

Table S1. Minimum Information for Publication of Quantitative Real-Time PCR Experiments

EXPERIMENTAL DESIGN	
Definition of experimental and control groups	The tests were carried out on 306 nasopharyngeal/oropharyngeal swabs collected between September 2020 and January 2021 into dedicated viral transport media (VTM) of three companies (UTM Universal Transport Medium, Copan, Virus Transport and Preservation Medium, Biocomma® and Virus Sample Stabilizer, Vazyme), each in a volume of 3 ml. The specimens were isolated from different patients or health care workers (one sample per patient). All samples have been submitted for routine diagnostics.
Number within each group	306 nasopharyngeal/oropharyngeal swabs.
Assay carried out by core lab or investigator's lab?	Investigator's lab.
Acknowledgement of authors' contributions	not applicable
SAMPLE	
Description	
Volume/mass of sample processed	BD MAX™ ExK™ TNA-3 (Becton Dickinson): 200 microliters QIAamp Viral RNA Mini Kit (QIAGEN) and QIAcube Connect device (QIAGEN): 140 microliters.
Microdissection or macrodissection	not applicable
Processing procedure	
If frozen - how and how quickly?	not applicable
If fixed - with what, how quickly?	not applicable
Sample storage conditions and duration (especially for FFPE samples)	The clinical samples stored at 4°C for 1 to 3 hours, according to UTM manufacturer's recommendations were used afterwards for RNA isolation with BD MAX™ ExK™ TNA-3 (Becton Dickinson). The clinical samples stored no longer than overnight at 4°C, according to VTM manufacturer's recommendations were used afterwards for RNA isolation with QIAamp Viral RNA Mini Kit (QIAGEN) and QIAcube Connect device (QIAGEN).
NUCLEIC ACID EXTRACTION	
Procedure and/or instrumentation	
Name of kit and details of any modifications	BD MAX™ ExK™ TNA-3 (Becton Dickinson) and QIAamp Viral RNA Mini Kit (QIAGEN).
Source of additional reagents used	not applicable
Details of DNase or RNase treatment	not applicable
Contamination assessment (DNA or RNA)	Negative controls in each run, incompatible reactions between methods repeated.
Nucleic acid quantification	
Instrument and method	BD MAX™ system (Becton Dickinson) and QIAcube Connect device (QIAGEN).
Purity (A260/A280)	not applicable
Yield	not applicable
RNA integrity method/instrument	
RIN/RQI or Cq of 3' and 5' transcripts	not applicable
Electrophoresis traces	not applicable
Inhibition testing (Cq dilutions, spike or other)	An inhibition of the PCR reaction excluded by the amplification of internal control.

In case of absence of internal control signal in sample it is recommended to repeat the assay diluting the sample 1:10 to check for possible problems of inhibition.

REVERSE TRANSCRIPTION

Complete reaction conditions

Amount of RNA and reaction volume	VIASURE SARS-CoV-2 <i>S gene</i> Real Time PCR Detection Kit (CerTest Biotec): data not provided by the manufacturer (closed molecular platform). Bosphore Novel Coronavirus (2019-nCoV) Detection Kit v1 (Anatolia Geneworks): 10 microliters. Vitassay qPCR SARS-CoV-2 (Vitassay Healthcare S.L.U.): 5 microliters.
Priming oligonucleotide (if using GSP) and concentration	data not provided by the manufacturer
Reverse transcriptase and concentration	data not provided by the manufacturer
Temperature and time	VIASURE SARS-CoV-2 <i>S gene</i> Real Time PCR Detection Kit (CerTest Biotec): 15 min at 45°C. Bosphore Novel Coronavirus (2019-nCoV) Detection Kit v1 (Anatolia Geneworks): 17 min at 50°C. Vitassay qPCR SARS-CoV-2 (Vitassay Healthcare S.L.U.): 15 min at 45°C.
Manufacturer of reagents and catalogue numbers	VIASURE SARS-CoV-2 <i>S gene</i> Real Time PCR Detection Kit (CerTest Biotec): BD REF 444212. Bosphore Novel Coronavirus (2019-nCoV) Detection Kit v1 (Anatolia Geneworks): ABCOW3. Vitassay qPCR SARS-CoV-2 (Vitassay Healthcare S.L.U.): 7081046.

Cqs with and without RT not applicable

Storage conditions of cDNA not applicable

qPCR TARGET INFORMATION

If multiplex, efficiency and LOD of each assay.	VIASURE SARS-CoV-2 <i>S gene</i> Real Time PCR Detection Kit (CerTest Biotec): has a detection limit of ≥ 24 cDNA copies per reaction (cp/rxn) with a positive rate of $\geq 95\%$. Bosphore Novel Coronavirus (2019-nCoV) Detection Kit v1 (Anatolia Geneworks): has a detection limit of ≥ 18 cDNA copies per reaction (cp/rxn). Vitassay qPCR SARS-CoV-2 (Vitassay Healthcare S.L.U.): has a detection limit of ≥ 10 cDNA copies per reaction (cp/rxn).
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Sequence accession number data not provided by the manufacturer

Location of amplicon

Amplicon length	data not provided by the manufacturer
<i>In silico</i> specificity screen (BLAST, etc)	data not provided by the manufacturer
Pseudogenes, retropseudogenes or other homologs?	not applicable
Sequence alignment	not applicable
Secondary structure analysis of amplicon	data not provided by the manufacturer

Location of each primer by exon or intron (if applicable) not applicable

What splice variants are targeted? data not provided by the manufacturer

qPCR OLIGONUCLEOTIDES

Primer sequences data not provided by the manufacturer

RTPrimerDB Identification Number	data not provided by the manufacturer
Probe sequences	data not provided by the manufacturer
Location and identity of any modifications	data not provided by the manufacturer
Manufacturer of oligonucleotides	data not provided by the manufacturer
Purification method	data not provided by the manufacturer

qPCR PROTOCOL

Complete reaction conditions

Reaction volume and amount of cDNA/DNA	VIASURE SARS-CoV-2 <i>S gene</i> Real Time PCR Detection Kit (CerTest Biotec): data not provided by the manufacturer (automatic method). Bosphore Novel Coronavirus (2019-nCoV) Detection Kit v1 (Anatolia Geneworks): reaction volume - 25 microliters. Vitassay qPCR SARS-CoV-2 (Vitassay Healthcare S.L.U.): reaction volume - 20 microliters.
Primer, (probe), Mg ⁺⁺ and dNTP concentrations	data not provided by the manufacturer
Polymerase identity and concentration	data not provided by the manufacturer
Buffer/kit identity and manufacturer	data not provided by the manufacturer
Exact chemical constitution of the buffer	data not provided by the manufacturer
Additives (SYBR Green I, DMSO, etc.)	data not provided by the manufacturer

Manufacturer of plates/tubes and catalog number	BD MAX TM PCR Cartridges (Becton Dickinson, Catalog No. 437519); LightCycler [®] 480 Multiwell Plate 96, white (Roche, Catalog No. 04729692001); SARS-CoV-2 strips (Catalog No. 7041S046/7042S046).
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Complete thermocycling parameters	VIASURE SARS-CoV-2 <i>S gene</i> Real Time PCR Detection Kit (CerTest Biotec): 2 min at 98°C and 45 cycles, each consisting of 10 sec at 95°C and 58 sec at 60°C. Bosphore Novel Coronavirus (2019-nCoV) Detection Kit v1 (Anatolia Geneworks): 6 min at 95°C and 38 cycles, each consisting of 15 sec at 97°C and 1 min 10 sec at 55°C. Vitassay qPCR SARS-CoV-2 (Vitassay Healthcare S.L.U.): 2 min at 95°C and 45 cycles, each consisting of 10 sec at 95°C and 50 sec at 60°C.
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Reaction setup (manual/robotic)	VIASURE SARS-CoV-2 <i>S gene</i> Real Time PCR Detection Kit – integrated robotic reaction setup; Bosphore Novel Coronavirus (2019-nCoV) Detection Kit v1 (Anatolia Geneworks) and Vitassay qPCR SARS-CoV-2 (Vitassay Healthcare S.L.U.) – manual reaction setup.
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Manufacturer of qPCR instrument	BD MAX TM System (Becton Dickinson); cobas z480 Instrument II (Roche).
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qPCR VALIDATION

Evidence of optimisation (from gradients)	data not provided by the manufacturer
Specificity (gel, sequence, melt, or digest)	data not provided by the manufacturer
For SYBR Green I, C_q of the NTC	data not provided by the manufacturer

Standard curves with slope and y-intercept

PCR efficiency calculated from slope	not applicable
Confidence interval for PCR efficiency or standard error	not applicable

r2 of standard curve	not applicable
Linear dynamic range	
Cq variation at lower limit	not applicable
Confidence intervals throughout range	not applicable
Evidence for limit of detection	data not provided by the manufacturer
If multiplex, efficiency and LOD of each assay.	VIASURE SARS-CoV-2 S gene Real Time PCR Detection Kit (CerTest Biotec): has a detection limit of ≥ 24 cDNA copies per reaction (cp/rxn) with a positive rate of $\geq 95\%$. Bosphore Novel Coronavirus (2019-nCoV) Detection Kit v1 (Anatolia Geneworks): has a detection limit of ≥ 18 cDNA copies per reaction (cp/rxn). Vitassay qPCR SARS-CoV-2 (Vitassay Healthcare S.L.U.): has a detection limit of ≥ 10 cDNA copies per reaction (cp/rxn).
DATA ANALYSIS	
qPCR analysis program (source, version)	BD MAX™ System Software version: V5.14 A; LightCycler® 480 Software release 1.5.1.62 SP3, Version 1.5.1.62.
Cq method determination	not applicable
Outlier identification and disposition	not applicable
Results of NTCs	Negative, set in each run of Bosphore Novel Coronavirus (2019-nCoV) Detection Kit v1 (Anatolia Geneworks) and Vitassay qPCR SARS-CoV-2 (Vitassay Healthcare S.L.U.).
Justification of number and choice of reference genes	not applicable
Description of normalisation method	not applicable
Number and concordance of biological replicates	not applicable
Number and stage (RT or qPCR) of technical replicates	not applicable
Repeatability (intra-assay variation)	not applicable
Reproducibility (inter-assay variation, %CV)	not applicable
Power analysis	not applicable
Statistical methods for result significance	ABS Quant/Fit Points
Software (source, version)	LightCycler® 480 Software release 1.5.1.62 SP3, Version 1.5.1.62
Cq or raw data submission using RDML	not applicable

Figure S1. Amplification curves obtained for the SARS-CoV-2 detection using Bosphore Novel Coronavirus kit (2019-nCoV) Detection Kit v1; positive results for six clinical samples (green lines) and positive controls (blue lines) are observed at the corresponding wavelength (A - amplification curves for the *ORF1ab* gene; B - amplification curves for the internal control in the wells detecting the *ORF1ab* gene; C - amplification curves for the *E* gene; D - amplification curves for the internal control in the wells detecting the *E* gene); red curves represent negative controls of the test.

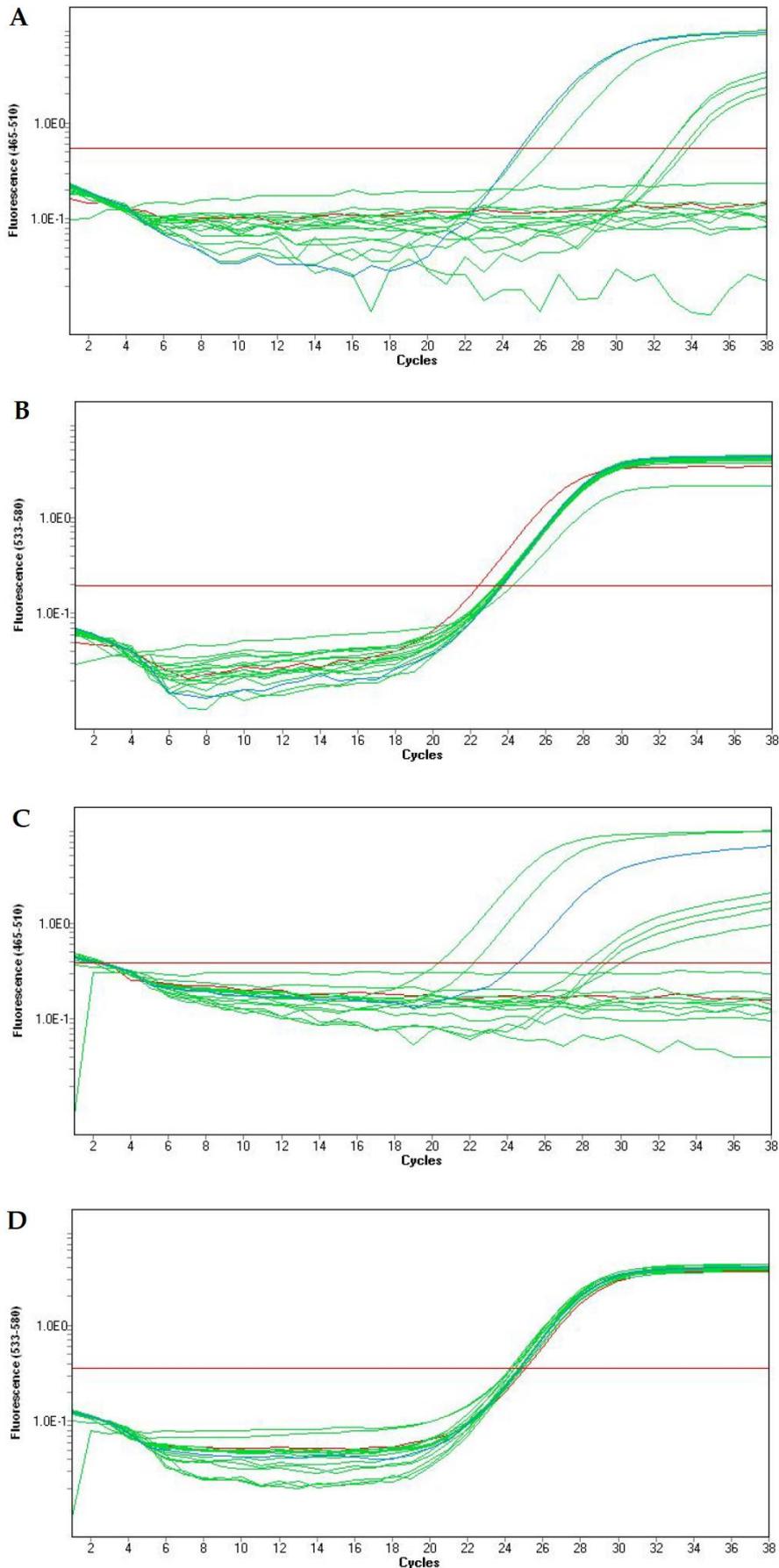


Figure S2. Amplification curves obtained for the SARS-CoV-2 detection using Vitassay qPCR SARS-CoV-2; positive results for one clinical sample (green lines) and positive controls (blue lines) are observed at the corresponding wavelength (A - amplification curves for the *ORF1ab* gene; B - amplification curves for the internal control in the wells detecting both *ORF1ab* and *N* genes; C - amplification curves for the *N* gene); red curves represent negative controls of the test.

