

**Title: Development and evaluation of a Duo SARS-CoV-2 RT-qPCR assay combining two assays approved by the World Health Organization targeting the envelope and the RNA-dependant RNA polymerase (RdRp) coding regions**

Authors: Laura Pezzi, Remi N. Charrel, Laetitia Ninove, Antoine Nougairede, Gregory Molle, Bruno Coutard, Guillaume Durand, Isabelle Leparc-Goffart, Xavier de Lamballerie, Laurence Thirion

## Supplementary data

Suppl. Dataset#1. Sequences of the two IVT RNA used in the study.

Highlighted in blue: primers

Highlighted in green: probe

**Bold underlined, T7 promoter sequence**

*Italicized, T7 termination sequence*

>IVT\_E gene

**TAATACGACTCACTATAAGGGAGA**ACATGAGGATACCCATGTACAGGTACGTTAATAGTTAATAGCGTACTTCTTTCTTATTATAGCGGCCGCTTATTAAAGTTACACTA

**GCCATCCTTACTGCGCTTCGATTTGTGTGCGTACTGCTGCAATATACATGAGGATCACCCATGT***gcaataactagcataacccttgggcctaaacgggtcttgagggttttgctga*

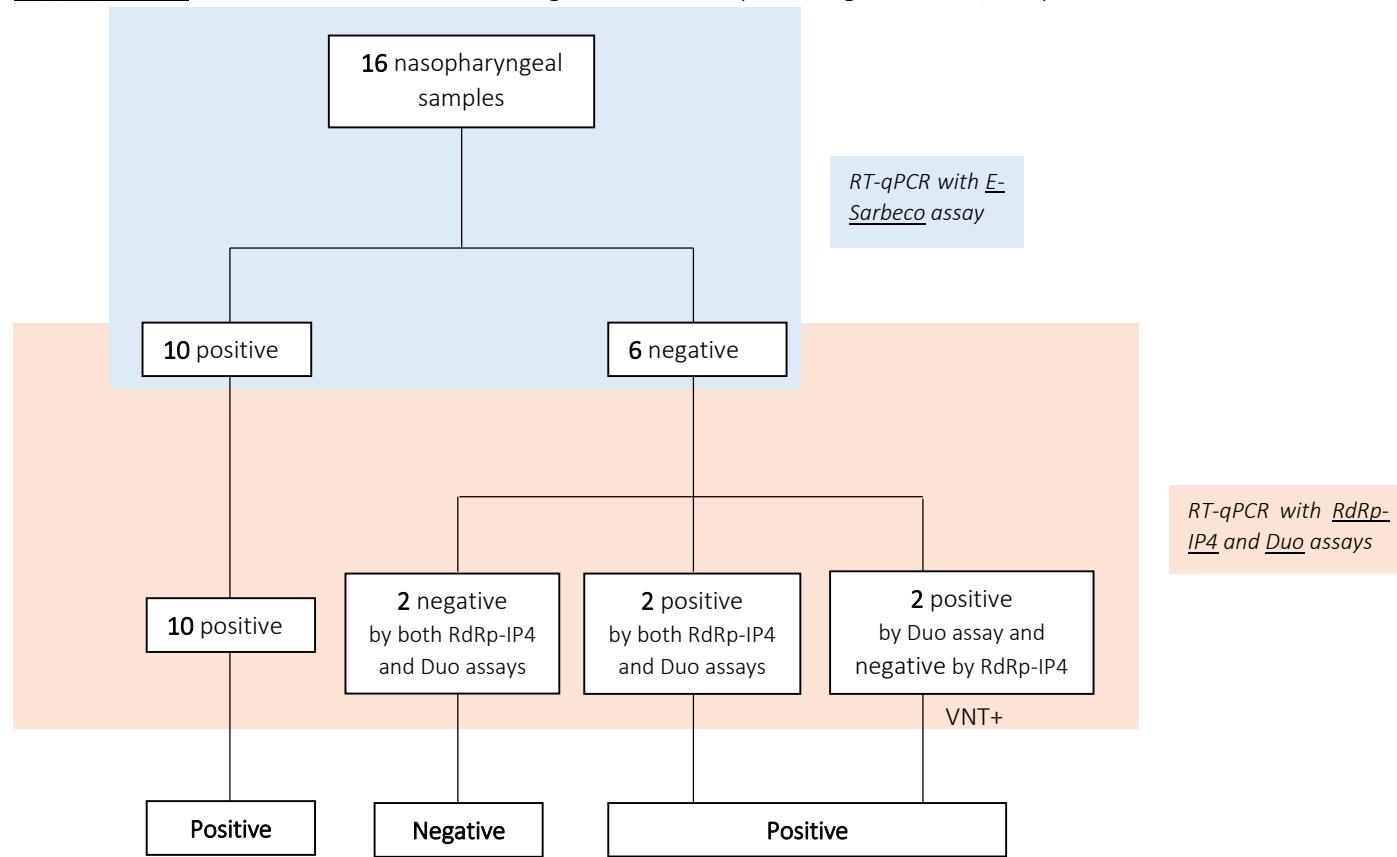
>IVT\_RdRp-IP4

**TAATACGACTCACTATAAGGGAGA**ACATGAGGATACCCATGTCAAGTATTGA**ATTATAGCGGCCGCTTATT**ACGAAATGCTGGTATTGTTGGTACTGACATTAG

**ATAATCAAGATCTCAATGGTAACTGGTATGATTCCGGTGATT****TCATACAAACCACGCCAGGTAGTGGAGTTCTGTTG**TAGATTCTTATTATTCAATTGTTAATGCC

**TATATTAACCTTGACCAGGGCTTACATGAGGATCACCCATGT***gcaataactagcataacccttgggcctaaacgggtcttgagggttttgctga*

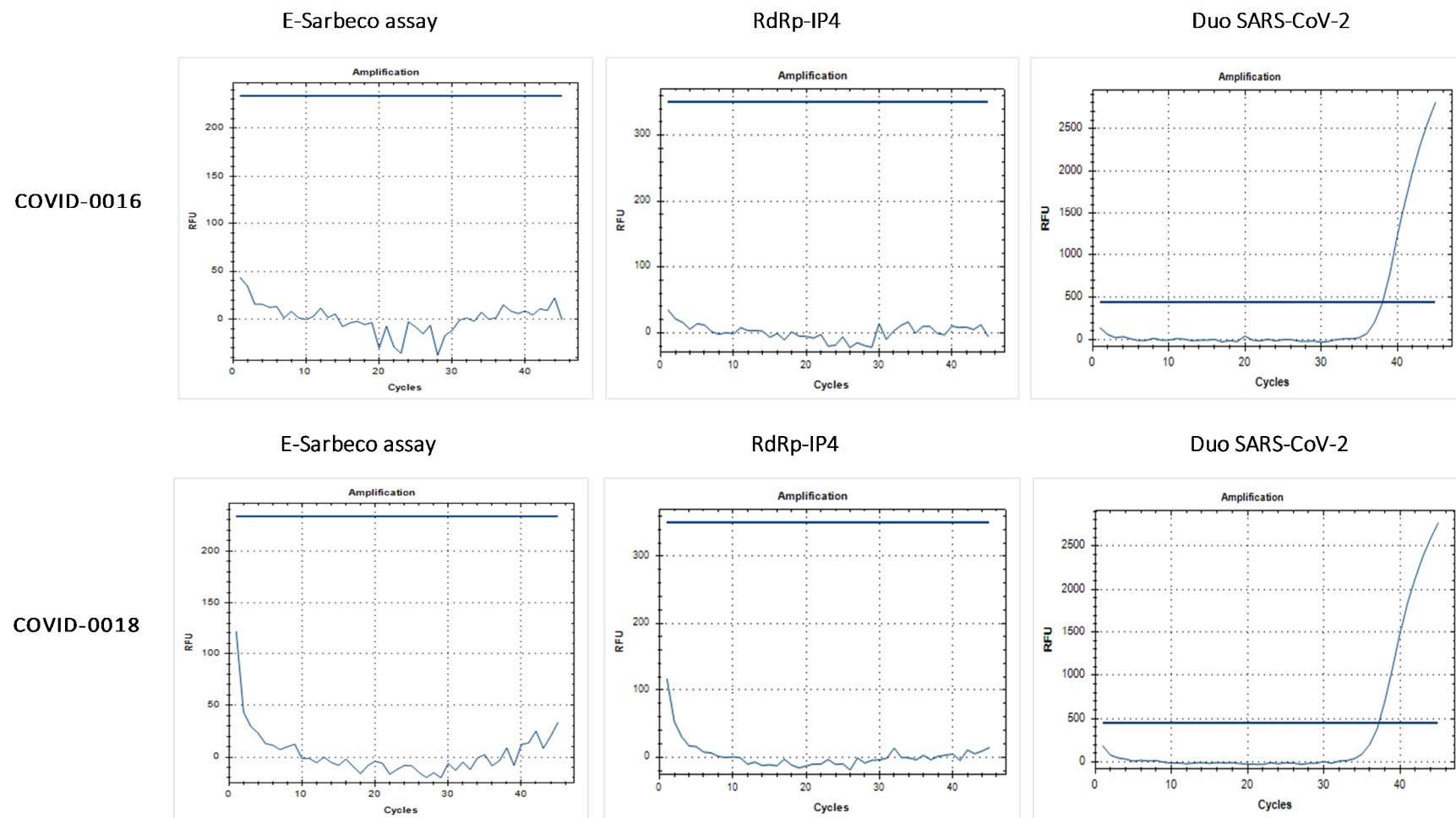
Suppl. Figure 1. Flowchart of molecular testing on clinical samples using E-Sarbeco, RdRp-IP4 and Duo SARS-CoV-2 assays.



**Table 1.** Results of RT-qPCR on clinical samples with E-Sarbeco, RdRp-IP4 and Duo SARS-CoV-2 assays. Highlighted in grey, samples with discrepant results obtained with the three assays.

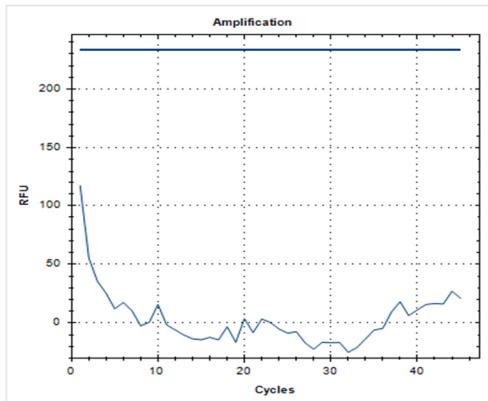
Sample nb	Sample name	Days between onset of symptoms and sampling (sera and nasopharyngeal swabs)	E-Sarbeco assay		RdRp-IP4 assay		Duo SARS-CoV-2		VNT	Titre VNT
1	COVID-0002	16	Positive	35,2	Positive	34,9	Positive	35,2	Positive	80
2	COVID-0004	9	Positive	31,4	Positive	31,2	Positive	31,2	Positive	>160
3	COVID-0006	11	Negative	>40	Negative	>40	Negative	>40	Negative	-
4	COVID-0008	13	Negative	>40	Negative	>40	Negative	>40	Positive	80
5	COVID-0010	13	Positive	31	Positive	30,4	Positive	30,8	Positive	80
6	COVID-0012	9	Positive	31	Positive	30,8	Positive	31	Positive	80
7	COVID-0016	17	Negative	>40	Negative	>40	Positive	38,1	Positive	>160
8	COVID-0018	15	Negative	>40	Negative	>40	Positive	37,2	Positive	>160
9	COVID-0020	12	Positive	35,4	Positive	37,4	Positive	35,8	Positive	>160
10	COVID-0022	16	Positive	32,8	Positive	34,2	Positive	33,1	Positive	40
11	COVID-0024	13	Positive	34	Positive	35,8	Positive	34,4	Positive	40
12	COVID-0026	15	Positive	37,1	Positive	36,4	Positive	35,9	Positive	40
13	COVID-0029	13	Positive	21,5	Positive	22,5	Positive	21,9	Positive	>160
14	COVID-0031	14	Positive	36,2	Positive	34,7	Positive	34,8	Negative	-
15	COVID-0033	7	Negative	>40	Positive	35,2	Positive	36,8	Positive	>160
16	COVID-0035	9	Negative	>40	Positive	36,6	Positive	38,9	Positive	80

Suppl Figure 2. Detection curves of samples with discrepant results obtained with the three RT-qPCR assays.

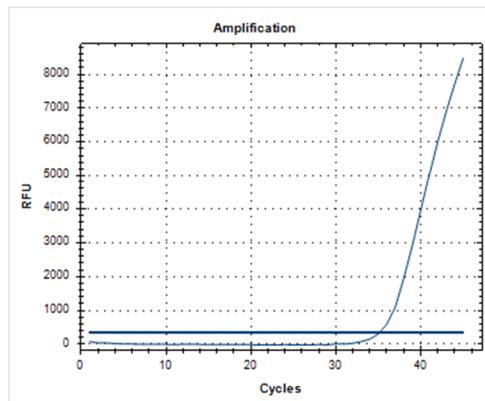


COVID-0033

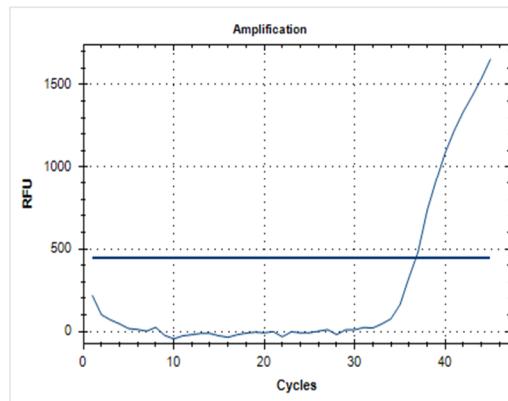
E-Sarbeco assay



RdRp-IP4

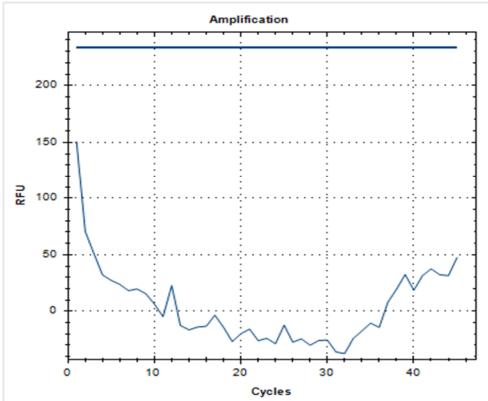


Duo SARS-CoV-2

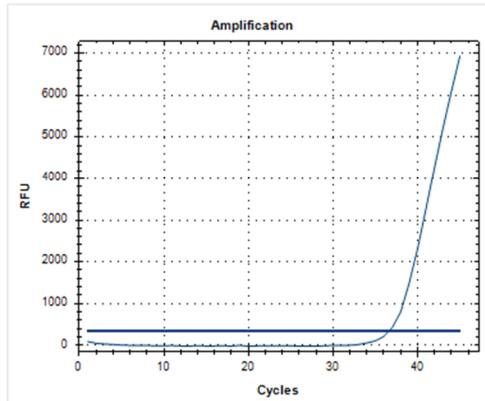


COVID-0035

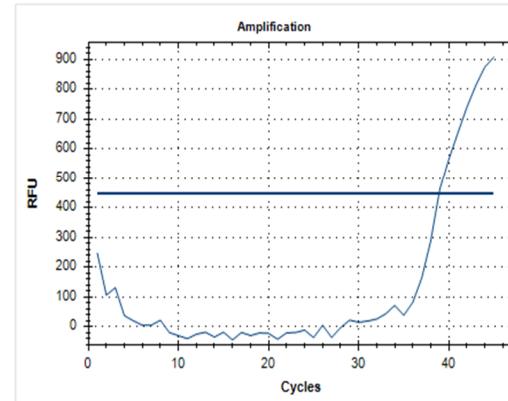
E-Sarbeco assay



RdRp-IP4



Duo SARS-CoV-2



Suppl. Dataset#2. *In silico* analysis of the genetic heterogeneity in the target regions of the assay included in the Duo SARS-CoV-2 assay

Analysis #A: Based on 14,277 complete genome sequences of SARS-CoV-2 from COG-UK Consortium (Regions: Scotland, England, Wales, Northern Ireland)

**A1. IP4-RdRp**

99,16% of sequences (14,156 sequences): no mismatch with IP4-RdRp primers and probe set

0,84% of sequences (121 sequences): 1 or more mismatches with IP4-RdRp primers and probe set

- 8 sequences: 1 or more mismatches with Fw primer
- 37 sequences: 1 or more mismatches with probe
- 76 sequences: 1 or more mismatches with Rev primer

**A2. E gene (Charité, Berlin)**

99,83% of sequences (14,252 sequences): no mismatch with E gene(Charité) primers and probe set

0,17% of sequences (25 sequences): 1 or more mismatches with E gene(Charité) primers and probe set

- 4 sequences: 1 or more mismatches with Fw primer
- 19 sequences: 1 or more mismatches with probe
- 2 sequences: 1 or more mismatches with Rev primer

**Analysis #B:** Based on 14,277 complete genome sequences of SARS-CoV-2 from COG-UK Consortium (Regions: Scotland, England, Wales, Northern Ireland)

### B1. E-Sarbco assay regions corresponding to the primers and the probe (73 sequences)

E Sarbco (Chart)	27.859	27.884	27.922	27.947	27.950	27.971	Position on the genome according to strain LC528232
NC_045121  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_Wuhan-Hu-11 complete_genome	-	-	-	-	-	-	
(C52996) Severe_acute_respiratory_syndrome_coronavirus_2_isolate_Wuhan-Hu-11 complete_genome	-	-	-	-	-	-	
MT139909  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_Wuhan-Hu-11 complete_genome	-	-	-	-	-	-	
LC528232  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_Wuhan-Hu-11 complete_genome	-	-	-	-	-	-	
MT135042  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(CHN/331/2020) complete_genome	-	-	-	-	-	-	
MT502082  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(BRA/SP02/2020) complete_genome	-	-	-	-	-	-	
MT773062  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(CAN/BC/19-035/2020) complete_genome	-	-	-	-	-	-	
MT192772  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(VNM/NCV-19-035/2020) complete_genome	-	-	-	-	-	-	
MT192773  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(VNM/NCV-19-035/2020) complete_genome	-	-	-	-	-	-	
MT176330  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(FRA/FL_593/2020) complete_genome	-	-	-	-	-	-	
MT304491  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(USA/TX_2967/2020) complete_genome	-	-	-	-	-	-	
MT159722  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_2019-CoV USA-Cruise-A-0/2020) complete_genome	-	-	-	-	-	-	
MT476667  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(USA/F-0/2020) complete_genome	-	-	-	-	-	-	
MT228072  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(USA/42-AU/936/2020) complete_genome	-	-	-	-	-	-	
MT390441  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(GRC/13/2020) complete_genome	-	-	-	-	-	-	
MT280395  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(GRC/13/2020) complete_genome	-	-	-	-	-	-	
MT398742  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(CHN/ME-H2021/2020) complete_genome	-	-	-	-	-	-	
MT066175  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 NTU01/TWN/human(2020) complete_genome	-	-	-	-	-	-	
MT207081  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_PCoV-FN-19-Jan-2020 partial_genome	-	-	-	-	-	-	
MT207082  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_PCoV-FN-19-Jan-2020 partial_genome	-	-	-	-	-	-	
MT281577  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(CHN/Fuyang_FV002/2020) complete_genome	-	-	-	-	-	-	
MT304047  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(KOR/BA-ACH_2634/2020) complete_genome	-	-	-	-	-	-	
MT476668  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(ESP/Barcelona/1/2020) complete_genome	-	-	-	-	-	-	
MT434775  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(USA-NY-CDC-SURV07NYC/2020) complete_genome	-	-	-	-	-	-	
MT106054  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_2019-CoV USA-TX/1/2020) complete_genome	-	-	-	-	-	-	
MT447156  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(THA/200383-NYT/2020) complete_genome	-	-	-	-	-	-	
MT771255  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(ESP/Barcelona/1/2020) complete_genome	-	-	-	-	-	-	
MT192759  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(TWN/GGMH-CGU-01/2020) complete_genome	-	-	-	-	-	-	
MT259229  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(CHN/Wuhan_18012802/2020) complete_genome	-	-	-	-	-	-	
MT438718  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(USA/CA-BALTIMORE/2020) complete_genome	-	-	-	-	-	-	
MT272688  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(NP/61/2020) complete_genome	-	-	-	-	-	-	
MT077125  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(ITA/MI/1/2020) complete_genome	-	-	-	-	-	-	
(L52996) Severe_acute_respiratory_syndrome_coronavirus_2_isolate_PCoV-FN-19-Jan-2020 partial_genome	-	-	-	-	-	-	
MT505043  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(ND/156/2020) complete_genome	-	-	-	-	-	-	
MT292572  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(ESP/Valladolid/11/2020) complete_genome	-	-	-	-	-	-	
MT451503  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(ESP/Barcelona/1/2020) complete_genome	-	-	-	-	-	-	
MT258377  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(USA/CZ-8057-005/2020) complete_genome	-	-	-	-	-	-	
MT355052  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(HKG/HKU-904/2020) complete_genome	-	-	-	-	-	-	
MT227745  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(TUR/TRAEGEM-001/2020) complete_genome	-	-	-	-	-	-	
MT272689  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(PKP/10/2020) complete_genome	-	-	-	-	-	-	
MT359865  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(ESP/0000113/2020) complete_genome	-	-	-	-	-	-	
MT385486  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(USA/CA-ZB016/2020) complete_genome	-	-	-	-	-	-	
MT435600  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(MD/CBC/3/2020) complete_genome	-	-	-	-	-	-	
MT451037  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(AUS/C131/2020) complete_genome	-	-	-	-	-	-	
MT300186  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(USA/UNC_20017/3/2020) complete_genome	-	-	-	-	-	-	
MT418983  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(ESP/Barcelona/1/2020) complete_genome	-	-	-	-	-	-	
MT396266  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2/mm/LD/1/2020) complete_genome	-	-	-	-	-	-	
MT451038  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(AUS/C134/2020) complete_genome	-	-	-	-	-	-	
MT451039  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(AUS/C134/2020) complete_genome	-	-	-	-	-	-	
MT446112  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(Guangzhou_GT05/2020) complete_genome	-	-	-	-	-	-	
MT419812  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV2 human(USA/PK-CDC-5/2020) complete_genome	-	-	-	-	-	-	
MT291526  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(CHN/Wuhan_IMF-WH-2019) complete_genome	-	-	-	-	-	-	
MT202091  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(HKG/HKU-1-P-8206/2020) complete_genome	-	-	-	-	-	-	
MT093571  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(SWE/01/2020) complete_genome	-	-	-	-	-	-	
MT007390  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_Australia/001/2020) complete_genome	-	-	-	-	-	-	
MT291536  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(CHN/Wuhan_IMF-BJ-2020) complete_genome	-	-	-	-	-	-	
MT418589  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(USA-QLS-0109/2020) complete_genome	-	-	-	-	-	-	
MT476670  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(CHN/Wuhan_IMF-BJ-2020) complete_genome	-	-	-	-	-	-	
MT215194  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(HKG_B5_VMX200286/2020) complete_genome	-	-	-	-	-	-	
MT451882  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(ND/BRC/75/2020) complete_genome	-	-	-	-	-	-	
MT285511  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(YAZ/NCB-1/2020) complete_genome	-	-	-	-	-	-	
MT451074  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(ND/BRC/10/2020) complete_genome	-	-	-	-	-	-	
MT412330  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV-2 human(USA-CT-UW-6385/2020) complete_genome	-	-	-	-	-	-	
MT452993  Severe_acute_respiratory_syndrome_coronavirus_2_isolate_SARS-CoV2 human(PAK/Mangai/2020) complete_genome	-	-	-	-	-	-	

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