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Protocols

SARS-CoV-2 detection in nasopharyngeal swabs: Performance characteristics of a real-time RT-qPCR and a droplet digital RT-PCR assay based on the exonuclease region (ORF1b, nsp 14)

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ABSTRACT

The emergence and spread of SARS-CoV-2 has led to a compelling request for accurate diagnostic tests. The aim of this study was assessing the performance of a real-time RT-qPCR (rt RT-qPCR) assay and of a droplet digital RT-PCR (dd RT-PCR) targeting the nsp14 genome region for the detection of SARS-CoV-2 in nasopharyngeal swabs.

A total of 258 nasopharyngeal swabs were analyzed with the nsp14 assays and, for comparison, with a reference assay targeting the RdRp and E genes. Conflicting results were further investigated by two additional protocols, the Centers for Disease Control and Prevention (CDC) real-time targeting N1/N2, and a nested RT-PCR for the spike region.

Agreement of results was achieved on 226 samples (156 positive and 70 negative), 8 samples were positive in the reference assay and in the nsp14 rt RT-qPCR but negative with the dd RT-PCR, and 24 samples provided different combinations of results with the three assays. Sensitivity, specificity and accuracy (95 %C.I.) of the nsp14 assays were: 100.0 % (97.4–100.0), 98.7 % (92.1–100.0), and 99.6 % (97.5–100.0) for the rt RT-qPCR; 92.4 % (87.4–95.6), 100.0 % (94.2–100.0), and 94.7 % (91.1–97.0) for the dd RT-PCR.

The results of the study support the use of the nsp14 real-time RT-qPCR and ddPCR for the detection of SARS-CoV-2 in nasopharyngeal swabs.

1. Introduction

Coronaviruses (CoV) belong to the Coronaviridae family, a group of enveloped, single-stranded RNA viruses responsible for either mild (CoV 229E, NL63, OC43, and HKU1) or severe respiratory infections (MERS-CoV, SARS-CoV, and SARS-CoV-2) in humans. SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) was first discovered in December 2019 in China (Zhou et al., 2020; World Health Organization (WHO, 2020a), and had subsequently been reported in several countries, until the recognition by the World Health Organization of COVID-19 (the disease induced by SARS-CoV-2) as a pandemic, on 11 March 2020 (World Health Organization (WHO, 2020b). As of November 7, 2021, almost 250 million COVID-19 cases and 5 million deaths have been reported world-wide (https://coronavirus.jhu.edu/map.html).

Nucleic acid amplification tests are considered the gold standard for

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SARS-CoV-2 detection and several real-time RT-PCR methods and commercial detection systems have been developed since the beginning of the outbreak [reviewed in Premraj et al. (2020) and Ravi et al. (2020)]. The most common targets comprise the ORF1ab region, which encodes for 16 non-structural proteins (nsp) mainly involved in the replication process, and that is the real-time PCR target in China's CDC (orf1ab) and University of Honk Kong (orf1b) SARS-CoV-2 detection protocols (Chu et al., 2020; Niu et al., 2020; Hong Kong University, Faculty of Medicine (HKU Med, 2020; China Centers for Disease Control (China CDC, 2020). This region also includes another target extensively used for detection of RNA viruses, the RNA-dependent RNA-polymerase (RdRp) gene (nsp12), adopted for confirmation of SARS-CoV-2 presence in clinical specimens in the protocols most widely applied in Europe, including those developed by the German Charité and by the Pasteur Institute (Corman et al., 2020) (Pasteur Institute, 2020). Further to these, a number of structural proteins such as the spike (S), the envelope (E), and the nucleocapsid (N) protein are extensively used worldwide (Chu et al., 2020; Corman et al., 2020; Niu et al., 2020; Shirato et al., 2020; Centers for Disease Control and Prevention (CDC, 2020a,b; China Centers for Disease Control (China CDC, 2020; Hong Kong University, Faculty of Medicine (HKU Med, 2020), with most of the available protocols simultaneously including more than one target from different genome regions.

In a previous study a real-time RT-qPCR was developed for the detection and quantitation of SARS-CoV-2 in urban wastewaters (La Rosa et al., 2021a). The target of this assay was located in the nsp14 region, which encodes for a bifunctional enzyme acting as a 3′-to-5′ exoribonuclease (ExoN) and a guanine-N7-methyltransferase (N7-MTase). The coded protein is part of the CoV replication-transcription complex (van Hemert et al., 2008) and ExoN, that is present in the amino-terminal part of nsp14, is a critical element of the RNA proofreading machinery, ensuring the high fidelity of CoV replication by removal of misincorporated nucleotides (Bouvet et al., 2012). Indeed, ExoN is encoded only in larger-sized members of the *Nidovirales* order, such as *Coronaviridae* and *Roniviridae*, being instead absent in families within smaller genomes (e.g. *Arteriviridae*) (Smith and Denison, 2012). Furthermore, mutations affecting the ExoN's catalytic site have been reported to significantly increase replication errors in other coronaviruses, including the murine hepatitis virus and SARS-CoV (Eckerle et al., 2010, 2007; Eskier et al., 2020).

Before the use for SARS CoV-2 detection in wastewaters, the real-time RT-qPCR assay targeting nsp14 had been characterized for specificity, using a panel of Alfa- and Beta-coronaviruses, nucleic acids from other viruses and bacteria as well as wastewater samples collected in the pre-epidemic period. Sensitivity was also assessed (limit of detection, LOD_{50} of 0.41 g.c./µl and limit of quantification, LOQ of 3.71 g.c./µl) on SARS-CoV-2 RNA (La Rosa et al., 2021a).

Aim of the present study was to assess the performance of the nsp14 real-time RT-qPCR (rt RT-qPCR) assay and of a droplet digital RT-PCR (dd RT-PCR) based on the same primers/probe combination for the detection of SARS-CoV-2 in nasopharyngeal swabs.

2. Material and methods

2.1. Sampling

The clinical specimens were taken within the GENCOVID study protocol "Health surveillance for the SARS-CoV-2 virus, responsible of Covid-19 pandemic in high risk population or in people in direct contact with positive patients", approved by the ethics committee of University of Naples "Federico II" (approval n° 141/20). Having provided their informed consent, each volunteer enrolled in the study was assigned a unique ID code associated to the nasopharyngeal (NP) swab. Data encryption was applied in order to ensure the protection of privacy on the web-based platform for the study management (https://covid.profila.io/).

Samples were collected with the Copan UTM-RT system (Universal Transport Medium for Viruses) with FLOQSwabs (Copan Diagnostics, CA, USA) and transported to the laboratory with triple packaging under controlled temperature (4 °C). Aliquots not used for immediate analysis were stored at −80 °C in the biobank of Istituto Zooprofilattico Sperimentale del Mezzogiorno (IZSMe).

A total of 258 NP swabs collected between April and July 2020 in Campania region, Italy, were selected for the study. NP swab selection was performed according to the following criteria: a) providing a large variability of SARS-CoV-2 viral loads, based on the threshold cycle (*Ct*) values obtained with the reference diagnostic RT-PCR assay (RdRp and E genes) used for case investigation (see below); b) including samples with inconclusive or unclear results. In detail, selected samples were as follows: 164 swabs testing positive for both RdRp and E genes, 15 swabs providing positive results with high Ct values only for one of the tested genes (either RdRp or E) and therefore classified as 'inconclusive', and 79 negative swabs. The 164 positive swabs were further subdivided in three categories based on *Ct* values: low ($\leq$25.99; n = 62), intermediate (between 26.00 and 33.99; n = 82) and high *Ct* values ($\geq$34.00; n = 20). To complete the test panel, 10 molecular biology grade water aliquots were randomly distributed among sample to act as blind negative controls.

2.2. RNA extraction

RNA was extracted from 200 µl of each sample using an automated platform (GeneQuality X120, AB Analitica, Italy) with a magnetic bead-based protocol, using the GeneQuality X120 Pathogen kit (AB Analitica) according to manufacturer's instructions, and RNA was eluted in 120 µl. RNA was kept at −80 °C until molecular assays.

2.3. Reference real-time RT-PCR assay

The analysis of NP swabs was performed on 10 µl of RNA using the RQ-2019-nCoV kit (AB Analitica), based on the RdRp and E target genes. The assay interpretation criteria were as follows: a sample was considered positive if amplification of both genes was achieved, negative in the absence of amplification for both targets, inconclusive when only one of the targets was amplified, and not compliant when a significant reaction inhibition was detected. The declared LOD for this analytical assay was 3.0 g.c./10 µl reaction. For both genes the *Ct* value was obtained after standardization of the interpretation of RT-PCR data on the instrument software (AriaDX, Agilent Technologies). The *Ct* of each analysis was considered for data comparison. In each PCR run, one negative and one positive control, provided by the kit, were always run in parallel with samples. Moreover, an RNA internal amplification control using MS2 phage genomic RNA (AbAnalitica) was included in the PCR set-up for each sample in order to rule out inhibition.

2.4. Real-time RT-qPCR (rt RT-qPCR) assay based on nsp14

Real-time RT-qPCR was performed as described in La Rosa et al. (2021a) with minor modifications. Briefly, the RT-qPCR mix (25 µl total volume) was prepared using the RNA UltraSense One-Step Quantitative RT-PCR System (Life Technologies), and 5 µl of sample RNA were analysed in reactions containing 1× RT-PCR buffer, 1 µl of enzyme mix, and 500 nM of forward primer 2297-CoV-2-F (5′-ACATGGCTTTGAGTTGACATCT-3′), 900 nM of reverse primer 2298-CoV-2-R (5′-AGCAGTGGAAAAGCATGTGG-3′) and 250 nM of probe 2299-CoV-2-P (FAM-CATAGACAACAGGTGCGCTC-MGBEQ; FAM: 6-carboxyfluorescein, MGBEQ: minor groove binder Eclipse quencher). Amplification conditions included reverse transcription at 50 °C for 30 min, inactivation at 95 °C for 5 min and 45 cycles of 15 s at 95 °C and 30 s at 60 °C. Standard curve was constructed using a synthetized fragment (BioFab Research, Italy) quantified by fluorometric measure (Qubit, Thermo Scientific). Tenfold dilutions were used for standard

curve construction (range 5×10^0–5×10^4 copies/μl) and quantifications were considered acceptable if curves displayed a slope between −3.1 and −3.6 and a $R^2 \geq 0.98$. Two negative controls (molecular biology grade water) were included in each run to check for reagent contamination and for environmental contamination, respectively. Amplifications were conducted on a Quant Studio 12 K Flex instrument (Thermo Scientific). For results interpretation, amplification plots were visually checked for exponential amplification, and a *Ct* cut-off value of 40 was applied. Analyses were conducted in blind.

2.5. Droplet digital RT-PCR (dd RT-PCR) assay based on nsp14

Droplet digital RT-PCR was performed on Bio-Rad's QX200 system (Bio-Rad Laboratory, Hercules, California, USA) using the same primers/probes targeting the nsp14 region used for RT-qPCR. The reaction mixture, containing 5 μl of RNA in a total volume 20 μl, was prepared as follows: 1× One-Step RT-ddPCR Supermix (BioRad), 1 μl of 300 mM DTT, 500 nM of forward primer, 900 nM of reverse primer, 250 nM of probe, and 2 μl of reverse transcriptase. Droplet generation was then performed according to manufacturer instruction and amplification was carried out on a C1000 Touch Thermal Cycler (Bio-Rad) with the following thermal profile: 50 °C for 60 min, 95 °C for 10 min followed by 45 cycles of 95 °C for 15 s and 60 °C for 45 s with a final stage at 98 °C for 10 min. QuantaSoft software v1.7 was used to count the PCR positive or negative droplets and to provide detection of the target sequence. The dd RT-PCR analyses were conducted in blind as for the rt RT-qPCR. Absolute quantification of the target, expressed as copies number per microliter of initial RNA, was also provided by dd RT-PCR but assessment of the accuracy of quantitative results was excluded from the analysis, being quantification out of the scope of the validation.

2.6. Analysis of conflicting results

All the NP samples providing discordant results between the reference RT-PCR assay and the nsp14 assays were further investigated by two other independent assays: a) the real-time RT-PCR detection of N1 and N2 genes according to the United States Center for Diseases Control (CDC) protocol (https://www.fda.gov/media/134922/download); b) a nested RT-PCR amplification of the spike region performed on 10 μl or RNA, using the primers and conditions described in Shirato et al. (2020), followed by sequencing for confirmation.

Results were interpreted as follows: a sample was assigned as positive if one of the following conditions occurred: i) positive result by both the reference assay (detection of RdRp and E genes) and at least one nsp14 assay; ii) positive result by the CDC real-time RT-PCR (amplification of both N1 and N2 target regions); iii) positive result by the nested amplification of the spike region followed by sequencing. A sample was assigned as negative when one the following results occurred: i) negative by both the reference assay (no detection of either RdRp or gene E) and by the nsp14 assay; ii) negative by the CDC real-time RT-PCR (no detection of either N1 and N2) and negative by the nested amplification of the spike region. A sample was not classifiable when discordant results were obtained between the reference RT-PCR assay and the nsp14 assays, and amplification was present for only one of the targets (N1 or N2) included in the CDC assay.

Samples still not classifiable after the analysis of conflicting results were excluded from statistical analyses.

2.7. Assessment of the performance characteristics of the nsp14 methods

Sensitivity, specificity and accuracy were calculated based on the number of true positive (TP), true negative (TN), false positive (FP) and false negative (FN) obtained by the assay on the total number of samples assigned as either positive or negative (i.e. number of tested samples minus samples not classifiable after the analysis of conflicting results).

Sensitivity (Se, proportion of positive samples correctly identified as positive: TP/(TP + FN)%), specificity (Sp, proportion of negative samples correctly identified as negative: TN/(TN + FP)%), and accuracy (Ac, proportion of correct assessments over the total number of assessments: (TP + TN)/(TP + TN + FP + FN)%) with corresponding 95 % confidence intervals (95 %C.I.) were calculated for nsp14-based assays on tested samples. Statistical calculations were done using the MedCalc Statistical Software v18 (MedCalc Software bvba, Ostend, Belgium).

3. Results

Table 1 summarizes the results obtained with the rt RT-qPCR and dd RT-PCR assay based on nsp14, the reference real-time RT-PCR assay (detection of RdRp and E genes), and the assays used to analyze the conflicting results (CDC N1/N2, and spike gene). The 10 blind negative controls (molecular biology grade water) provided negative results in all the assays.

Agreement of results between the two assays based on nsp14 and the reference assays (RdRp/E genes) was achieved on 226 samples (156 positive and 70 negative). Thirty-two samples provided discordant results. Of these, 8 samples were positive in the reference assay and in the nsp14 rt RT-qPCR, while gave a negative result with the dd RT-PCR assay. Twenty-four samples provided different combinations of results with the three assays (see Table 1) and were therefore further investigated by the two other independent assays based on N gene detection (real-time CDC protocol) and nested amplification of the spike region. This analysis confirmed that 8 samples contained SARS-CoV-2 RNA (2 samples were amplified with the CDC protocol, 2 with the nested for the spike region and 4 with both protocols) and that 5 samples were negative (no amplification by either the CDC or the spike protocols). Finally, 11 samples could not be resolved as either negative or positive, as amplification was present in only one (N1 or N2) of the CDC real-time targets. Overall, the 258 samples were classified as follows: 172 samples were positive, 75 negatives, and 11 not classifiable. Following exclusion of not classifiable samples, the performance characteristics of the nsp14-based methods were assessed on the 247 samples clearly assigned as either positive or negative. The calculated performance characteristics (95 %C.I.) were: Se 100.0 % (97.4–100.0), Sp 98.7 % (92.1–100.0), and Ac 99.6 % (97.5–100.0) for the rt RT-qPCR nsp14 assay, and Se 92.4 % (87.4–95.6), Sp 100.0 % (94.2–100.0), and Ac 94.7 % (91.1–97.0) for the dd RT-PCR assay.

Compared to the reference assay, the nsp14 rt RT-qPCR identified 8 additional positive samples, 4 testing negative in the reference assay, and 4 from inconclusive samples. Further to this, 4 inconclusive samples were correctly classified as negative by the nsp14 assay. Overall, *Ct* values of the nsp14 rt RT-qPCR were higher than for reference diagnostic assay (median value 31.55 *vs*. 29.13 of RdRp rection and 27.98 of gene E reaction in the reference assay). Based on the nsp14 rt RT-qPCR, quantitative results in positive samples ranged from an estimated amount of 0.1 genome copies (g.c.)/μl of RNA to 5.3×10^5 g.c./ μl of RNA. Discordant results between the nsp14 rt RT-qPCR and the reference diagnostic assay were all related to samples displaying viral concentrations ranging between undetected and an estimated amount of 1.7 g.c./ μl of RNA (median: 0.3 g.c./μl), while discordant results between the rt RT-qPCR and the dd RT-PCR targeting the nsp14 region were associated to samples with concentrations equal or below 1.1 g.c./ μl of RNA (data not shown).

Sequences obtained on the spike region were submitted to GenBank under the accession numbers: MW287562-MW287567.

4. Discussion

Since its first reporting in Wuhan (China) in December 2019, COVID-19 quickly spread worldwide, setting significant challenges for the global health system governance. Clinical and public health laboratories were early faced with the need to improve their testing capacity, ensuring the accurate molecular detection of SARS-CoV-2 (Younes et al.,

Table 1
Summary of the study results.

N° of samples[a]	RT-qPCR nsp14	dd RT-PCR nsp14	Reference diagnostic RT-PCR assay		Assessment following analysis of conflicting results		
			RdRp	gene *E*	Positive[b]	Negative[c]	Not classifiable[d]
156	+	+	+	+	156		
70	−	−	−	−		70	
8	+	−	+	+	8		
7	+	−	−	−	2[e], 1[f]	1	1[h], 2[i]
2	+	+	−	−	1[e]		1[h]
2	+	−	+	−	1[f]		1[i]
2	+	+	+	−	1[e], 1[g]		
4	−	−	+	−		1	1[h], 2[i]
1	−	+	+	−			1[i]
5	−	−	−	+		3	1[h], 1[i]
1	+	−	−	+	1[g]		
258	178	161	173	170	172	75	11
TP	172	159		164			
TN	74	75		71			
FP	1	0		0			
FN	0	13		4			
Inconclusive	−	−		8			
Total	247	247		247			

[a] The 10 blind negative controls (molecular biology grade water) were excluded from calculations.
[b] Assigned as positive if at least one of the following conditions occurred: i) positive resul by both the reference diagnostic assay (combined detection of RdRp and gene E) and by the nsp14 assay, ii) positive by the CDC real-time RT-PCR (combined detection of N1 and N2), iii) positive by the nested amplification of the spike region followed by sequencing.
[c] Assigned as negative if at least one of the following conditions occurred: i) negative by both the reference diagnostic assay (no detection of either RdRp or gene E) and by the nsp14 assay, ii) negative by the CDC real-time RT-PCR (no detection of either N1 and N2) and negative by the nested amplification of the spike region.
[d] Not classifiable: positive only with one (either N1 or N2) of the CDC real-time RT-PCR assays and negative by the nested amplification of the spike region. Not classifiable samples were excluded from calculations.
[e] Positive by the CDC real-time RT-PCR and the nested amplification of the spike region followed by sequencing.
[f] Positive by the nested amplification of the spike region followed by sequencing.
[g] Positive by the CDC real-time RT-PCR.
[h] Positive by the N1 CDC real-time RT-PCR.
[i] Positive by the N2 CDC real-time RT-PCR.

2020). According to the WHO recommendations, there is a continuous and compelling request of timely and accurate diagnostic tests (World Health Organization (WHO, 2020c). Currently, real-time RT-PCR on nasopharyngeal and respiratory specimens represents the gold standard for the qualitative and quantitative detection of SARS-CoV-2 virus as primary or confirmatory diagnosis (Böger et al., 2021; Goudouris, 2021).

The most common target genes, included in the majority of the available commercial real-time RT-PCR kits, are mainly based on previous testing experiences with other beta-Coronaviruses as SARS-CoV and MERS-CoV epidemic (Chan et al., 2015, 2020; Cheng et al., 2007) or on protocols developed for SARS-CoV-2 by national public health institutes or authorities as for the US CDC and the German Charitè methods (Corman et al., 2020; Centers for Disease Control and Prevention (CDC, 2020a,b). Nevertheless, given its evolutionary rate, the SARS-CoV-2 virus displays a not negligible capacity for RNA mutation (Li et al., 2020), and if mutations occur on detected target genes a decrease of the assay sensitivity may occur. Thus, also considering the emerging public health threat arise around the expression of novel Variants of Concern (VOC) (European Centre for Disease Prevention and Control, World Health Organization (ECDC/WHO, 2021), it becomes fundamental the identification of alternative and reliable target genes for virus detection.

Data shown in the present study assess the performance in SARS-CoV-2 detection in NP swabs of a real-time RT-qPCR and a dd RT-PCR protocol based on nsp14 genomic region, a target previously developed for SARS-CoV-2 monitoring in wastewaters (La Rosa et al., 2021a, 2021b, 2021c; De Giglio et al., 2021), and then successfully used for SARS-CoV-2 detection in other complex environmental matrices in which high sensitivity is required, as solid waste (Di Maria et al., 2021) and air particulate matter (Pivato, personal communication). Nsp14 region is known to be crucial in SARS-CoV-2 replication through the ExoN domain and its proofreading activity, able to maintain the genome integrity, preventing and repairing mutations (Ogando et al., 2020). Moreover, the N7-MTase domain of nsp14 is involved in the viral mRNA cap synthesis and is linked with ExoN through a flexible hinge region highly conserved across Coronaviruses (Romano et al., 2020). Interestingly, among RNA viruses, ExoN is encoded only in eight families of the *Nidovirales* order (*Coronaviridae, Tobaniviridae, Roniviridae, Medioniviridae, Euroniviridae, Mesoniviridae, Abyssoviridae,* and *Mononiviridae*) and in the *Arenaviridae* family (Cruz-González et al., 2021), a condition that reduce the risk of primer cross-reactivity with non-target viruses.

In our study, on a total of 247 samples unequivocally ascertained as either positive (n = 172) or negative (n = 75), the two assays designed on nsp14 provided correct results in 246 samples (172 positive and 74 negative) tested by the real-time RT-qPCR assay, and in 234 samples (159 positive and 75 negative) for the dd RT-PCR. Interestingly, all the discordant results obtained in the comparison of the nsp14 real-time RT-qPCR assay with the CE IVD marked reference diagnostic assay based on RdRp and gene E regions were associated to samples with low concentrations of the viral target, ranging from undetected to and estimated amount of 1.7 g.c./µl and with a median value of 0.3 g.c./µl of RNA. Based on manufacturer's data, the declared LOD for the reference assay used in this study is 3.0 g.c./10 µl reaction (equivalent to 0.3 g.c./µl), while the nsp14 real-time RT-qPCR protocol characterized on SARS-CoV-2 RNA from cell cultured virus showed a LOD of 0.41 g.c./µl and a LOQ of 3.71 g.c./µl (La Rosa et al., 2021a). Thus, most of the conflicting results in this in-house validation study were obtained on samples with target concentrations close to the analytical detection limit of the two assays.

Overall, the rt RT-qPCR nsp14 assay revealed better performances than the dd RT-PCR in terms of sensitivity (100.0 % vs 92.4 %) and

accuracy (99.6 % vs 94.7 %). It should be considered, however, that – as for the comparison with the reference assay – the discordant results between the rt RT-qPCR and the dd RT-PCR targeting the nsp14 region were all associated with samples with low concentrations (below an estimated amount of 1.1 g.c./ µl of RNA) in which higher variability of results associated to target distribution may be expected.

Significantly, a systematic review and meta-analysis of 16 studies, performed on a total of 3818 patients using different clinical specimens (nasopharyngeal swabs, throat swabs, sputum, etc.), estimated a pooled sensitivity for RT-PCR assays of 87.8 % (95 %C.I.: 81.5 %–92.2 %) (Jarrom et al., 2020). Similarly, a systematic review by the Cochrane COVID-19 Diagnostic Test Accuracy Group focusing on point-of-care rapid RT-PCR commercial assays (13 studies, 1179 confirmed SARS-CoV-2 samples), showed an average sensitivity of 95.2 % (95 %C.I.: 86.7 %–98.3 %; range: 68 %–100 %) and an average specificity of 98.9 % (95 %C.I.: 97.3 %–99.5 %; range: 92 %–100 %) (Dinnes et al., 2020). Therefore, the performance characteristics shown in this study by the two nsp14 assays are comparable with those reported for other SARS-CoV-2 detection systems. In particular, the results of the real-time RT-PCR (sensitivity 100.0 %, specificity 98.7 % and accuracy 99.6 %) demonstrate a performance of this assay above average and highlight that nsp14 should be considered a reliable target for SARS-CoV-2 detection.

5. Conclusion

In conclusion, the results of the study support the use of the nsp14 target region in real-time RT-qPCR and dd RT-PCR assays for the detection of SARS-CoV-2 in nasopharyngeal swabs. The integration of the validated target in a multiplex real-time RT-PCR assay incorporating an internal process control (e.g. the human ribonuclease P) to differentiate samples affected by inappropriate sampling or high PCR inhibition, or characterized by a low concentration of the viral target, could likely improve the identification of positive samples and help solving cases of inconclusive diagnosis.

Data availability

No data was used for the research described in the article.
Data will be made available on request.
All data is within the manuscript and figure
The authors do not have permission to share data.

CRediT authorship contribution statement

BP, YTRP, GLR, and ES conceived the study. AM, SG, LV, GLC, MT, MCC, DDC, TV, LC, SDP, FB performed the experiments. FC, PC, BP, YTRP, GLR, and ES administered the project and analysed data. All of the authors contributed to the writing of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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