A new Syber Green real time PCR to detect SARS-CoV-2

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Abstract

Phylogenetic analyses demonstrated that etiologic agent of pandemic outbreak is a betacoronavirus named SARS-CoV-2. For public health interventions, a diagnostic test with high sensitivity and specificity is required. The gold standard protocol for diagnosis by WHO is the RT-PCR. To detect low viral load and large-scale screening a low-cost diagnostic test becomes necessary. Here we develop a cost-effective test capable of to detect the new coronaviruses. We validated an auxiliary protocol for molecular diagnosis with RT-PCR SYBR Green methodology to successfully screen negative cases of SARS-CoV-2. Our results demonstrated that a set of primers with high specificity, and no homology with other viruses from Coronovideae family or human respiratory tract pathogenic viruses. Optimization of annealing temperature and polymerization time led to an high specificity in the PCR products. We have developed a more affordable and swift methodology for negative SARS-CoV-2 screening. This methodology can be applied on large scale populational to soften panic and economic burden through guidance for isolation strategies.

Introduction

In December of 2019, an outbreak of pneumonia with unknown etiology was identified in Wuhan, China. The outbreak, which probably originated in a seafood market, occurred as a result of zoonotic transmission. In January 2020, the pneumonia outbreak progressed to a nationwide epidemic, which has now become a pandemic. Patients may present with fever, dyspnea, cough, and lung lesions and infiltrates. The clinical picture of patients is like diseases caused by coronavirus-type agents such as the Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). Phylogenetic analyses have proven that the etiologic agent of the Wuhan pneumonia outbreak is a betacoronavirus called SARS-CoV-2.

The Wuhan pneumonia outbreak has become a pandemic, with confirmed cases on all continents and thousands of deaths around the world. Since we are facing a pathogen for which there is no effective treatment or vaccine, public health measures are necessary to contain the spread of the virus. Isolation and social distancing have been the main tools in the fight to interrupt the chain of viral transmission. For social distancing to be the effective, it is necessary to quarantine all individuals who carry the virus. However, some individuals may be asymptomatic, which make difficult to diagnose the pathology, and since they are not isolated, eventually they will spread the virus.

To corroborate the effects of public health interventions, laboratory diagnosis of individuals with SARS-CoV-2 is necessary. However, for proper laboratory diagnosis, techniques with high sensitivity and specificity are necessary, considering that patients may have a low viral load when first infected. Molecular techniques have been designed to address this need. According to the World Health Organization (WHO), the gold standard to detect the coronavirus is the real-time polymerase chain reaction (RT-PCR) using TaqMan probes, which precisely detect the presence of the virus. However, due
to the intensive labor required by the technique and the reagents involved, as well as the limited availability of kits, many diagnoses are based only on late-stage symptoms \(^{11}\). Early diagnosis, even when asymptomatic, is vital to prevent the spread of the virus, as well as for initial prophylaxis with emerging treatments \(^{12}\), since the spectrum of this disease in humans is not yet fully understood. Thus, low-cost diagnostic tests must be developed for large-scale patient screening to confirm positive and/or negative cases of the new coronavirus.

To this end, we developed an auxiliary protocol for molecular diagnosis by RT-PCR with SYBR Green methodology to detect negative cases of SARS-CoV-2. This protocol will maximize the cost-benefit of viral detection and accelerate availability by the use of conventional kits on a large scale in molecular biology laboratories.

**Methods**

**Clinical samples.**

This study was approved by the Research Ethics Committee of Pontifical Catholic University of Rio Grande do Sul (PUCRS) trough of number 3.977.510. All participants provided written informed consent prior to inclusion in this study. All methods were performed in accordance with relevant guidelines and regulations. To determine the specificity of the primers set, the detection curve and possible cross-reactions, a sample obtained from the Clinical Analyses Laboratory of the Hospital de Clínicas de Porto Alegre, RS, Brazil was used. The sample was collected from a patient attended in the São Lucas da PUCRS hospital from inpatients with suspected COVID-19. The sample was sent to the Laboratório Central de Saúde Pública do Rio Grande do Sul (LACEN) and Fleury Laboratory for determined to be positive for SARS-CoV-2 through the Center of Control Disease and Prevention (CDC) protocol.

**In silico study of primer specificity.**

Two pairs of primers complementary to two different regions of the viral RNA sequence of the isolated coronavirus (Whuan-H1/NC_045512.2) were tested (Table 1). First, the primers were input in Beacon Designer Software (Premier Biosoft International) to search their molecular structures based on test type. The SYBR Green option was selected and the parameters were adjusted accordingly. The primers were then input into the Prime-BLAST platform (NCBI) and evaluated for their specificity with the sequence of SARS-CoV-2 genetic material. Searches were also made for similarities within regions of the human genome and the main respiratory and opportunistic viruses and pathogens in order to determine whether the primers flanked the regions of interest and amplified only the SARS-CoV-2 genetic material. The input SARS-CoV-2 primers were compared with the genomes of the microorganisms showed on Tables 1 and 2.
RNA extraction and reverse transcription for first-strand DNA synthesis.

RNA was extracted from 300 μL of patient cheek or nasal swab samples using the SV-Total RNA kit (Promega, Madison, Wisconsin, USA). Reverse transcriptase first-strand DNA synthesis was performed by two methods: (1) a random primer technique using GoScript Reverse Transcription Mix, Random Primers (Promega, Madison, WI, USA), and (2) the 3’ primer technique using M-MLV reverse transcriptase (Thermo Fisher Scientific, Waltham, MA, USA) with two distinct reverse primers. After the transcription reaction, the product was quantified in a NanoDrop fluorometer (Thermo Fisher Scientific, Waltham, MA, USA).

Sample preparation and concentration curve.

To determine the specificity and sensitivity of SARS-CoV-2 detection, after reverse transcription, different concentration curves were obtained for RNA extracted from the SARS-CoV-2-positive sample. The ssDNA was produced through the random primer technique, amplification was performed using 100 ng, 50 ng and 10 ng per reaction. The first strand DNA synthesis using the 3’ primer technique, 500 ng, 100 ng and 50 ng per reaction were used. Additionally, RNA samples extracted from human skin and brain tissue, previously collected, in 2018, were reverse transcribed with random primers to use as a negative control (to ensure potential SARS-CoV-2 negativity).

Real-time PCR (RT-PCR).

For each reaction, 60 ng of cDNA from the negative controls were used. The samples were amplified using two different master mix compositions, with and without uracil DNA glycosylase (UDG) activation. The GoTaq qPCR master mix kit (Promega) was used for the methodology without UDG activation, while the PowerUp SYBR Green Master Mix kit (Thermo) was used for the methodology with UDG activation. The thermal cycles followed manufacturer recommendations and were optimized according to the size of the PCR product and the primers annealing temperature (Table 3). The real-time PCR reaction was performed using StepOne Plus equipment (Thermo Fisher Scientific, Waltham, MA, USA).

Confirmation of SARS-CoV-2 derived amplicons in a 2% agarose gel electrophoresis.

The RT-PCR products were separated under electrophoresis in a 2% agarose gel containing ethidium bromide at 100 V for 30 minutes and analyzed in an automated Gel Doc™ EZ Gel Imager (Bio-Rad Laboratories, Inc.).

Dilution and detection curve
A dilution curve was performed using the quantifiable SARS-CoV Control (iDT - Integrated DNA Technologies). Serial dilutions of 20,000, 2,000, 200, 20 and 2 copies of the virus were tested. To establish detection capacity, serial dilutions of ssDNA of a positive sample for SARS-Cov-2 were performed in the following concentrations: 200 ng, 20 ng, 2 ng and 0.2 ng.

Protocol validation.

After establishing the best concentration criteria and methodology for diagnosis, the validation step used samples from 15 patients treated at the Hospital São Lucas with suspected SARS-CoV-2 infection. Reverse transcriptase using random primers followed by RT-PCR methodology with UDG activation, and sample concentrations above 100 ng of per reaction (Table 3) were applied to confirm the specificity of this new method. The protocol was validated using a Basic Validation of Qualitative Tests through WestgardQC software (https://www.westgard.com/validating-qualitative-tests.htm).

Results

Validation of the primer’s sequences.

The Beacon Designer platform have shown that there were none secondary structures, no hairpin, no homodimers, and not even cross dimers formation were observed in the primers set sequences. The Primer-BLAST analysis of both, the hCOVassay1 and the hCOVassay2, primer sequences showed that they are present only in the target SARS-CoV-2 genome. When bases complementarity of the primers set where searched against the human genome, there were no similarity, reinforces that the viral genome alone would be amplified. In addition, we verified the primers’ ability to anneal in the genomes from other airways circulating opportunistic microorganisms. No relevant homologies were found, i.e., the primers will not generate unspecific amplifications. The searches are shown in Tables 1 and 2. The primers from hCOVassay1 and hCOVassay2 have proven to be specific only for the Sars-CoV-2 genome.

RNA extraction and reverse transcription optimization.

RNA was extracted using a total RNA extraction kit. The results were below 1 ng/μL in NanoDrop quantification. The values of the total RNA extracted were on the pg scale, below the equipment’s detection threshold.

After reverse transcription, the mean amount of ssDNA obtained was 1,394 ng/μL (SD = 26.9) for the hCOVassay1 primer, 1,327 ng/μL (DP = 107.6) for the hCOVassay2 primer (3’ primer methodology), and 727 ng/μL (SD = 27.3) for the random primers methodology. The ssDNA purity levels were very similar in both techniques. The random primers technique was chosen to produce ssDNA in the first validation stage, since it is easier and quicker than the 3’ primer methodology.
Template DNA concentration curve for quantitative PCR detection.

The manufacturer recommended temperatures, time per cycle, and number of cycles were applied to amplify the targeted DNA. The amplifications with the 3’ primer technique and master mix without UDG activation using the hCOVassay1 primer, and DNA dilutions of 500 ng, 100 ng and 50 ng had yield cycle threshold (CT) values of 34, 35 and 37, respectively. Amplifications with the hCOVassay2 3’ primer generate CT values of 35, 37 and 37, respectively. Amplifications of ssDNA reverse transcribed with random primers, and master mix without UDG activation on hCOVassay1 with dilutions of 100 ng, 50 ng and 10 ng had yield CT values of 32, 33 and 36, respectively. When the hCOVassay2 primer were used to amplify the same dilutions of the template DNA the CT values were as follow: 33, 34 and 36, respectively. The CT cutoff for the negative controls was 36 for the hCOVassay1 primer set, and 38 for the hCOVassay2 primer set (Figure 1).

When master mix with UDG activation were used to amplify dilutions of the template DNA with 500 ng, 100 ng and 50 ng, the CT values yielded for the hCOVassay1 primer set were 34, 34 and 37, respectively. The hCOVassay2 primer set generated CT values of 31, 34 and 37, respectively. For the reverse transcriptase technique using the random primer methodology and master mix without UDG activation, dilutions of the template DNA with 100 ng, 50 ng and 10 ng had yielded CT values of 29, 30 and 32, respectively, for the hCOVassay1 primer set. When the primer set of the hCOVassay2 were used the CT values were 30, 31 and 33, respectively (Figure 2).

Melt curve analysis.

The melt curve of all amplified SARS-CoV-2-positive samples, with both set of primers, have produce a similar pattern. A clearly distinct curve was obtained for negative control samples, suggesting that any positive PCR signal is related to some nonspecific signals or the formation of primer dimers (Figures 3 and 4).

Visualization of RT-PCR amplicons

The RT-PCR amplicons were separated by electrophoresis, and the products were analyzed under UV to confirm the negative and positive samples. Figure 5 shows the 102 bp amplicon corresponding to SARS-CoV-2-positive samples while the last two lanes shows negative samples. When master mix with or without UDG activation was used, the negative control samples did not have a 111 bp band for the hCOVassay1 primer set, or a 102 bp band for the hCOVassay2 primer set (Figure 5: lanes 7 and 8).
Optimization of amplification parameters

The amplification parameters for each primer were modified (primers annealing temperature and time, and Taq DNA polymerization time), which improved the capture signal during the real-time RT-PCR assay, mainly when the master mix with UDG activation was used. The parameters alterations reduced possible nonspecific annealing, and hinder the formation of double strands larger than 200 bp.

The Figure 6 shows that the amplification curves of RT-PCR where the master mix without UDG activation was used produced a faint SYBR signal in negative controls for SARS-CoV-2, although for both primers set no amplicons can be visualized after separation by electrophoresis.

The SYBR Green master mix with UDG activation proved to be very efficient and reliable in terms of unspecific signals. The master mix with UDG activation is more appropriate to avoid false positives in uninfected human cDNA samples, maintaining the specificity of amplification signal (Figure 7).

Table 3 shows the pre-established parameters, as recommended by the manufacturer, and alterations made to improve the detection method here described.

Quantifiable control and detection curve

It was possible to detect the presence of the virus by observing the amplification curve from 20 copies of SARS-CoV-2 for both primers. Regarding the detection capacity, total ssDNA concentrations after transcription using 3’ primer less than 20 ng did not generate a considerably safe amplification curve for virus detection. Values equal to or greater than 200 ng of ssDNA were capable of being detected with great reliability. The comparison of the amplification curves showed that 200 ng of ssDNA corresponds to approximately 200 copies of the SARS-CoV-2 virus in the sample. (Figure 8)

Protocol validation using unknown samples

In order to validate all variables that improved the distinction between the positive from the negative samples, 15 samples from inpatients suspected of COVID-19 infection who were being treated at the Hospital São Lucas were used. From this 41 samples, 33 were negative and 8 were positive for this new protocol. All samples were also tested through CDC protocol for SARS-CoV-2. All 33 negative samples were confirmed, and 7 positive samples were confirmed by CDC protocol. Only one positive sample from SyBr Green protocol was negative in CDC protocol.

The samples used in the validation phase were tested with the both, random and 3’ primers to generate the first strand DNA and the real-time RT-PCR reaction were performed with a master mix with UDG activation.
The mean concentration of first strand DNA after reverse transcription with random primer methodology was of 793 ng/μL (SD = 141.6). The hCOVassay1 primer set produced amplification signals in 4 samples, and with the hCOVassay2 primer set amplifications signals were present in all samples. The amplicons separated on a 2% agarose gel electrophoresis showed only bands incompatible with the amplicon.

The mean concentration of first strand DNA after reverse transcription with the 3’ primer was of 1009.3 ng/μL (SD = 31.1) for the hCOVassay1 3’ primer, and of 1027.5 ng/μL (SD = 103.8) for the hCOVassay2 3’ primer. No amplification curve signals were produced for the hCOVassay1 primer. The signal was incompatible with the expected PCR product for this reaction, which was due to a CT greater than 39, a melt curve incompatible with the positive control curve and a lack of bands in the gel (Figure 9).

Comparative analysis of the real-time RT-PCR signal profile, comparison of the signal melt curve with positive controls, and comparison of the size of amplicons after separation by electrophoresis allowed a proper threshold adjustment to CT above to 37 that discriminate negatives from positives samples when both set of primers were used.

The results of Basic Validation of Qualitative Tests were 100% for positive agreement, 97.1% for negative agreement and 96.7% for overall agreement.

**Discussion**

The SYBR Green and TaqMan techniques are routinely used in real time PCR. Due to its simpler design, easy configuration and low cost, the SYBR Green detection methodology is predominantly used for the detection and amplification of nucleic acids. However, the TaqMan methodology uses an additional labeled probe, increasing significantly the sensitivity and specificity of the assay due to the reporter dye’s conjugated to the specific oligonucleotide sequence of the probes, capable of emitting fluorescence. The TaqMan assay is considered the main method to detect and quantify human pathogens with a low copy number, including viruses.

Nonspecific primer binding, which results in the production of unwanted PCR products, and the formation of primer dimers, can significantly influence the sensitivity and reliability of the PCR reaction signals. Therefore, the choice of specific primers and their *in silico* validation, followed by the real-time RT-PCR reaction parameters optimization, plays a fundamental role in the success of a real-time RT-PCR assay when SYBR Green is used.

The hCOVassay1 primer set used in this study, being complementary to the SARS-Cov-2 sequence, had a high specificity index and generated a 111 bp PCR amplicon. This primer has no homology with the genomic sequence of other viruses from the *Coronaviridae* family or other human respiratory viruses known, except rhinovirus/enterovirus, that possess a 5 base-pair mismatch on the forward primer and 4 distinct nucleotides on the reverse primer, but would generate an amplicon of 409 bp. The forward primer also have a five nucleotides homologous to the *Legionella* spp. genome, and four mismatches on the reverse primer, capable to generate an amplicon of 3,591 bp. The hCOVassay2 primer set have four and
tree nucleotides homologous with *Legionella* spp. genome, in the forward primer and the reverse primer, respectively. This set of primers can produce amplicons of 3,243 bp and 500 bp, respectively.

The primer annealing temperature and TAQ DNA polymerization time were optimized based on information obtained in the Primer Blast (NCBI) data for each sequence, which allowed to increase amplification specificity for the PCR products.

Annealing temperatures of 54°C were calculated according to the number of CG/AT bases in each primer pair. In addition, the 20-second polymerization time reduced the possibility of unspecific signals generated by partial primer annealing and/or primer dimers.

The standard WHO methodology uses the TaqMan probe technique to detect the new coronavirus. This methodology is extremely effective and can even accurately discriminate between SARS-CoV-2 and SARS-COV infections. The Charité Protocol, developed at the University of Charité, Berlin, uses four different probes to identify SARS-CoV-2. This test is very expensive, which limits its large-scale application. The U.S. Centers for Disease Control and Prevention (CDC) also has a standardized protocol with four different TaqMan probes that can accurately determine SARS-CoV-2 infection. Both the Charité and the CDC protocols involve reagents and probes that are now scarce and expensive on the world market.

The present study established a screening strategy for SARS-CoV-2 through a molecular assay using SYBR Green methodology. The proposed method harbors a lower cost with potential for large-scale screening of symptomatic and/or asymptomatic individuals. This screening strategy will allow communities or companies to screen for SARS-CoV-2-negative individuals in large-scale. However, when the test shows a positive result, it is advised to follow molecular tests established by the WHO or CDC, or even a serological test after the period required to detect antibodies, seven to fourteen days. Therefore, the most scarce and expensive tests could be applied only in a fraction of the target population (Figure 10).

**Conclusion**

In this study we have established a fast and low-cost method for negative SARS-CoV-2 screening. We suggest that the primers’ double negativity in RT-PCR signal analysis in linear mode can certify the absence of the virus. Samples whose signal is compatible with positive control in one of the two tested primers must be repeated and/or treated as indeterminate. Double-positive samples for the tested primers should be confirmed according to WHO or CDC protocols.

Double-negative patients can resume normality regarding social isolation, while patients who are positive for one or both proposed primers should be immediately tested according to recommended protocols or wait seven to fourteen days for serological test, also known by rapid test.
Due to its low cost and processing speed, this methodology can be applied on a large scale, providing peace of mind for those being tested and their peers, as well as guidance for social isolation protocols.

We aimed to develop a low-cost diagnostic test capable of detecting the SARS-CoV-2 virus in oropharyngeal mucosa of both symptomatic and asymptomatic patients to help reestablish normal work routines, as well as to remove SARS-CoV-2-negative individuals from isolation (with appropriate medical follow-up).

Declarations

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AUTHORS CONTRIBUTIONS.

DRM designed the protocols, conducted most experiments and wrote the manuscript. GGZ helped with RNA extraction and reverse transcription optimization; FR participated in the experiments of PCR; MVCG assisted in the assays of PCR; AA helped in in silico assays; DCM aided in the design of all experiments and contributed with reagents; JCC coordinated the research project. All authors carefully reviewed the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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**Tables**

[Please see the supplementary files section to view the tables.]

**Figures**
Figure 1

Amplification curves of reverse transcription in real time using master mix without uracil DNA glycosylase (UDG) activation. A) Dilutions of ssDNA (500 ng, 100 ng and 50 ng) produced by 3’ primer with the hCOVassay1 primer. B) Three different dilutions of the ssDNA (500 ng, 100 ng and 50 ng) amplified with the 3’ primer of the hCOVassay2 set. C and D) The three dilutions of ssDNA (100 ng, 50 ng and 10 ng) produced with the random primers methodology and amplified with the (C) hCOVassay1 and (D) hCOVassay2 primers. E) Amplified curves of negative controls produced using the two primers set.
Figure 2

Amplification curves of reverse transcription in real time using master mix without uracil DNA glycosylase (UDG) activation. A) Three dilutions of ssDNA produced by 3’ primer for the hCOVassay1 primer. B) Three dilutions of ssDNA amplified with the hCOVassay2 primer. C) Three dilutions of ssDNA produced by the random methodology and amplified with the hCOVassay1 and hCOVassay2 primers (D). E) Amplified negative controls for the two tested primer pairs.
Figure 3

Representation of melt curves for amplification using a master mix without UDG activation. The melt curves showed a similar pattern for all SARS-CoV-2-positive samples for the hCOVassay1 (A) and hCOVassay2 (B) primers. The melt curve of negative samples was quite different from that of the positive samples (arrows).
Figure 4

Melt curves obtained after PCR amplification using master mix with UDG activation. The melt curves showed a similar pattern for all SARS-CoV-2-positive samples for the hCOVassay1 (A) and hCOVassay2 (B) primers set. The melt curve of negative samples was clearly distinct (arrows).

Figure 5

Real time PCR amplicons of SARS-CoV2 positive samples after separation on a 2% agarose gel electrophoresis. The amplicon generated by RT-PCR with the hCOVassay1 primer set of 111 bp (upper figures), and amplicon of 102 bp generated with the hCOVassay2 primer set (lower figures). A) Amplicons produced by RT-PCR without UDG activation. B) Amplicons produced by RT-PCR with UDG activation.
Lanes 1-3: 500 ng, 100 ng and 50 ng of the first strand DNA synthesized using the 3’ primer followed by PCR amplification, respectively. Lanes 4-6: 100 ng, 50 ng and 10 ng of the first strand DNA followed by RT-PCR amplification. Lanes: 7 and 8 are negative control.

Figure 6

Real time PCR amplicons of SARS-CoV2 positive samples without UDG activation followed by separation on a 2% agarose gel electrophoresis. A) Amplification curve produced using the hCOVassay1 primer of SARS-CoV-2-positive (red line) and negative control (green and yellow line). B) Amplification curve produced using the hCOVassay2 primer of SARS-CoV-2-positive (green) and negative control (indigo and light blue lines) samples. C) Amplicons after separation on a 2% agarose gel by electrophoresis. Lane 1: amplicons of a positive for SARS-CoV-2 using hCOVassay1 primer (111 bp). Lane 2: SARS-CoV-2 negative sample amplified using hCOVassay1 primer. Lane 3: SARS-CoV-2 positive sample amplified using the hCOVassay2 primer (102 bp). Lane 4: SARS-CoV-2 negative sample amplified using the hCOVassay2 primer.
Figure 7

Real time PCR amplification curves and amplicons of SARS-CoV2 positive samples with UDG activation followed by separation on a 2% agarose gel electrophoresis. A) Amplification curve of a SARS-CoV-2-positive samples using the hCOV assay1 primer set (arrow). B) Amplification curve for SARS-CoV-2 positive samples using the hCOV assay2 primer set (arrow). For both primers (A and B) no amplification or cycle threshold curves were produced for SARS-CoV-2-negative control samples. C) Real time PCR amplicons. Lane 1: amplicon of SARS-CoV-2-positive sample using the hCOV assay1 primer set (111 bp). Lane 2: amplicon of SARS-CoV-2-negative sample using the hCOV assay1 primer set (111 bp). Lane 3: SARS-CoV-2-positive sample for hCOV assay2 primer (102 bp). Lane 4: SARS-CoV-2-negative sample for hCOV assay2 primer (102 bp). D) Amplicons of 4 samples and 4 controls for both primers tested in 3 different dilutions of reverse transcriptase produced by the random primer method. Lanes 1, 2 and 3: SARS-CoV-2-positive sample at concentrations 100 ng, 50 ng, and 10 ng, respectively, for hCOV assay1 primer. Lanes 7, 8 and 9: SARS-CoV-2-positive sample at concentrations 100 ng, 50 ng, and 10 ng, respectively, for hCOV assay2 primer. Lanes 4, 5, 6, 10, 11 and 12: SARS-CoV-2-negative samples amplified using the two primer pairs.
Real-time PCR amplification for quantifiable control and detection curves. Four different concentrations of quantification control for SARS-CoV-2 using the hCOVassay1 (A) and hCOVassay2 (B) primer set. The detection curve showed possible detection with 200 ng of ssDNA in each primer set (C and D). E.: The comparison between the quantification control and the quantified sample showed that 200 ng of ssDNA in the sample corresponds to $2.10^3$ copies of the SARS-CoV-2 virus.

Figure 8
Figure 9

Real time PCR amplification curves and amplicons of SARS-CoV2 positive samples with UDG activation followed by separation on a 2% agarose gel electrophoresis. Amplification curve using hCOVassay1 (A) and hCOVassay2 (B) primer set. The red arrow on B shows two amplification signals far from the agarose 2%. C.: Lanes 1-3: primer hCOVassay1. Lanes: 4-6: primer hCOVassay2. Lanes 7-9: negative controls amplified using hCOVassay1 primer set, and 10-12 with hCOVassay2 primer set. D.: Examples of PCR amplification curves graphics for 10 samples (8 negative samples, 2 positive sample – yellow and green, and positive control – red).

Figure 10
Illustration of large-scale negative SARS-CoV-2 screening. Individuals from a given population can be screened through the SYBR molecular test presented in this study. All individuals who test negative can immediately resume their work routine (i.e. those belonging to essential work groups). Those who test positive should be tested according to WHO or CDC protocols or else wait in isolation for seven days to be diagnosed with a rapid test. For those who test positive according to WHO or CDC protocols or rapid testing, medical care and isolation should be applied according to disease severity. For those who test negative in the rapid test, social isolation with medical monitoring should continue only for 7 days, not 14 days recommended for suspected cases.

Supplementary Files

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- Tables.docx