Rapid isothermal amplification detection of buccal SARS-CoV-2 for ambulatory screening of COVID-19.

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Abstract. A commercially-available isothermal amplification of SARS-CoV-2 RNA was applied to auto-sampled saliva using dry dental cotton rolls, swallowed for 02 minutes. Among 212 tests, isothermal amplification yielded 3 (0.14%) invalid, 120 (56.6%) positive and 89 (42%) negative tests. Compared to reference RT-PCR assays routinely performed on nasopharyngeal swabs in parallel, excluding the 03 isothermal amplification invalid assays and 01 RT-PCR invalid assay, these figures indicated 119/123 (96.7%) samples were positive in the two methods and 85/85 samples were negative in the two methods. Four buccal swabs missed by the isothermal amplification, exhibited Ct values of 26-34 in reference RT-PCR assays. Positive isothermal amplification detection was achieved in < 10 minutes. Supervision of the auto-sampling was a key to achieve these performances. These data support the proposal to use herein reported protocol including supervised buccal auto-sampling, for the screening of people suspected of COVID-19 patients at the point-of-care.

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 minutes at the point-of-care (POC) [4, 5]. Alternative posterior oropharyngeal saliva swabs collected by an investigator, yielded similar detection rate of SARS-CoV-2 RNA as nasopharyngeal swabs suggesting that oral fluid could be of interest for the diagnosis of COVID-19 [6]. One step forwards, RT-PCR has been favorably evaluated on auto-sampled saliva samples which are more comfortable to practice and were found as effective as nasopharyngeal swabs and stable for several days without the use of any preservative [7-12]. In parallel to 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 performances [13]. Furthermore, isothermal amplification applied to saliva samples also proved promising on a limited series of COVID-19 patients [14].

Here, we evaluated the performances of one such commercially-available isothermal molecular test for the rapid detection of SARS-CoV-2 RNA detection in standardized buccal auto-sampling in order to achieve unprecedented sensibility and specificity of isothermal amplification SARS-CoV-2 RNA detection, compared to the gold standard RT-PCR, in less than 10 minutes.

Ambulatory people presenting to the Institut Hospitalier-Universitaire Méditerranée Infection for confirmation of a previous COVID-19 diagnosis, or for COVID-19 follow-up, were instructed to swallow a dry dental cotton roll (3.8 X 0.8 mm; GACD, Paris, France) over the four buccal quadrants and eventually to mass the swab on the gingiva; for exactly two minutes. Sampling was supervised by one of

us and the swallowed dental cotton collected by the patient himself was immediately triturated for 20 sec. in the lysis buffer contained in the kit purchased by the supplier (ID NOW™, Abbott, Scarborough, USA)(Supplementary Vidéo); followed by isothermal amplification of the RdRp gene performed following the supplier's instructions (Abbott). In parallel, 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, 15]. Isothermal amplification yielded 3/212 (0.14%) invalid, 120/212 (56.6%) positive and 89/212 (42%) negative tests. Compared to reference RT-PCR assays routinely performed on nasopharyngeal swabs in parallel, excluding the 03 isothermal amplification invalid assays and 01 RT-PCR invalid assay, these figures indicated 119 samples were positive in the two methods and 85 samples were negative in the two methods for a 100% specificity. More precisely, 04 RT-PCR-positive samples were missed by isothermal amplification. They had Ct values of 26, 28, 33 and 34. These 04 samples have been later confirmed in the two methods. While the manipulation included 2-min. sampling, 3-min. heating of the lysis buffer, 20 sec. to triturate the buccal swab in the heated lysis buffer and 3.2-min. amplification and detection, the measured duration of analysis was 10 minutes for negative results and 3.2 minutes ± 0.7 minute [range, 2-6 minutes] for positives (data from 67 measures). A second group of people were further instructed to follow the same protocol, yet in these patients sampling was not supervised. In this group of 68 people, isothermal amplification was positive in 34/68 (50%) people and negative in 34/68 (50%). Compared to the conventional RT-PCR performed in parallel on nasopharyngeal swab, sensitivity of the isothermal amplification was of 60.7% and specificity was 100%.

Recently, investigation of a series of 44 people suspected of COVID-19 in Japan, using buccal sample yielded a sensitivity of 82.6% over 23 definite COVID-19 patients; and results were obtained in 45 minutes [14]. Further investigations using the same commercially-available isothermal amplification technology that we evaluated herein (ID NOW™, Abbott, Scarborough, USA) showed a 94% positive percent agreement (90/96 nasopharyngeal swabs) in one study [16] and 91% positive percent agreement (30/33 nasopharyngeal swabs) in another study [13]. Here, a larger series yielded results in less than 10 minutes and sensitivity was of 96.7% when buccal sampling was supervised, versus 60.7% when buccal sampling was not supervised. Our interpretation is that standardization of the sample which collected not only saliva, but also buccal cells; and its supervision by a trained personal, were keys to success. The fact that we selected a population enriched in patients already diagnosed with SARS-CoV-2 infection followed in our Institute as part of their medical care, explained the high prevalence of positives allowing to appreciate the positive agreement between ID NOW and RT-PCR routine analysis; which may have biased data. Also, isothermal amplification yields non-quantitative results which needs confirmation by quantitative RT-PCR and genotyping.

In conclusion, the data here reported support the use of isothermal amplification detection of the SARS-CoV-2 RNA in the buccal sample, for the screening of COVID-19 patients at the POC. Accordingly, the instrument was easy to use, requiring minimal hands-on time and no specialized staff; and reagents were stored at room temperature, facilitating their deployment. Furthermore, herein proposed protocol could be incorporated in zero-COVID-19 strategies in spaces accommodating gathered people. Standardized buccal sampling and supervision of this auto-sampling were keys to achieve unprecedented sensitivity of detection,

using a less aggressive and more comfortable sampling than the usual nasopharyngeal swabbing.

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Table 1. Performances of a commercially-available isothermal amplification performed on buccal swabs compared with RT-PCR performed on nasopharyngeal swabs (standard reference), in 212 successive ambulatory people presenting at the IHU Méditerranée Infection, Marseille, February 2021. In 04 people, assays were non interpretable resulting in comparison in 208 people.

		RT-PCR		
		POS	NEG	TOTAL
Isothermal amplification	POS	119	0	119
	NEG	4	85	89
	TOTAL	123	85	208

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CONFLICT OF INTERESTS

AB is employed by POCRAMé, a IHU Méditerranée Infection-based start-up which commercializes POC solutions for the diagnosis of COVID-19. DR and MD are among cofounders and share-holders of POCRAMé.