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Rapid Isothermal Amplification for the Buccal Detection SARS-CoV-2 in the Context of Out-Patient COVID-19 Screening

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Abstract: A commercially available isothermal amplification of SARS-CoV-2 RNA was applied to self-collected saliva samples using dry dental cotton rolls, which were held in the mouth for two minutes. Of 212 tests, isothermal amplification yielded three (0.14%) invalid results, 120 (56.6%) positive results and 89 (42%) negative results. Compared to reference RT-PCR assays routinely performed simultaneously on nasopharyngeal swabs, excluding the three invalid isothermal amplification assays and one RT-PCR invalid assay, these figures indicated that 119/123 (96.7%) samples were positive in both methods and 85/85 samples were negative in both methods. Four positive buccal swabs which were missed by the isothermal amplification, exhibited Ct values of 26–34 in reference RT-PCR assays. Positive isothermal amplification detection was achieved in less than 10 min. Supervision of the self-sampling procedure was key to achieve these performances. These data support the proposal to use the protocol reported in this paper, including supervised buccal self-sampling, to screen people suspected of having COVID-19 at the point of care.

Keywords: SARS-CoV-2; COVID-19; point of care; diagnosis; saliva; isothermal amplification

1. Introduction

Direct diagnosis of SARS-CoV-2 infection, referred as COVID-19 [1], is routinely performed by the reverse-transcription polymerase chain reaction (RT-PCR) detection of viral RNA in nasopharyngeal swabs [2,3], with results obtained in less than 25 min at the point of care (POC) [4,5]. Alternative posterior oropharyngeal saliva swabs were collected by an investigator and yielded similar detection rates of SARS-CoV-2 RNA as the nasopharyngeal swabs, suggesting that oral fluid could be of interest for the diagnosis of COVID-19 [6]. RT-PCR has been favorably evaluated on self-collected saliva samples, which are more comfortable to carry out and were found to be as effective as nasopharyngeal swabs, as well as being stable for several days without the use of any preservative [7–12]. Alongside RT-PCR, isothermal amplification recently emerged as an alternative technique for detecting SARS-CoV-2 RNA yet reports of its application to nasopharyngeal swabs yielded contradictory data regarding its clinical performance [13]. Among this emerging technology, loop-mediated amplification (LAMP) and Nicking enzyme-assisted reaction (NEAR) have been used in point of care, far from central laboratories to identify both symptomatic

and asymptomatic individuals [14]. Furthermore, isothermal amplification applied to saliva samples has also proved promising on a limited series of COVID-19 patients [15]. Moreover, it was recently demonstrated that saliva collection with a roll cotton improved significantly the molecular detection of SARS-CoV-2 compared to nasopharyngeal swab (NPS) specimens [16].

In this study, we evaluated the performance of one such commercially available isothermal molecular test for the rapid detection of SARS-CoV-2 RNA detection in standardized buccal self-collected samples in order to achieve unprecedented sensitivity and specificity of isothermal amplification of SARS-CoV-2 RNA detection, compared to the gold standard RT-PCR, in less than 10 min.

2. Materials and Methods

During the period of 16 February 2020 to 22 February 2020, individuals admitted to the Institut Hospitalo-Universitaire (IHU) Méditerranée Infection (Marseille, France), for SARS-CoV-2 routine diagnosis, follow-up or confirmation of a previous COVID-19 diagnosis were invited to enroll in this study. The inclusion criteria were all individuals on demand of SARS-CoV-2 detection using NPSs, accepting in parallel saliva collection. No individuals were excluded.

Participants were instructed to hold one dry dental cotton roll (3.8 × 0.8 mm; GACD, Paris, France) in their mouth over the four buccal quadrants and then to rub the cotton over the gums, for exactly two min. Sampling was supervised by one of us and the dental cotton rolls, retrieved by the patient themselves were triturated for 20 s in the lysis buffer contained in the kit purchased by IHU Méditerranée Infection from the supplier (ID NOW™, Abbott, Scarborough, ME, USA) (Supplementary Video). This was followed by NEAR isothermal amplification of the RdRp gene, performed according to the supplier's instructions (Abbott). Simultaneously, a nasopharyngeal swab was taken for each patient to perform RT-PCR analysis targeting the envelope protein (E)-encoding gene or the nucleocapsid protein (N)-encoding gene, as previously described [4,17]. The two technologies RT-PCR and isothermal amplification used in this study were able to detect the SARS-CoV-2 variants including the Marseille 4 variant [18] and the variant N501Y circulating in Marseille at the time of the study [19].

3. Results

A total of 280 sample pairs of NPSs and saliva samples were collected. The median age was 53 years (range, 5–99 years), 134/280 individuals (48%) were men and 146/280 (52%) women and 263/280 (94%) were adults.

Isothermal amplification yielded 3/212 (0.14%) invalid results, i.e., 209 interpretable results, including 120/209 (57.4%) positive results and 89/209 (42.6%) negative results. Compared to reference RT-PCR assays routinely performed simultaneously on nasopharyngeal swabs, excluding the three invalid isothermal amplification assays and one invalid RT-PCR assay, these figures indicated 120 samples were positive in both methods and 85 samples were negative in both methods, for 100% specificity (Table 1). More precisely, four RT-PCR-positive samples were missed by isothermal amplification. These samples had Ct values of 26, 28, 33 and 34 (Ct range of positive samples in the RT-PCR platform are shown in Figure 1). The four discordant samples were later confirmed by both methods.

The manipulation included two minutes sampling, three minutes heating the lysis buffer, 20 s triturating the dental cotton in the heated lysis buffer, and 3.2 min amplification and detection. The measured duration of analysis was 10 min for negative results and 3.2 min ± 0.7 min (range, 2–6 min) for positive results (data from 67 measures). A second group of people were further instructed to follow the same protocol, with no supervision. In this group of 68 people, isothermal amplification was positive in 34/68 (50%) of people and negative in 34/68 (50%). Compared to the conventional RT-PCR, which was performed in parallel on a nasopharyngeal swab, the sensitivity of the isothermal amplification was 60.7% and specificity was 100%.

Table 1. Comparison between isothermal amplification/saliva and RT-PCR/NPS detection of SARS-CoV-2 on 209 supervised samplings giving interpretable NEAR results.

	RT-PCR +	RT-PCR −	Total
Isothermal amplification +	120	0	120
Isothermal amplification −	4	85	89
Total	124	85	209
Sensitivity	96.77% (95% CI: 91.95% to 99.11%)		
Specificity	100% (95% CI: 95.75% to 100.00%)		
Accuracy	98.17% (95% CI: 95.24% to 99.48%)		

(+): positive, (−): negative.

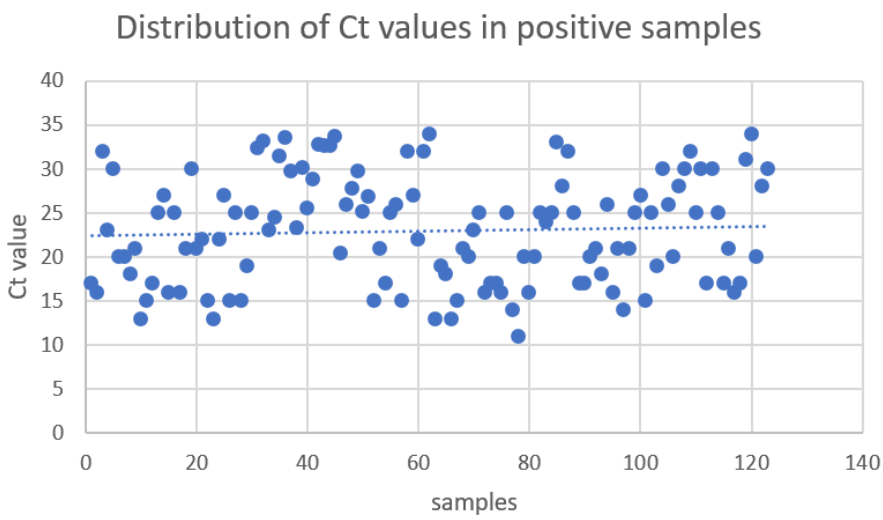

Figure 1. Distribution of Ct values of positive samples.

4. Discussion

Recently, an investigation into a series of 44 people in Japan suspected of having COVID-19, using a buccal sample yielded a sensitivity of 82.6% over 23 definite COVID-19 patients. Results were obtained in 45 min [15]. Further investigations using the same commercially available isothermal amplification technology that we evaluated here (ID NOWTM, Abbott, Scarborough, ME, USA) showed 94% positive percent agreement (90/96 nasopharyngeal swabs) in one study [20] and 91% positive percent agreement (30/33 nasopharyngeal swabs) in another study [13]. Here, a larger series yielded results in less than 10 min and sensitivity was 96.7% when buccal sampling was supervised, compared to 60.7% when buccal sampling was not supervised. In fact, two min were required to appropriately collect saliva and mucosal buccal material [16]; a duration which could not be ensured in non-supervised procedure. Our interpretation is that standardization of the sample; and its supervision by trained personal, are the keys to success. The fact that we selected a population enriched in patients who had already been diagnosed with SARS-CoV-2 infection and who were being treated in our Institute as part of their medical care, explained the high prevalence of positives and allowed us to reveal the positive agreement between ID NOW and RT-PCR routine analysis, which may have biased data. Furthermore, isothermal amplification yields non-quantitative results which needs confirmation by quantitative RT-PCR and genotyping.

5. Conclusions

In conclusion, the data here reported support the use of isothermal amplification detection of the SARS-CoV-2 RNA in buccal samples, to screen COVID-19 patients at the POC. The instrument was easy to use, requiring minimal hands-on time and no special-

ized staff, and reagents were stored at room temperature, facilitating their deployment. Furthermore, the protocol proposed in this study could be incorporated in zero-COVID-19 strategies in places where people are gathered together. Standardized buccal sampling and supervision of self-collection of samples were key to achieving unprecedented sensitivity of detection, using a less aggressive and more comfortable sampling procedure than standard nasopharyngeal swabbing.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/jcm10122643/s1, Video S1: Sampling and handling for the buccal detection SARS-CoV-2.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of IHU Méditerranée Infection (protocol code 2021-014, date 30 April 2021).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: The suppliers cited in this paper had strictly no role in the study and did not interfere in any way with the design, realization, interpretation and writing. A.B. is employed by POCRAMé, a IHU Méditerranée Infection-based start-up which commercializes POC solutions for the diagnosis of COVID-19. D.R., P.Y.-L. and M.D. are among the cofounders and are shareholders in POCRAMé.

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