.7	99.8	C	T
	nCoV-773	9,951,190	19.98	99.8	C	T
	nCoV-777	8,808,756	26.93	99.8	C	T
	nCoV-781	15,688,460	35.5	99.9	C	T
	nCoV-C32	2,772,158	88.5	100	C	T

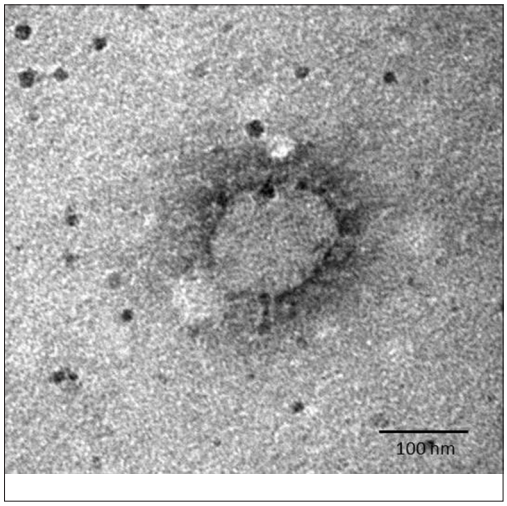

Fig. 3. Transmission electron microscopy imaging of SARS-CoV-2. A negative-stained SARS-CoV-2 viral particle, demonstrating spike morphology of glycoprotein along with peplomeric projections, a feature typical to the family *Coronaviridae*, is seen.

Next-generation sequencing was performed on SARS-CoV-2 positive clinical samples (100 µl) included in the study and the tissue culture fluid (50 µl) of virus isolates at PID-3 as described earlier[14,15]. Reference-based mapping as implemented in the CLC genomics workbench 11.0 (CLC, Qiagen) was used to retrieve the sequence of the SARS-CoV-2. BLAST (*https://blast.ncbi.nlm.nih.gov/Blast.cgi*) identification of the viral genome sequences retrieved from the clinical samples and their isolates had 99.98 per cent identity with the SARS-CoV-2 isolate Wuhan-Hu-1 (Accession No. NC_045512). Details of the sequences obtained including the per cent of the reads mapped, total reads and the per cent of genome coverage recovered for the clinical samples and the isolates are provided in Table II. Partial sequences were retrieved from the clinical samples (nCoV-C 132 and nCoV-C 31) and were not included in the analysis.

MEGA software version 7.0.11[16] was used for the multiple alignments of the sequences retrieved in this study and the sequences from the Global Initiative on Sharing All Influenza Data (GISAID) database (*https://www.gisaid.org/*) (Supplementary Table (available from *http://www.ijmr.org.in/articles/2020/151/2/images/IndianJMedRes_2020_151_2_244_282559_sm7.pdf*)). A neighbour-joining tree was generated using the best substitution model (Kimura 2-parameter model) with a bootstrap of 1000 replicates. As per Tang *et al*[17], the circulating SARS-CoV-2 can be grouped into two types (S and L type) based on the two different single-nucleotide polymorphisms (SNPs) at positions 8782 and 28144 in the genome. The S type possesses TC SNPs while the L type possesses CT SNPs at positions 8782 and 28144, respectively. In the present study, it was observed that two sequences from clinical samples (nCoV-763 and nCoV-770) had TT SNPs, while the other sequences

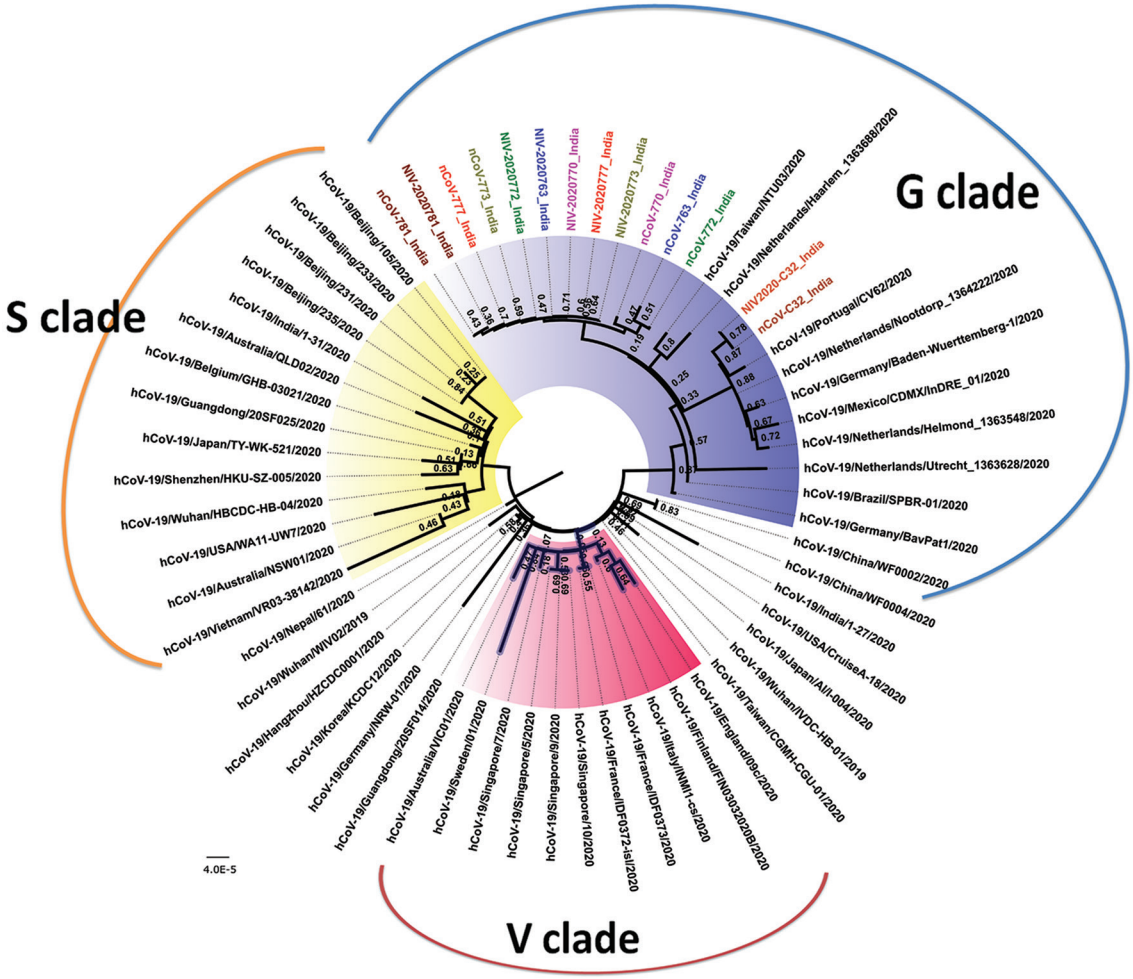

Fig. 4. Neighbour-joining tree of SARS-CoV-2. The phylogenetic tree is generated using the best substitution model. A bootstrap of 1000 replicates was used to assess the statistical robustness of the tree. Same colours are used for sequences derived from a clinical sample and the respective isolate. Clinical samples are labelled with initials as nCoV while the isolates are labelled with initials as NIV. The clades are represented by different colours in the core region (S - yellow, V - pink, G - blue and unclassified - not coloured).

had CT as the SNP (L type) (Table II). The TT SNPs have been observed in few of the GISAID sequences, including one of the Kerala genome sequences (nCoV-19/India/31 January 2020) submitted by us earlier. All the isolates of the clinical samples were of L type. Specific amino acid mutations in the nsp3 region, spike protein and ORF8, in general, lead to the formation of V, G and S genetic variants/clades, respectively, as per the recent classification followed by GISAID. It was observed that the clinical samples, as well as the isolates, had the mutation D614G in the spike protein, classifying the study samples and isolates into the G clade (Table II and Fig. 4). No specific substitutions were observed in any of the isolate sequences with respect to the corresponding clinical sample sequences, as these were sequences from a low passage. The sequences of the clinical samples and the isolate from the contact of the infected Delhi-based individual, who returned from Italy, further showed two mutations, R203K and G204R in the nucleocapsid protein (*N*). Although all strains demonstrated 99.6 per cent identity with the original Wuhan Hu-1 sequence, the role of unique SNPs and mutations in identifying the source of infection needs to be explored.

After the first isolation of the virus in the human airway epithelial cells reported by China[5], countries such as Australia[18], Korea[19], Germany[20] and the USA[21] have also isolated the SARS-CoV-2 strain. In India, initial attempts to isolate the virus from the first three cases did not succeed due to low titres in the clinical specimens. This is the first successful virus isolation of SARS-CoV-2 in the Vero CCL-81 cells in India from nasal and throat swabs of persons with a travel

history from Italy and their contacts. Isolation of SARS-CoV-2 from clinical samples will be helpful to address key questions of correlating the differential cell line susceptibility and viral replication efficiency, especially important for clinical samples with low viral titres. Isolation of the virus in such a pandemic situation would help to develop indigenously designed reagents such as positive controls, virus antigen and antibodies, which could lead to the indigenous development of sero-diagnostic assays. These assays would be critical for conducting population-based serosurveys. Propagation in culture will also facilitate antiviral susceptibility studies and vaccine efforts in India.

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***Conflicts of Interest*:** None.

Prasad Sarkale[1], Savita Patil[1], Pragya D. Yadav[1,*], Dimpal A. Nyayanit[1], Gajanan Sapkal[2], Shrikant Baradkar[1], Rajen Lakra[1], Anita Shete-Aich[1], Sharda Prasad[3], Atanu Basu[3], Lalit Dar[6], Veena Vipat[4], Sidhartha Giri[7], Varsha Potdar[4], Manohar Lal Choudhary[4], Ira Praharaj[7], Amita Jain[8], Bharati Malhotra[9], Pranita Gawande[1], Kaumudi Kalele[1], Nivedita Gupta[7], Sarah S. Cherian[5] & Priya Abraham[†]

[1]Maximum Containment Laboratory, [2]Diagnostic Virology Group, [3]Electron Microscopy & Histopathology Group, [4]Influenza Group, [5]Bioinformatics & Data Management Group, [†]ICMR-National Institute of Virology, Pune 411 001, Maharashtra, [6]Department of Microbiology, All India Institute of Medical Sciences, New Delhi 110 029, [7]Division of Epidemiology & Communicable Diseases, Indian Council of Medical Research, New Delhi 110 029, [8]King George's Medical University, Lucknow 226 003, Uttar Pradesh & [9]Department of Microbiology, SMS Medical College, Jaipur 302 004, Rajasthan, India

[*]*For correspondence*: hellopragya22@gmail.com

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Supplementary Table: Acknowledgement for the list of the sequences downloaded from GISAID database that were used in the study

Accession ID	Virus name	Location	Collection date	Originating laboratory	Submitting laboratory	Authors
EPI_ ISL_413014	hCoV-19/Canada/ ON-PHL2445/2020	North America/ Canada/Ontario	2020-01-25	Public Health Ontario Laboratory	Ontario Agency for Health Protection and Promotion (OAHPP)	Alireza Eshaghi, Samir N Patel, Jonathan B Gubbay, Vanessa G Allen, Christine Frantz, Aimin Li, Sandeep Nagra
EPI_ ISL_402119	hCoV-19/Wuhan/ IVDC-HB-01/2019	Asia/China/Hubei/ Wuhan	2019-12-30	National Institute for Viral Disease Control and Prevention, China CDC	National Institute for Viral Disease Control and Prevention, China CDC	Wenjie Tan Xiang ZhaoWenling Wang Xuejun Ma, Yongzhong Jiang, Roujian Lu, Ji Wang, Weimin Zhou, Peihua Niu, Peipei Liu, Faxian Zhan, Weifeng Shi, Baoying Huang, Jun Liu, Li Zhao, Yao Meng, Xiaozhou He, Fei Ye, Na Zhu, Yang Li, Jing Chen, Wenbo Xu, George F. Gao, Guizhen Wu
EPI_ ISL_413904	hCoV-19/Iran/ Qom255194/2020	Asia/Iran/Qom	2020-02-09	Iran National Influenza Center	Iran National Influenza Center	Nazanin Zahra Shafiei Jandaghi, Jila Yavarian, Fatemeh AjamiNejad, Nastaran Ghavvami, Kaveh Sadeghi and Talat Mokhtari Azad
EPI_ ISL_412973	hCoV-19/Italy/CDG1/2020	Europe/Italy/ Lombardy	2020-02-20	Department of Infectious Diseases, Istituto Superiore di Sanità, Roma , Italy	Virology Laboratory, Scientific Department, Army Medical Center	Paola Stefanelli, Stefano Fiore, Antonella Marchi, Eleonora Benedetti, Concetta Fabiani, Giovanni Faggioni, Antonella Fortunato, Riccardo De Santis, Silvia Fillo, Anna Anselmo, Andrea Ciammaruconi, Stefano Palomba, Florigio Lista
EPI_ ISL_408667	hCoV-19/Japan/ TY-WK-521/2020	Asia/Japan/Tokyo	2020-01-31	Dept. of Virology III, National Institute of Infectious Diseases	Pathogen Genomics Center, National Institute of Infectious Diseases	Tsuyoshi Sekizuka, Shutoku Matsuyama, Naganori Nao, Kazuya Shirato, Makoto Takeda, Makoto Kuroda
EPI_ ISL_408668	hCoV-19/Vietnam/ VR03-38142/2020	Asia/Vietnam/Thanh Hoa	2020-01-24	National Influenza Center - National Institute of Hygiene and Epidemiology (NIHE)	National Influenza Center - National Institute of Hygiene and Epidemiology (NIHE)	Ung Thi Hong Trang, Hoang Vu Mai Phuong, Nguyen Le Khanh Hang, Nguyen Vu Son, Le Thi Thanh, Vuong Duc Cuong, Nguyen Phuong Anh, Pham Thi Hien, Tran Thu Huong, Le Thi Quynh Mai,

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Accession ID	Virus name	Location	Collection date	Originating laboratory	Submitting laboratory	Authors
EPI_ ISL_410546	hCoV-19/Italy/ INMI1-cs/2020	Europe/Italy/Rome	2020-01-31	INMI Lazzaro Spallanzani IRCCS	Laboratory of Virology, INMI Lazzaro Spallanzani IRCCS	Maria R. Capobianchi, Cesare E. M. Gruber, Martina Rueca, Fabrizio Carletti, Barbara Bartolini, Francesco Messina, Emanuela Giombini, Francesca Colavita, Concetta Castilletti, Eleonora Lalle, Emanuele Nicastri, Giuseppe Ippolito.
EPI_ ISL_410536	hCoV-19/ Singapore/5/2020	Asia/Singapore	2020-02-06	Singapore General Hospital, Molecular Laboratory, Division of Pathology	Programme in Emerging Infectious Diseases, Duke-NUS Medical School	Danielle E Anderson, Martin Linster, Yan Zhuang, Jayanthi Jayakumar, Kian Sing Chan, Lynette LE Oon, Shirin Kalimuddin, Jenny GH Low, Yvonne CF Su, Gavin JD Smith
EPI_ ISL_407313	hCoV-19/Hangzhou/ HZCDC0001/2020	Asia/China/Zhejiang/ Hangzhou	2020-01-19	Hangzhou Center for Disease Control and Prevention	Hangzhou Center for Disease Control and Prevention	Jun Li, Haoqiu Wang, Hua Yu, Lingfeng Mao, Xinfen Yu, Zhou Sun, Qingxin Kong, Xin Qian, Shuchang Chen, Xuchu Wang
EPI_ ISL_413589	hCoV-19/Netherlands/ Utrecht_1363628/2020	Europe/Netherlands/ Utrecht	2020-03-01	MHC Utrecht	Erasmus Medical Center	David Nieuwenhuijse Bas Oude Munnink, Reina Sikkema, Claudia Schapendonk, Irina Chestakova, Anne vander Linden, Mark Pronk, Pascal Lexmond, Corien Swaan, Manon Haverkate, Madelief Mollers, Mart Stein, Sandra Kengne Kamga Mobou, Jeroen van Kampen, Jolanda Voermans, Aura Timen, Corine GeurtsvanKessel, Annemiek van der Eijk, Richard Molenkamp, Marion Koopmans, on behalf of the Dutch national COVID-19 response team

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Accession ID	Virus name	Location	Collection date	Originating laboratory	Submitting laboratory	Authors
EPI_ ISL_413572	hCoV-19/Netherlands/ Haarlem_1363688/2020	Europe/Netherlands/ Haarlem	2020-03-01	MHC Kennemerland	Erasmus Medical Center	David Nieuwenhuijse, Bas Oude Munnink, Reina Sikkema, Claudia Schapendonk, Irina Chestakova, Anne van der Linden, Mark Pronk, Pascal Lexmond, Corien Swaan, Manon Haverkate, Madelief Mollers, Mart Stein, Sandra Kengne Kamga Mobou, Jeroen van Kampen, Jolanda Voermans, Aura Timen, Corine GeurtsvanKessel, Annemiek van der Eijk, Richard Molenkamp, Marion Koopmans, on behalf of the Dutch national COVID-19 response team.
EPI_ ISL_413574	hCoV-19/Netherlands/ Helmond_1363548/2020	Europe/Netherlands/ Helmond	2020-02-29	MHC West-Brabant	Erasmus Medical Center	David Nieuwenhuijse, Bas Oude Munnink, Reina Sikkema, Claudia Schapendonk, Irina Chestakova, Anne van der Linden, Mark Pronk, Pascal Lexmond, Corien Swaan, Manon Haverkate, Madelief Mollers, Mart Stein, Sandra Kengne Kamga Mobou, Jeroen van Kampen, Jolanda Voermans, Aura Timen, Corine GeurtsvanKessel, Annemiek van der Eijk, Richard Molenkamp, Marion Koopmans, on behalf of the Dutch national COVID-19 response team.

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EPI_ ISL_413579	hCoV-19/Netherlands/ Nootdorp_1364222/2020	Europe/Netherlands/ Nootdorp	2020-03-03	MHC Haaglanden	Erasmus Medical Center	David Nieuwenhuijse, Bas Oude Munnink, Reina Sikkema, Claudia Schapendonk, Irina Chestakova, Anne van der Linden, Mark Pronk, Pascal Lexmond, Corien Swaan, Manon Haverkate, Madelief Mollers, Mart Stein, Sandra Kengne Kamga Mobou, Jeroen van Kampen, Jolanda Voermans, Aura Timen, Corine GeurtsvanKessel, Annemiek van der Eijk, Richard Molenkamp, Marion Koopmans, on behalf of the Dutch national COVID-19 response team.
EPI_ ISL_413592	hCoV-19/Taiwan/ NTU03/2020	Asia/Taiwan/Taipei	2020-03-02	Department of Laboratory Medicine, National Taiwan University Hospital	Microbial Genomics Core Lab, National Taiwan University Centers of Genomic and Precision Medicine	Shiou-Hwei Yeh, You-Yu Lin, Ya-Yun Lai, Chiao-Ling Li, Shan-Chwen Chang, Pei-Jer Chen, Sui-Yuan Chang
EPI_ ISL_406844	hCoV-19/Australia/ VIC01/2020	Oceania/Australia/ Victoria/Clayton	2020-01-25	Monash Medical Centre	Collaboration between the University of Melbourne at The Peter Doherty Institute for Infection and Immunity, and the Victorian Infectious Disease Reference Laboratory	Caly, L., Seemann, T., Schultz, M., Druce, J. and Taiaroa, G
EPI_ ISL_406862	hCoV-19/Germany/ BavPat1/2020	Europe/Germany/ Bavaria/Munich	2020-01-28	Charité Universitätsmedizin Berlin, Institute of Virology; Institut für Mikrobiologie der Bundeswehr, Munich	Charité Universitätsmedizin Berlin, Institute of Virology	Victor M Corman, Julia Schneider, Talitha Veith, Barbara Mühlemann, Markus Antwerpen, Christian Drosten, Roman Wölfel
EPI_ ISL_413520	hCoV-19/Beijing/233/2020	Asia/China/Beijing	2020-01-28	unknown	Infectious Disease Control Center	Li, J., Li, L., Li, Z., Qiu, S., Song, H., Li, P. and Li, P.
EPI_ ISL_413521	hCoV-19/Beijing/235/2020	Asia/China/Beijing	2020-01-28	unknown	Infectious Disease Control Center	Li, J., Li, L., Li, Z., Qiu, S., Song, H., Li, P. and Li, P.
EPI_ ISL_413522	hCoV-19/India/1-27/2020	Asia/India/Kerala	2020-01-27	Indian Council of Medical Research - National Institute of Virology	National Influenza Center, Indian Council of Medical Research - National Institute of Virology	Potdar V, Yadav PD, Choudhary ML, Shete-Aich A

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Accession ID	Virus name	Location	Collection date	Originating laboratory	Submitting laboratory	Authors
EPI_ ISL_413523	hCoV-19/India/1-31/2020	Asia/India/Kerala	2020-01-31	Indian Council of Medical Research-National Institute of Virology	National Influenza Center, Indian Council of Medical Research-National Institute of Virology	Potdar V, Yadav PD, Choudhary ML, Shete-Aich A
EPI_ ISL_413518	hCoV-19/Beijing/105/2020	Asia/China/Beijing	2020-01-26	unknown	Infectious Disease Control Center	Li, J., Li, L., Li, Z., Qiu, S., Song, H., Li, P. and Li, P.
EPI_ ISL_413519	hCoV-19/Beijing/231/2020	Asia/China/Beijing	2020-01-28	unknown	Infectious Disease Control Center	Li, J., Li, L., Li, Z., Qiu, S., Song, H., Li, P. and Li, P.
EPI_ ISL_413562	hCoV-19/USA/ WA11-UW7/2020	North America/USA/ Washington	2020-03-02	UW Virology Lab	UW Virology Lab	Pavitra Roychoudhury, Hong Xie, Keith Jerome, Alexander Greninger
EPI_ ISL_411951	hCoV-19/Sweden/01/2020	Europe/Sweden	2020-02-07	unknown	Unit for Laboratory Development and Technology Transfer, Public Health Agency of Sweden	Bengner, M., Palmerus, M., Lindsjo, O., Lind Karlberg, M., Monteil, V., Appelberg, S., Brave, A., Muradrasoli, S. and Tegmark-Wisell, K.
EPI_ ISL_412872	hCoV-19/South Korea/ KCDC12/2020	Asia/South Korea/ Gyeonggi-do	2020-02-01	Division of Viral Diseases, Center for Laboratory Control of Infectious Diseases, Korea Centers for Diseases Control and Prevention	Division of Viral Diseases, Center for Laboratory Control of Infectious Diseases, Korea Centers for Diseases Control and Prevention	Jeong-Min Kim, Yoon-Seok Chung, Namjoo Lee, Mi-Seon Kim, Sang Hee Woo, Hye-Jun Jo, Sehee Park, Heui Man Kim, Myung Guk Han
EPI_ ISL_411915	hCoV-19/Taiwan/ CGMH-CGU-01/2020	Asia/Taiwan/ Taoyuan	2020-01-25	Laboratory Medicine	Department of Laboratory Medicine, Lin-Kou Chang Gung Memorial Hospital, Taoyuan, Taiwan.	Kuo-Chien Tsao, Yu-Nong Gong, Shu-Li Yang, Yi-Chun Li, Chung-Guei Huang, Yhu-Chering Huang, Shin-Ru Shih
EPI_ ISL_407893	hCoV-19/Australia/ NSW01/2020	Oceania/Australia/ New South Wales/ Sydney	2020-01-24	Centre for Infectious Diseases and Microbiology Laboratory Services	NSW Health Pathology - Institute of Clinical Pathology and Medical Research; Westmead Hospital; University of Sydney	Eden J-S, Carter I, Rahman H, Holmes EC, Rockett R, O'Sullivan MV, Sintchenko V, Chen SC, Maddocks S, Kok J and Dwyer DE for the 2019-nCoV Study Group

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EPI_ ISL_407896	hCoV-19/Australia/ QLD02/2020	Oceania/Australia/ Queensland/Gold Coast	2020-01-30	Pathology Queensland	Public Health Virology Laboratory	Ben Huang, Alyssa Pyke, Amanda De Jong, Andrew Van Den Hurk, Carmel Taylor, David Warrilow, Doris Genge, Elisabeth Gamez, Glen Hewitson, Ian Maxwell Mackay, Inga Sultana, Jamie McMahon, Jean Barcelon, Judy Northill, Mitchell Finger, Natalie Simpson, Neelima Nair, Peter Burtonclay, Peter Moore, Sarah Wheatley, Sean Moody, Sonja Hall-Mendelin, Timothy Gardam, and Frederick Moore.
EPI_ ISL_406597	hCoV-19/France/ IDF0373/2020	Europe/France/ Ile-de-France/Paris	2020-01-23	Department of Infectious and Tropical Diseases, Bichat Claude Bernard Hospital, Paris	National Reference Center for Viruses of Respiratory Infections, Institut Pasteur, Paris	Mélanie Albert, Marion Barbet, Sylvie Behillil, Méline Bizard, Angela Brisebarre, Flora Donati, Vincent Enouf, Maud Vanpeene, Sylvie van der Werf, Yazdan Yazdanpanah, Xavier Lescure.
EPI_ ISL_413692	hCoV-19/China/ WF0002/2020	Asia/China	2020-01	Weifang Center for Disease Control and Prevention	Weifang Center for Disease Control and Prevention & BGI-Shenzhen	Qing Nie, Xingguang Li, Erik M Volz, Han Fu, Haowei Wang, Xiaoyue Xi, Wei Chen, Dehui Liu, Yingying Chen, Mengmeng Tian, Wei Tan, Junjie Zai, Wanying Sun, Jiandong Li, Junhua Li
EPI_ ISL_413694	hCoV-19/China/ WF0004/2020	Asia/China	2020-01	Weifang Center for Disease Control and Prevention	Weifang Center for Disease Control and Prevention & BGI-Shenzhen	Qing Nie, Xingguang Li, Erik M Volz, Han Fu, Haowei Wang, Xiaoyue Xi, Wei Chen, Dehui Liu, Yingying Chen, Mengmeng Tian, Wei Tan, Junjie Zai, Wanying Sun, Jiandong Li, Junhua Li
EPI_ ISL_413623	hCoV-19/USA/ CruiseA-18/2020	North America/USA	2020-02-24	unknown	Pathogen Discovery, Respiratory Viruses Branch, Division of Viral Diseases, Centers for Dieases Control and Prevention	Clinton R. Paden, Ying Tao, Krista Queen, Anna Uehara, Jing Zhang, Yan Li, Haibin Wang, Shifaq Kamili, Xiaoyan Lu, Brian Lynch, Senthil Kumar K. Sakthivel, Brett L. Whitaker, Lijuan Wang, Janna' R. Murray, Jasmine Padilla, Justin Lee, Susan I. Gerber, Stephen Lindstrom, Suxiang Tong

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EPI_ ISL_413603	hCoV-19/Finland/ FIN03032020B/2020	Europe/Finland/ Helsinki	2020-03-03	Department of Virology and Immunology, University of Helsinki and Helsinki University Hospital, Huslab Finland	Department of Virology, Faculty of Medicine, University of Helsinki, Helsinki, Finland	Teemu Smura, Hannimari Kallio-Kokko, Olli Vapalahti
EPI_ ISL_412912	hCoV-19/Germany/ Baden-Wuerttemberg-1/2020	Europe/Germany/ Baden-Wuerttemberg	2020-02-25	State Health Office Baden-Wuerttemberg	Charité Universitätsmedizin Berlin, Institute of Virology	Victor M Corman, Julia Schneider, Barbara Mühlemann, Talitha Veith, Jörn Beheim-Schwarzbach, Terry Jones, Rainer Oehme, Silke Fischer, Christian Drosten
EPI_ ISL_412972	hCoV-19/Mexico/ CDMX-InDRE_01/2020	North America/ Mexico/Mexico City	2020-02-27	Instituto Nacional de Enfermedades Respiratorias	Instituto de Diagnostico y Referencia Epidemiologicos (INDRE)	Ramirez-Gonzalez Ernesto, Garces-Ayala Fabiola, Araiza-Rodriguez Adnan, Mendieta-Condado Edgar, Rodriguez-Maldonado Abril, Wong-Arambula Claudia, Vazquez-Perez Joel, Martinez Arturo, Boukadida Celia, Munoz-Medina Esteban, Sanchez Alejandro, Isa Pavel, Taboada Blanca, Lopez Susana, Arias Carlos, Barrera-Badillo Gisela, Hernandez-Rivas Lucia, Lopez-Martinez Irma
EPI_ ISL_412964	hCoV-19/Brazil/ SPBR-01/2020	South America/ Brazil/Sao Paulo/Sao Paulo	2020-02-25	Hospital Israelita Albert Einstein	Instituto Adolfo Lutz Interdisciplinary Procedures Center Strategic Laboratory	Jaqueline Goes de Jesus, Claudio Tavares Sacchi, Daniela Bernardes Borges da Silva, Ingra Morales Claro, Flávia Cristina da Silva Sales, Claudia Regina Gonçalves, Joshua Quick, Maria do Carmo, Sampaio Tavares Timenetsky, Nicholas James Loman, Andrew Rambaut, Ester Cerdeira Sabino, Nuno Rodrigues Faria
EPI_ ISL_410301	hCoV-19/Nepal/61/2020	Asia/Nepal/ Kathmandu	2020-01-13	National Influenza Centre, National Public Health Laboratory, Kathmandu, Nepal	The University of Hong Kong	Ranjit Sah , Runa Jha, Daniel Chu, Haogao Gu, Malik Peiris, Anup Bastola, Alfonso J. Rodriguez-Morales, Bibek Kumar Lal, Basu Dev Pandey, Leo Poon

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EPI_ ISL_412980	hCoV-19/Wuhan/ HBCDC-HB-04/2020	Asia/China/Hubei/ Wuhan	2020-01-18	Union Hospital of Tongji Medical College, Huazhong University of Science and Technology	Hubei Provincial Center for Disease Control and Prevention	Bin Fang, Xiang Li, Xiao Yu, Linlin Liu, Bo Yang, Faxian Zhan, Guojun Ye, Xixiang Huo, Junqiang Xu, Bo Yu, Kun Cai, Jing Li, Yongzhong Jiang.
EPI_ ISL_407976	hCoV-19/Belgium/ GHB-03021/2020	Europe/Belgium/ Leuven	2020-02-03	KU Leuven, Clinical and Epidemiological Virology	KU Leuven, Clinical and Epidemiological Virology	Bert Vanmechelen, Elke Wollants, Annabel Rector, Els Keyaerts, Lies Laenen, Marc Van Ranst, and Piet Maes
EPI_ ISL_410720	hCoV-19/France/ IDF0372-isl/2020	Europe/France/ Ile-de-France/Paris	2020-01-23	Department of Infectious and Tropical Diseases, Bichat Claude Bernard Hospital, Paris	National Reference Center for Viruses of Respiratory Infections, Institut Pasteur, Paris	Mélanie Albert, Marion Barbet, Sylvie Behillil, Méline Bizard, Angela Brisebarre, Flora Donati, Vincent Enouf, Maud Vanpeene, Sylvie van der Werf, Yazdan Yazdanpanah, Xavier Lescure.
EPI_ ISL_410716	hCoV-19/ Singapore/10/2020	Asia/Singapore	2020-02-04	National Public Health Laboratory, National Centre for Infectious Diseases	National Centre for Infectious Diseases, National Centre for Infectious Diseases	Octavia S, Mak TM, Cui L, Lin RTP
EPI_ ISL_410715	hCoV-19/ Singapore/9/2020	Asia/Singapore	2020-02-04	National Public Health Laboratory, National Centre for Infectious Diseases	National Public Health Laboratory, National Centre for Infectious Diseases	Octavia S, Mak TM, Cui L, Lin RTP
EPI_ ISL_410713	hCoV-19/ Singapore/7/2020	Asia/Singapore	2020-01-27	National Public Health Laboratory, National Centre for Infectious Diseases	National Public Health Laboratory, National Centre for Infectious Diseases	Octavia S, Mak TM, Cui L, Lin RTP
EPI_ ISL_413647	hCoV-19/Portugal/ CV62/2020	Europe/Portugal	2020-03-01	Centro Hospital do Porto, E.P.E. - H. Geral de Santo Antonio	Instituto Nacional de Saude (INSA)	Raquel Guiomar, Inês Costa, Pedro Pechirra, Joana Mendonça, Luís Vieira, Helena Ramos, Joana Isidro, Vítor Borges, João Paulo Gomes
EPI_ ISL_412046	hCoV-19/Shenzhen/ HKU-SZ-005/2020	Asia/China/ Shenzhen	2020-01	unknown	University of Hong Kong-Shenzhen Hospital	Chan, J.F.-W., Yuan, S., Kok, K.H., To, K.K.-W., Chu, H., Yang, J., Xing, F., Liu, J., Yip, C.C.-Y., Poon, R.W.-S., Tsai, H.W., Lo, S.K.-F., Chan, K.H., Poon, V.K.-M., Chan, W.M., Ip, J.D., Cai, J.P., Cheng, V.C.-C., Chen, H., Hui, C.K.-M. and Yuen, K.Y.

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EPI_ ISL_403935	hCoV-19/ Guangdong/20SF025/2020	Asia/China/ Guangdong/ Shenzhen	2020-01-15	Guangdong Provincial Center for Diseases Control and Prevention; Guangdong Provincial Public Health	Department of Microbiology, Guangdong Provincial Center for Diseases Control and Prevention	Min Kang, Jie Wu, Jing Lu, Tao Liu, Baisheng Li, Shujiang Mei, Feng Ruan, Lifeng Lin, Changwen Ke, Haojie Zhong, Yingtao Zhang, Lirong Zou, Xuguang Chen, Qi Zhu, Jianpeng Xiao, Jianxiang Geng, Zhe Liu, Jianxiong Hu, Weilin Zeng, Xing Li, Yuhuang Liao, Xiujuan Tang, Songjian Xiao, Ying Wang, Yingchao Song, Xue Zhuang, Lijun Liang, Guanhao He, Huihong Deng, Tie Song, Jianfeng He, Wenjun Ma
EPI_ ISL_403934	hCoV-19/ Guangdong/20SF014/2020	Asia/China/ Guandong/Shenzhen	2020-01-15	Guangdong Provincial Center for Diseases Control and Prevention; Guangdong Provincial Public Health	Department of Microbiology, Guangdong Provincial Center for Diseases Control and Prevention	Min Kang, Jie Wu, Jing Lu, Tao Liu, Baisheng Li, Shujiang Mei, Feng Ruan, Lifeng Lin, Changwen Ke, Haojie Zhong, Yingtao Zhang, Lirong Zou, Xuguang Chen, Qi Zhu, Jianpeng Xiao, Jianxiang Geng, Zhe Liu, Jianxiong Hu, Weilin Zeng, Xing Li, Yuhuang Liao, Xiujuan Tang, Songjian Xiao, Ying Wang, Yingchao Song, Xue Zhuang, Lijun Liang, Guanhao He, Huihong Deng, Tie Song, Jianfeng He, Wenjun Ma
EPI_ ISL_407084	hCoV-19/Japan/AI/ I-004/2020	Asia/Japan/Aichi	2020-01-25	Department of Virology III, National Institute of Infectious Diseases	Pathogen Genomics Center, National Institute of Infectious Diseases	Tsuyoshi Sekizuka, Shutoku Matsuyama, Naganori Nao, Kazuya Shirato, Shinji Watanabe, Makoto Takeda, Makoto Kuroda
EPI_ ISL_413488	hCoV-19/Germany/ NRW-01/2020	Europe/Germany/ North Rhine Westphalia/ Heinsberg District	2020-02-28	Center of Medical Microbiology, Virology, and Hospital Hygiene, University of Duesseldorf	Center of Medical Microbiology, Virology, and Hospital Hygiene, University of Duesseldorf	Ortwin Adams, Marcel Andree, Alexander Dilthey, Torsten Feldt, Sandra Hauka, Torsten Houwaart, Björn-Erik Jensen, Detlef Kindgen-Milles, Malte Kohns Vasconcelos, Klaus Pfeffer, Tina Senff, Daniel Strelow, Jörg Timm, Andreas Walker, Tobias Wienemann

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Accession ID	Virus name	Location	Collection date	Originating laboratory	Submitting laboratory	Authors
EPI_ ISL_412116	hCoV-19/ England/09c/2020	Europe/United Kingdom/England	2020-02-09	Respiratory Virus Unit, Microbiology Services Colindale, Public Health England	Respiratory Virus Unit, Microbiology Services Colindale, Public Health England	Monica Galiano, Shahjahan Miah, Angie Lackenby, Omolola Akinbami, Tiina Talts, Leena Bhaw, Richard Myers, Steven Platt, Kirstin Edwards, Jonathan Hubb, Joanna Ellis, Maria Zambon
EPI_ ISL_405839	hCoV-19/Shenzhen/ HKU-SZ-005/2020	Asia/China/ Guangdong/ Shenzhen	2020-01-11	The University of Hong Kong - Shenzhen Hospital	Li Ka Shing Faculty of Medicine, The University of Hong Kong	Chan, J.F.-W., Yuan, S., Kok, K.H., To, K.K.-W., Chu, H., Yang, J., Xing, F., Liu, J., Yip, C.C.-Y., Poon, R.W.-S., Tsai, H.W., Lo, S.K.-F., Chan, K.H., Poon, V.K.-M., Chan, W.M., Ip, J.D., Cai, J.P., Cheng, V.C.-C., Chen, H., Hui, C.K.-M. and Yuen, K.Y.