

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

| EXPERIMENTAL DESIGN | |
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| 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. |

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| | 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). |
| 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 |

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| 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™ 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). |
| 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. |
| 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. |
| Manufacturer of qPCR instrument | BD MAX™ System (Becton Dickinson); cobas z480 Instrument II (Roche). |
| 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, Cq 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 |

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| r ² 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. | <p>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 $\geq 95\%$.</p> <p>Bosphore Novel Coronavirus (2019-nCoV) Detection Kit v1 (Anatolia Geneworks): has a detection limit of ≥ 18 cDNA copies per reaction (cp/rxn).</p> <p>Vitassay qPCR SARS-CoV-2 (Vitassay Healthcare S.L.U.): has a detection limit of ≥ 10 cDNA copies per reaction (cp/rxn).</p> |
| DATA ANALYSIS | |
| qPCR analysis program (source, version) | BD MAX TM 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.

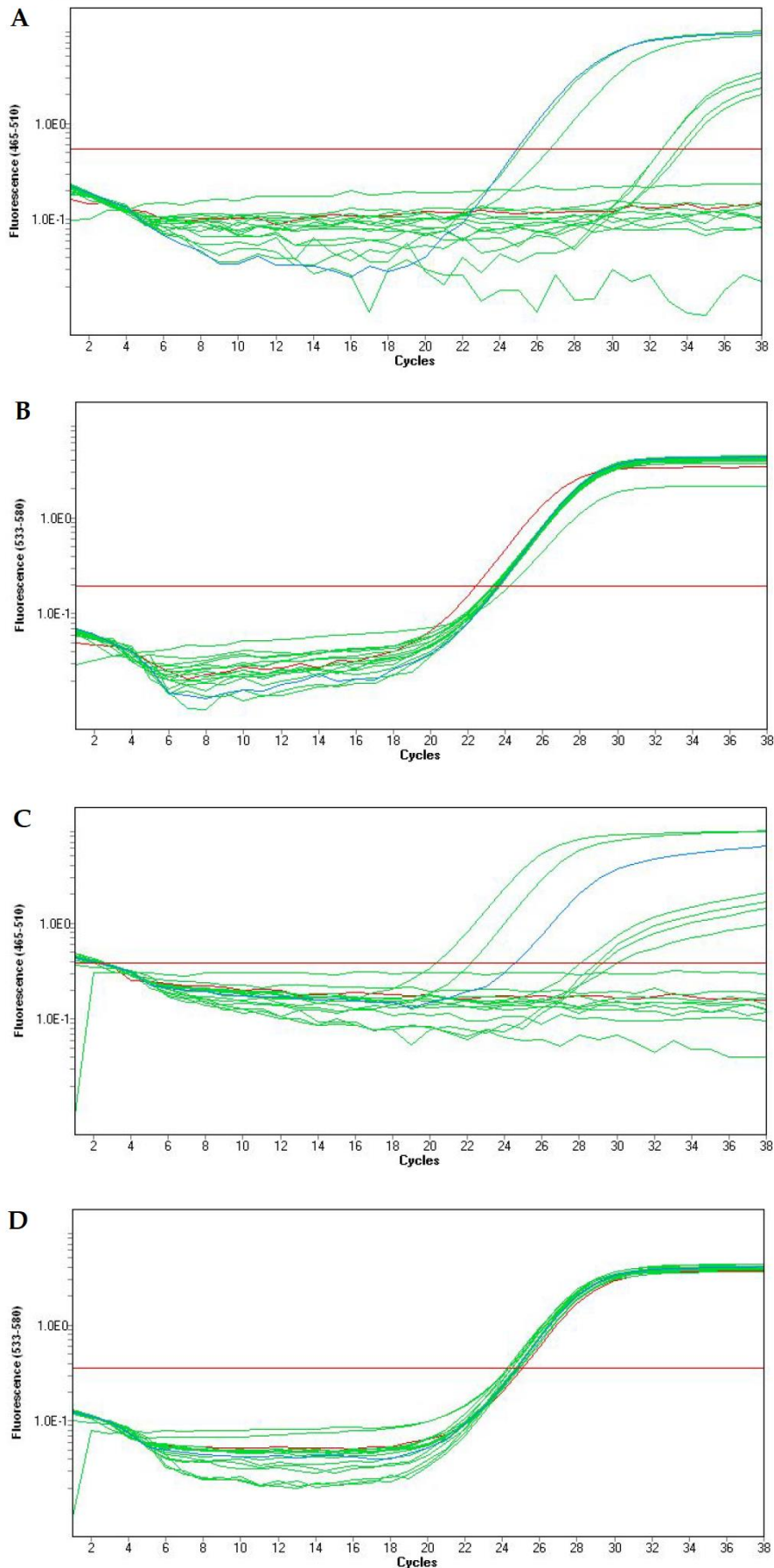


Figure S2. Amplification curves obtained for the SARS-CoV-2 detection using Vitassay qPCR SARS-CoV-2; positive results for one clinical sample (green lines) and positive controls (blue lines) are observed at the corresponding wavelength (A - amplification curves for the *ORF1ab* gene; B - amplification 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.

