



HAL
open science

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

Amar Bouam, Jean-Jacques Vincent, Elisabeth Le Glass, Lionel Almeras, Pierre-Yves Levy, Hervé Tissot-Dupont, Jean-Christophe Lagier, Pierre-Edouard Fournier, Didier Raoult, Michel Drancourt

► To cite this version:

Amar Bouam, Jean-Jacques Vincent, Elisabeth Le Glass, Lionel Almeras, Pierre-Yves Levy, et al.. Rapid Isothermal Amplification for the Buccal Detection SARS-CoV-2 in the Context of Out-Patient COVID-19 Screening. *Journal of Clinical Medicine*, 2021, 10 (12), pp.2643. 10.3390/jcm10122643 . hal-03333759

HAL Id: hal-03333759

<https://amu.hal.science/hal-03333759>

Submitted on 10 Sep 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License



Communication

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

Amar Bouam^{1,2}, Jean-Jacques Vincent³, Elisabeth Le Glass³, Lionel Almeras^{4,5} , Pierre-Yves Levy³, Hervé Tissot-Dupont^{1,3}, Jean-Christophe Lagier^{1,3}, Pierre-Edouard Fournier^{3,4}, Didier Raoult^{1,3} and Michel Drancourt^{1,3,*}

¹ Aix-Marseille-Université, IRD, MEPHI, IHU Méditerranée Infection, 13005 Marseille, France; amarbouam@yahoo.fr (A.B.); herve.tissot-dupont@mediterranee-infection.com (H.T.-D.); jean-christophe.lagier@ap-hm.fr (J.-C.L.); didier.raoult@gmail.com (D.R.)

² POCRAMé, 13005 Marseille, France

³ IHU Méditerranée Infection, 13005 Marseille, France; jean-jacques.vincent@ap-hm.fr (J.-J.V.); elisabeth.leglass@gmail.com (E.L.G.); pylevy@wanadoo.fr (P.-Y.L.); pierre-edouard.fournier@univ-amu.fr (P.-E.F.)

⁴ Aix-Marseille-Université, IRD, VITROME, IHU Méditerranée Infection, 13005 Marseille, France; almeras.lionel@gmail.com

⁵ Unité Parasitologie et Entomologie, Département Microbiologie et Maladies Infectieuses, Institut de Recherche Biomédicale des Armées, 13005 Marseille, France

* Correspondence: michel.drancourt@univ-amu.fr



Citation: Bouam, A.; Vincent, J.-J.; Le Glass, E.; Almeras, L.; Levy, P.-Y.; Tissot-Dupont, H.; Lagier, J.-C.; Fournier, P.-E.; Raoult, D.; Drancourt, M. Rapid Isothermal Amplification for the Buccal Detection SARS-CoV-2 in the Context of Out-Patient COVID-19 Screening. *J. Clin. Med.* **2021**, *10*, 2643. <https://doi.org/10.3390/jcm10122643>

Academic Editors: Philippe Parola, Corneliu Petru Popescu and Bahi Takkouche

Received: 7 May 2021

Accepted: 10 June 2021

Published: 16 June 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

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.

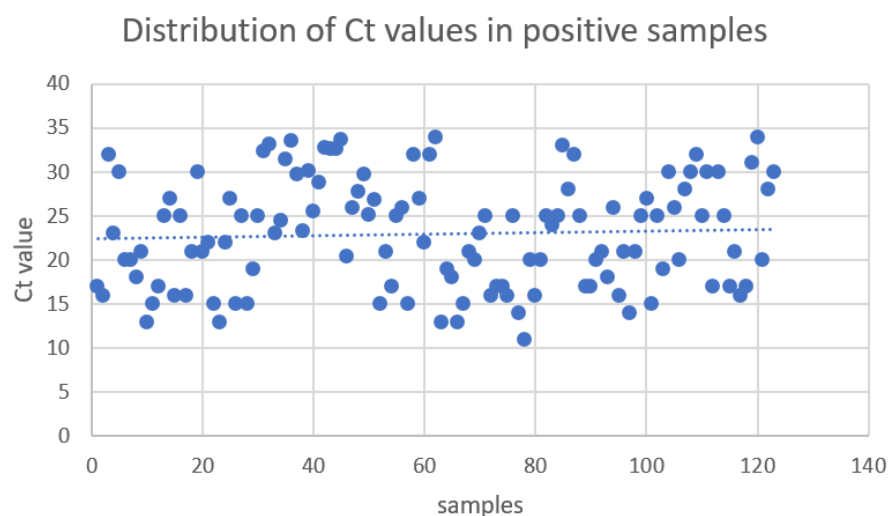
Isothermal amplification yielded 3/212 (0.14%) invalid results, i.e., 209 interpretable results, including 120/209 (57.4%) positive results and 89/209 (42.6%) negative results. Compared to reference RT-PCR assays routinely performed simultaneously on nasopharyngeal swabs, excluding the three invalid 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 $3.2 \text{ min} \pm 0.7 \text{ min}$ (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 NOW™, 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.

Author Contributions: Conceptualization, M.D., D.R.; methodology, A.B., J.-J.V., E.L.G.; validation, M.D.; formal analysis, A.B., J.-J.V., E.L.G.; investigation, A.B., J.-J.V., E.L.G., L.A., P.-Y.L., H.T.-D., J.-C.L., P.-E.F.; writing—original draft preparation, A.B., M.D.; writing—review and editing, A.B., J.-J.V., E.L.G., L.A., P.-Y.L., H.T.-D., J.-C.L., P.-E.F., D.R., M.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the French Government under the «Investissements d’avenir» (Investments for the Future) program managed by the Agence Nationale de la Recherche (ANR, fr: National Agency for Research) (reference: Méditerranée Infection 10-IAHU-03). This work was supported by Region Sud (Provence Alpes Cote d’Azur) and European funding FEDER PRIMMI.

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.

Acknowledgments: The authors would like to thank the residents who participated in this study and the technical assistance of Gérard Aboudharam and Céline Gazin.

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 POGRAMÉ, 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 POGRAMÉ.

References

1. Zhu, N.; Zhang, D.; Wang, W.; Li, X.; Yang, B.; Song, J.; Zhao, X.; Huang, B.; Shi, W.; Lu, R.; et al. China Novel Coronavirus Investigating and Research Team. A novel coronavirus from patients with pneumonia in China. *N. Engl. J. Med.* **2019**, *382*, 727–733. [[CrossRef](#)] [[PubMed](#)]
2. Corman, V.M.; Landt, O.; Kaiser, M.; Molenkamp, R.; Meijer, A.; Chu, D.K.; Bleicker, T.; Brünink, S.; Schneider, J.; Schmidt, M.L.; et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Eurosurveillance* **2020**, *25*, 2000045. [[CrossRef](#)] [[PubMed](#)]
3. Ciotti, M.; Angeletti, S.; Minieri, M.; Giovannetti, M.; Benvenuto, D.; Pascarella, S.; Sagnelli, C.; Bianchi, M.; Bernardini, S.; Ciccozzi, M. COVID-19 Outbreak: An Overview. *Chemotherapy* **2019**, *64*, 215–223. [[CrossRef](#)] [[PubMed](#)]
4. Fournier, P.E.; Zandotti, C.; Ninove, L.; Prudent, E.; Colson, P.; Gazin, C.; Million, M.; Tissot-Dupont, H.; Fenollar, F. Contribution of VitaPCR SARS-CoV-2 to the emergency diagnosis of COVID-19. *J. Clin. Virol.* **2020**, *133*, 104682. [[CrossRef](#)] [[PubMed](#)]
5. Bouam, A.; Boualam, M.; Levy, P.Y.; Honoré, S.; La Scola, B.; Fournier, P.E.; Raoult, D.; Drancourt, M. Point-of-care diagnosis of COVID-19 at IHU Méditerranée Infection, Marseille, France. *Clin. Infect. Dis.* **2021**, submitted.
6. Chen, J.H.; Yip, C.C.; Poon, R.W.; Chan, K.H.; Cheng, V.C.; Hung, I.F.; Chan, J.F.; Yuen, K.Y.; To, K.K. Evaluating the use of posterior oropharyngeal saliva in a point-of-care assay for the detection of SARS-CoV-2. *Emerg. Microbes Infect.* **2020**, *9*, 1356–1359. [[CrossRef](#)] [[PubMed](#)]
7. Wyllie, A.L.; Fournier, J.; Casanovas-Massana, A.; Campbell, M.; Tokuyama, M.; Vijayakumar, P.; Warren, J.L.; Geng, B.; Muenker, M.C.; Moore, A.J.; et al. Saliva or Nasopharyngeal Swab Specimens for Detection of SARS-CoV-2. *N. Engl. J. Med.* **2020**, *383*, 1283–1286. [[CrossRef](#)] [[PubMed](#)]
8. Teo, A.K.J.; Choudhury, Y.; Tan, I.B.; Cher, C.Y.; Chew, S.H.; Wan, Z.Y.; Cheng, L.T.E.; Oon, L.L.E.; Tan, M.H.; Chan, K.S.; et al. Saliva is more sensitive than nasopharyngeal or nasal swabs for diagnosis of asymptomatic and mild COVID-19 infection. *Sci. Rep.* **2021**, *11*, 3134. [[CrossRef](#)] [[PubMed](#)]

9. Herrera, L.A.; Hidalgo-Miranda, A.; Reynoso-Noverón, N.; Meneses-García, A.A.; Mendoza-Vargas, A.; Reyes-Grajeda, J.P. Saliva is a reliable and accessible source for the detection of SARS-CoV-2. *Int. J. Infect. Dis.* **2021**, *10*, S1201–S9712. [[CrossRef](#)]
10. Azzi, L.; Carcano, G.; Gianfagna, F.; Grossi, P.; Gasperina, D.D.; Genoni, A.; Fasano, M.; Sessa, F.; Tettamanti, L.; Carinci, F.; et al. Saliva is a reliable tool to detect SARS-CoV-2. *J. Infect.* **2020**, *81*, e45–e50. [[CrossRef](#)] [[PubMed](#)]
11. Bastos, M.L.; Perlman-Arrow, S.; Menzies, D.; Campbell, J.R. The Sensitivity and Costs of Testing for SARS-CoV-2 Infection with Saliva versus Nasopharyngeal Swabs: A Systematic Review and Meta-analysis. *Ann. Intern. Med.* **2021**, *12*, M20–M6569. [[CrossRef](#)]
12. Butler-Laporte, G.; Lawandi, A.; Schiller, I.; Yao, M.C.; Dendukuri, N.; McDonald, E.G.; Lee, T.C. Comparison of Saliva and Nasopharyngeal Swab Nucleic Acid Amplification Testing for Detection of SARS-CoV-2: A Systematic Review and Meta-analysis. *JAMA Intern. Med.* **2021**, *15*, e208876. [[CrossRef](#)]
13. Cradic, K.; Lockhart, M.; Ozbolt, P.; Fatica, L.; Landon, L.; Lieber, M.; Yang, D.; Swickard, J.; Wongchaowart, N.; Fuhrman, S.; et al. Clinical Evaluation and Utilization of Multiple Molecular In Vitro Diagnostic Assays for the Detection of SARS-CoV-2. *Am. J. Clin. Pathol.* **2020**, *154*, 201–207. [[CrossRef](#)] [[PubMed](#)]
14. James, A.S.; Alawneh, J.I. COVID-19 Infection Diagnosis: Potential Impact of Isothermal Amplification Technology to Reduce Community Transmission of SARS-CoV-2. *Diagnostics* **2020**, *10*, 399. [[CrossRef](#)] [[PubMed](#)]
15. Yamazaki, W.; Matsumura, Y.; Thongchankaew-Seo, U.; Yamazaki, Y.; Nagao, M. Development of a point-of-care test to detect SARS-CoV-2 from saliva which combines a simple RNA extraction method with colorimetric reverse transcription loop-mediated isothermal amplification detection. *J. Clin. Virol.* **2021**, *136*, 104760. [[CrossRef](#)] [[PubMed](#)]
16. Melo Costa, M.; Benoit, N.; Dormoi, J.; Amalvict, R.; Gomez, N.; Tissot-Dupont, H.; Million, M.; Pradines, B.; Granjeaud, S.; Almeras, L. Salivette, a relevant saliva sampling device for SARS-CoV-2 detection. *J. Oral Microbiol.* **2021**, *13*, 1920226. [[CrossRef](#)] [[PubMed](#)]
17. Amrane, S.; Tissot-Dupont, H.; Doudier, B.; Eldin, C.; Hocquart, M.; Mailhe, M.; Dudouet, P.; Ormières, E.; Ailhaud, L.; Parola, P.; et al. Rapid viral diagnosis and ambulatory management of suspected COVID-19 cases presenting at the infectious diseases referral hospital in Marseille, France,—January 31st to March 1st, 2020: A respiratory virus snapshot. *Travel Med. Infect. Dis.* **2020**, *36*, 101632. [[CrossRef](#)] [[PubMed](#)]
18. Fournier, P.E.; Colson, P.; Levasseur, A.; Devaux, C.A.; Gautret, P.; Bedotto, M.; Delerce, J.; Brechard, L.; Pinault, L.; Lagier, J.C.; et al. Emergence and outcomes of the SARS-CoV-2 ‘Marseille-4’ variant. *Int. J. Infect. Dis.* **2021**, *106*, 228–236. [[CrossRef](#)] [[PubMed](#)]
19. Leung, K.; Shum, M.H.; Leung, G.M.; Lam, T.T.; Wu, J.T. Early transmissibility assessment of the N501Y mutant strains of SARS-CoV-2 in the United Kingdom, October to November 2020. *Eurosurveillance* **2021**, *26*, 2002106. [[CrossRef](#)] [[PubMed](#)]
20. Rhoads, D.D.; Cherian, S.S.; Roman, K.; Stempak, L.M.; Schmotzer, C.L.; Sadri, N. Comparison of Abbott ID Now, DiaSorin Simplexa, and CDC FDA Emergency Use Authorization Methods for the Detection of SARS-CoV-2 from Nasopharyngeal and Nasal Swabs from Individuals Diagnosed with COVID-19. *J. Clin. Microbiol.* **2020**, *58*, e00760-20. [[CrossRef](#)] [[PubMed](#)]