An isothermal lab-on-phone test for easy molecular diagnosis of SARS-CoV-2 near patients and in less than 1 hour

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ABSTRACT

Objectives: The performance of a new point-of-care CE-IVD-marked isothermal lab-on-phone COVID-19 assay was assessed in comparison to a gold standard real-time reverse transcriptase–PCR method.

Methods: The study was conducted following a nonprobability sampling of ≥16-year-old volunteers from three different laboratories, using direct mouthwash (N = 24) or nasopharyngeal (N = 191) clinical samples.

Results: The assay demonstrated 95.19% sensitivity and 100% specificity for detection of SARS-CoV-2 in direct nasopharyngeal crude samples and 78.95% sensitivity and 100% specificity in direct mouthwash crude samples. It also successfully detected currently predominant SARS-CoV-2 variants of concern (Beta B.1.351, Delta B.1.617.2, and Omicron B.1.1.529) and demonstrated to be inert against potential cross-reactions of other common respiratory pathogens that cause infections that present similar symptoms to COVID-19.

Conclusion: This lab-on-phone pocket-sized assay relies on an isothermal amplification of SARS-CoV-2's N and E genes, taking just 50 minutes from sample to result, with only 2 minutes of hands-on time. It presents good performance when using direct nasopharyngeal crude samples, enabling a low-cost, real-time, rapid, and accurate identification of SARS-CoV-2 infections at the point of care, which is important for both clinical management and population screening, as a tool to break the chain of transmission of COVID-19 pandemic, especially in low-resources environments.

Introduction

The world has been facing the COVID-19 pandemic, which is a respiratory disease caused by the novel coronavirus SARS-CoV-2 that was first detected in China in December 2019 (Wu and Mc-Googan, 2020). This virus can spread from an infected individual's mouth or nose in small liquid particles when they cough, sneeze, speak, sing, or breathe, spreading more easily indoors and in crowded settings. COVID-19 is associated with a variety of clinical outcomes, including asymptomatic infection, mild upper respiratory infection, severe lower respiratory disease, including pneumonia and respiratory failure, and, in some cases, death. Older

individuals and individuals of all ages with severe chronic medical conditions, such as heart disease, lung disease, diabetes, and those who are immunosuppressed, such as patients with transplants and oncologic patients (Fung and Babik, 2021), seem to be at a higher risk of developing serious COVID-19 illness and even death (Ribas et al., 2021). The most common symptoms are fever, dry cough, and fatigue, arising from an incubation period of approximately 5.2 days from infection; whereas the period from the onset of COVID-19 symptoms to death ranges from 6 to 41 days, with a median of 14 days (Rothan and Byrareddy, 2020).

Regular testing, aside from the COVID-19 vaccine rollout and current social distancing guidelines, is an essential strategy to break the chain of transmission of the pandemic, especially when, on average, around one of three cases of COVID-19 show no symptoms (Sah et al., 2021). To date, there are over 2046 COVID-19 in vitro diagnostic tests with CE-marking available in the market, 819 of which are rapid tests (European Commission, 2021). These tests are based on different methods, which can be usually convened into two main groups: immunoassays and nucleic acid amplification tests (NAATs), where the latter usually present much better performances (Kahn et al., 2021). Among NAATs, there are real-time reverse transcriptase–PCR (rRT-PCR), Digital PCR, loop-mediated isothermal amplification (LAMP), sequencing, and CRIPSR-based methods that are being used for COVID-19 diagnostic tests. Although reverse transcriptase–PCR is considered the gold standard test for COVID-19, the LAMP method offers more advantages in reaching similar performances, having even been considered one of the best candidates to replace the PCR method (Keikha, 2018). In particular, LAMP offers a fast and reliable isothermal amplification of DNA or RNA templates in only 5-50 minutes, without requiring a thermocycler, and being more robust and tolerant to inhibitors that frequently affect PCR it allows for the use of crude samples, such as direct nasopharyngeal samples collected in viral transportation medium (Ganguli et al., 2020) or even direct samples of raw sewage (Ongerth and Danielson, 2020). In early stages of the COVID-19 pandemic, a publication from Zhang et al. (2020a) first demonstrated the viability of using reverse transcriptase-LAMP (RT-LAMP) as a fast colorimetric point-of-care (PoC) diagnostic tool for SARS-CoV-2 detection. This assay was later optimized to improve its performance (Zhang et al., 2020b). Even though the colorimetric LAMP-based assays do offer a convenient, simple visual readout, which is particularly useful for PoC testing, they are easily prone to color misinterpretations from the end user or interferences by highly buffered sample inputs or acid samples and can ultimately lead to false-positives when low-pH samples are used, such as saliva samples (Uribe-Alvarez et al., 2021). In contrast, LAMP-based assays rely on fluorescence, measuring target amplification using an intercalating dye or using specific fluorescent probes, enabling multiplexing capabilities (Zhang and Tanner, 2021) and offering a more reliable and sensitive solution to assess LAMP target amplification.

STAB VIDA has developed a fluorescent isothermal RT-LAMP-based PoC COVID-19 CE-IVD-marked diagnostic assay that is automatically processed in real-time in an inexpensive, portable, and reusable device that is controlled 100% through a user-friendly mobile app (see Figure 1).

Herein, we assessed the clinical performance of such a pocket-sized diagnostic assay, in a head-to-head comparison to gold standard rRT-PCR, using direct mouthwash or nasopharyngeal samples, to automatically and reliably detect the presence of SARS-CoV-2's E and N gene within 50 minutes (from sample to result), with only 2 minutes hands-on time.

Moreover, the capacity to detect currently predominant SARS-CoV-2 variants of concern (as of December 16, 2021) (European Centre for Disease Prevention and Control, 2021), namely Delta and Omicron variants, and the potential cross-reaction with other respiratory viruses that cause infections that present very similar symptoms to COVID-19, such as other coronaviruses, respiratory syncytial virus (RSV), or influenza, was also assessed.

Materials and methods

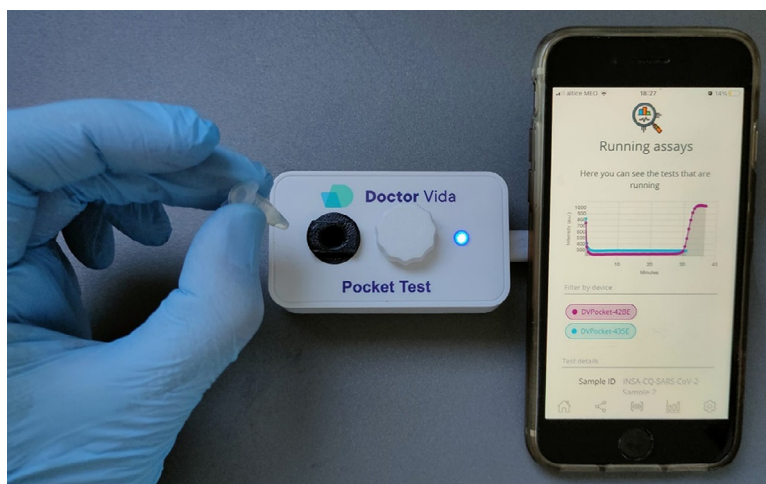### Data collection and ethical considerations

The clinical validation study was conducted following a non-probability sampling of $\geq$16-year-old volunteers from two independent laboratories: Garcia de Orta Hospital (HGO) (Portugal) and King Saud bin Abdulaziz's University for Health Sciences (KSAU-HS) (Saudi Arabia) and from STAB VIDA's certified COVID-19 diagnostic service (Portugal). Ethical approvals were assessed and granted by relevant ethics committees. Nasopharyngeal swab (N = 191) and mouthwash samples (N = 24), also known as mouth/throat washes, were collected from patients who volunteered and were visiting the previously mentioned clinical services for SARS-CoV-2 testing, after a signed written informed consent was obtained. All samples were anonymized after being collected.

rRT-PCR

As a reference method, rRT-PCR for SARS-CoV-2 was performed on nasopharyngeal swab samples, following the laboratory routines of each clinical service. Briefly, for HGO, samples were collected with Abbott's multi-Collect Specimen Collection Kit (Abbott, USA). The samples were processed in either Alinity m or m2000 systems (Abbott, USA), which detect RdRp e N genes, or GeneXpert system (Cepheid, USA), which detects E and N2 genes. All of these tests are automated, use an internal control for each sample, and were analyzed following manufacturer instructions. For KSAU-HS, the DIOS-RT-qPCR Screening kit (Institute of Applied Biotechnologies, Czech Republic) was used as a reference test, together with a Lightcycler 480 instrument (Roche, Switzerland), following the protocol provided by the test manufacturer. For all other samples analyzed at STAB VIDA's certified COVID-19 diagnostic laboratory, the nasopharyngeal swab collection was collected in ATL buffer (Qiagen) or viral preservation medium (VPM) (Jiangsu Kangjian Medical Apparatus Co Ltd), RNA extracted using automated Maxwell RSC system (Promega) following manufacturer instructions, and SARS-CoV-2's N, E, and S gene targets, as well as human RNAseP gene as internal control, were analyzed using QuantStudio-5 Real-Time PCR System (Thermo Fisher), following the certified internal service procedure.

Isothermal lab-on-phone COVID-19 assay

In parallel to rRT-PCR assays, the isothermal lab-on-phone COVID-19 assays (namely, Doctor Vida® pocket COVID-19 assay, Cat no. 133001001, STAB VIDA Lda) were performed on direct crude nasopharyngeal swab and mouthwash samples, following manufacturer instructions. Briefly, nasopharyngeal swab samples were collected in 3 ml VPM. In contrast, mouthwash samples were collected by gargling with 5 ml of sterile 0.9% saline solution (Omega Pharma International) for 20 seconds and mixing this with 3 ml VPM. All samples were incubated at room temperature for at least 10 minutes after collection. In both cases, 10 µl of the final VPM sample mix was directly added to the reaction tube of the isothermal lab-on-phone COVID-19 assay and incubated at room temperature for 5 minutes before running the assay. Results were transferred and stored in real-time to an API server, through a mobile app (namely, "Dr Vida Pocket PCR" app available for free at Google Play Store: https://play.google.com/store/apps/details?id=com.stabvida.dvpocket and Apple app Store: https://apps.apple.com/us/app/doctorvida-pocket/id1522700987), and automatically analyzed after 40 minutes to deliver a final result that

Figure 1. The CE-IVD pocket-sized PCR-like device for isothermal amplification of COVID-19 at point of care.

is automatically presented to end user through the mobile app and simultaneously sent to a registered email address. Similar to rRT-PCR's cycle threshold (Ct) value, the isothermal lab-on-phone COVID-19 assay relies on a time-to-positive value, which is defined as the time of the assay at which the fluorescent signal exceeds the threshold set for a positive result (i.e., exceeds background level).

SARS-CoV-2 variant(s), cross-reactions, and limit of detection (LoD)

SARS-CoV-2's Beta (B.1.351) and Delta (B.1.617.2) variants were also tested with the isothermal lab-on-phone COVID-19 assay, using a commercially available heat-inactivated SARS-CoV-2 lineages (respectively, South Africa/KRISPK005325/2020 and USA/PHC658/2021 isolates) from ZeptoMetrix. SARS-CoV-2's Omicron variant (B.1.1.529) was tested with the isothermal lab-on-phone COVID-19 assay, using a fully sequenced RNA sample that was kindly provided by the National Health Institute Doutor Ricardo Jorge (INSA), National Reference Laboratory and National Health Observatory for COVID-19 in Portugal. Moreover, nasopharyngeal swab samples from two different male patients (aged 29 and 30 years), infected with Delta and Omicron variants (based on the local prevalence of these strains at collection time and rRT-PCR results for three different SARS-CoV-2 genes), respectively, were collected and analyzed daily from day 1 of first symptoms (mild-symptoms: cough and fever) and up to 14 days.

For cross-reactions assessment, the commercially available Respiratory Verification Panel 2 from ZeptoMetrix (Cat no. NATRVP2-QIA) was used. For LoD determination, chemically inactivated SARS-CoV-2 viral particles from lineage USA-WA1/2020 from ZeptoMetrix's SARS-CoV-2 External Run (Cat No. NATSARS(CoV2)-ERC1) were used at different concentrations (5-300 viral particles/reaction, i.e., 0.082-4.918 viral particles per μl, considering a final reaction volume of 61 μl), using VPM as sample diluent.

External quality assessment

The isothermal lab-on-phone COVID-19 assay performance was also assessed with blind samples from an external quality assessment (EQA) program (SARS-CoV-2, PCR 2/2021) for the detection of the SARS-CoV-2 virus, organized by the Portuguese National Influenza Reference Laboratory (LNRVG), belonging to the Department of Infectious Diseases of the National Institute of Health Doutor Ricardo Jorge (INSA). A total of 77 organizations partici-

Table 1
Isothermal lab-on-phone COVID-19 assay LoD study for direct viral particle crude samples

Target level(per reaction)	SARS-CoV-2 (N/E gene) Detection Rate
5 viral particles	0% (0/3)
10 viral particles	33% (1/3)
50 viral particles	67% (2/3)
75 viral particles	100% (3/3)
100 viral particles	100% (3/3)
300 viral particles	100% (3/3)

LoD: limit of detection

pated in this exercise, to which five nucleic acid samples were sent for blind testing.

Statistics

Categoric data were expressed as numbers, and differences between data were analyzed using the chi-square test. Namely, Fisher's exact test was used when comparing the values of two tests to each other. The sensitivities and specificities were calculated and presented with 95% confidence intervals (CIs), as estimated using the Clopper-Pearson exact method.

Results

For analytic validation, the LoD and cross-reactivity were determined. Inactivated SARS-CoV-2 viral particles at five levels of concentrations, ranging from 5-300 viral particles/reaction in triplicate, were used to determine the LoD of the isothermal lab-on-phone COVID-19 assay, using such crude samples directly (i.e., without any RNA extraction or other pretreatment). The lowest target level demonstrating >95% detection rate of SARS-COV-2 was found for 75 viral particles per reaction (i.e., 1.23 viral particles/μl) (see Table 1).

Cross-reactivity assessment for the isothermal lab-on-phone COVID-19 assay was determined using intact bacterial cells and viral particles from respiratory pathogens, which cause infections that present similar symptoms to COVID-19 that have been chemically modified to render them noninfectious (see Table 2).

The results from clinical validation for the isothermal lab-on-phone COVID-19 assay using mouthwash and nasopharyngeal direct crude samples are summarized in Table 3, in comparison to the reference method, rRT-PCR with extracted RNA. The clinical performance of the isothermal lab-on-phone COVID-19 assay was

Table 2

Cross-reactivity analysis with the isothermal lab-on-phone COVID-19 assay for direct crude samples

Pathogen	SARS-CoV-2 (N/E gene) detection rate
Adenovirus 3	0% (0/2)
B. pertussis (A639)	0% (0/2)
C. pneumoniae (CWL-029)	0% (0/2)
Coronavirus 229E	0% (0/2)
Coronavirus HKU-1 (Recombinant)	0% (0/2)
Coronavirus NL63	0% (0/2)
Coronavirus OC43	0% (0/2)
Influenza A 2009 H1N1pdm (A/NY/02/09)	0% (0/2)
Influenza A H1N1 A/New (Caledonia/20/99)	0% (0/2)
Influenza A H3N2 (A/Brisbane/10/07)	0% (0/2)
Influenza B (B/Panama/45/90)	0% (0/2)
M. pneumoniae (M129)	0% (0/2)
Metapneumovirus 8 (Peru6-2003)	0% (0/2)
Parainfluenza virus Type 1	0% (0/2)
Parainfluenza virus Type 2	0% (0/2)
Parainfluenza virus Type 3	0% (0/2)
Parainfluenza virus Type 4	0% (0/2)
Rhinovirus 1A	0% (0/2)
RSV A	0% (0/2)

RSV: respiratory syncytial virus

Table 3

Clinical performance of the isothermal lab-on-phone COVID-19 assay using direct mouthwash or nasopharyngeal crude samples in comparison to reference method rRT-PCR (using extracted RNA), considering a N/E gene Ct <30 or Ct <35.

		Nasopharyngeal samples						Mouthwash samples		
		$Ct < 30$ (N/E gene)			$Ct < 35$ (N/E gene)			$Ct < 35$ (N/E gene)		
		Reference method - rRT-PCR			Reference method - rRT-PCR			Reference method - rRT-PCR		
		POS	NEG	TOTAL	POS	NEG	TOTAL	POS	NEG	TOTAL
Isothermal lab-on-phone –	POS	80	0	**80**	99	0	**99**	15	0	**15**
COVID-19	NEG	2	87	**89**	5	87	**92**	4	5	**9**
TOTAL		**82**	**87**	**169**	**104**	**87**	**191**	**19**	**5**	**24**

Isothermal lab-on-phone – COVID-19 assay performance (CI$_{95\%}$)			
Coincidence rate of positive (Sensitivity)	97.56% (91.47-99.70%)	95.19% (89.14-98.42%)	78.95% (54.43-93.95%)
Coincidence rate of negative (Specificity)	100.00% (95.85-100.00%)	100.00% (95.85-100.00%)	100.00% (47.82-100.00%)
Total coincidence rate (Accuracy)	98.82% (95.79-99.86%)	97.38% (94.00-99.14%)	83.33% (62.62-95.26%)
Theoretical coincidence rate	0.50	0.50	0.57
Kappa coefficient	0.98	0.95	0.61
Positive Predictive Value[a]	100.00%	100.00%	100.00%
Negative Predictive Value[a]	97.75% (91.71-99.42%)	94.57% (88.09-97.61%)	55.56% (34.35-74.91%)
Disease prevalence[a]	48.52% (40.77-56.32%)	54.45% (47.10-61.66%)	83.33% (62.62-95.26%)

CI: confidence interval; Ct: cycle threshold; NEG: negative; POS: positive; rRT- PCR: real-time reverse transcription–PCR

[a] values dependent of disease prevalence

analyzed and calculated, considering a 95% CI and an rRT-PCR N/E gene Ct <30 or Ct <35.

SARS-CoV-2 variants' Beta (B.1.351), Delta (B.1.617.2), and Omicron (B.1.1.529) samples were tested with the isothermal lab-on-phone COVID-19 assay (see results in Figure 2).

Moreover, the evolution of two patients carrying two different variants of SARS-CoV-2 (Delta and Omicron) was followed up by daily diagnosing of both patients using rRT-PCR and the isothermal lab-on-phone COVID-19 assay in parallel for up to 14 days (see Figure 3).

The isothermal lab-on-phone COVID-19 assay was submitted to a blind nucleic acid sample exercise as part of the 2021 EQA exercise, promoted by National Health Institute Doutor Ricardo Jorge (INSA) and results were compared with rRT-PCR as well as the other 76 participants (see Table 4).

Discussion

The pocket-sized isothermal lab-on-phone COVID-19 assay was demonstrated to detect as low as 75 viral particles per reaction (i.e., 1.23 viral particles/μl), which represents an equivalent LoD to gold standard rRT-PCR methods. Moreover, based on cross-reactivity analysis, the assay manages to specifically detect SARS-CoV-2 without suffering from any cross-reaction from other common respiratory pathogens that cause infections that present similar symptoms to COVID-19, such as RSV, influenza, and other related coronaviruses (see Table 2).

The combined clinical study results of three independent sites showed that using direct nasopharyngeal swabs as crude samples, the overall relative sensitivity of the isothermal lab-on-phone COVID-19 assay was 95.19% (95% CI 89.14-98.42%) and the overall

Table 4
Results from EQA exercise promoted by National Health Institute Doutor Ricardo Jorge (INSA) using purified RNA samples

| Sample ID	Expected result	STABVIDA's routine rRT-PCR			Isothermal lab-on-phone – COVID-19 assay		EQA results match rate		
		Result	Ct (cycles)		Result	Time-to-positive (mins)	STABVIDA's routine rRT-PCR	Isothermal lab-on-phone – COVID-19 assay	All participants
			N gene	E gene		N/E gene			
2121	Positive	Positive	32.9	32.8	Positive	36.5	100%	100%	96.7%
2221	Negative	Negative	-	-	Negative	-	100%	100%	95.6%
2321	Positive	Positive	31.7	31.0	Positive	25.5	100%	100%	98.9%
2421	Positive	Positive	35.3	34.6	Positive	31.0	100%	100%	68.1%
2521	Negative	Negative	-	-	Negative	-	100%	100%	94.5%

Ct: cycle threshold; EQA: external quality assessment; rRT-PCR: real-time reverse transcription–PCR

relative specificity was 100% (95% CI 95.85-100.00%), which represents an accuracy of 97.38% (95% CI 94.00-99.14%) compared with rRT-PCR using purified RNA samples. In a total of 191 tested crude nasopharyngeal samples, five failed to be detected for SARS-CoV-2 N and E gene by the isothermal lab-on-phone COVID-19 assay, with the rRT-PCR Ct values for SARS-CoV-2 target N and E genes of the overall tested samples ranging from 26-34. In the case of the samples that led to a false-negative, two had a Ct value (for N/E gene) of 27 and 29, and the remaining were all above 30. Moreover, these two false-negative cases with a Ct value below 30 had in common that the samples were collected in the later stages of the disease (day 8-9 after first symptoms), which could lead to the hypothesis that such crude samples may include higher levels of potentially interfering substances that may develop further on with the disease (e.g., mucus, IgA, etc.) and that could somehow decrease the performance of the isothermal lab-on-phone COVID-19 assay. In addition, considering that for rRT-PCR, which uses purified RNA as samples, these substances would not be presenting an issue, given that they should have been removed during the RNA extraction process. To further understand and validate this hypothesis, further investigations should be carried out in future research. In contrast, when assessing a target limit of Ct <30, considering that higher than this value, it has been found that only less than 3% of clinical samples led to a cultivable virus (Piralla *et al.*, 2021); the performance of the isothermal lab-on-phone COVID-19 assay increased to 97.56% sensitivity and 100% specificity with an accuracy of 98.82% (N = 169). Overall, the performance of the isothermal lab-on-phone COVID-19 assay for direct nasopharyngeal samples can be considered very good, especially given that it detects SARS-CoV-2 in crude samples (i.e., without any pretreatment required). In contrast, in the case of rRT-PCR, the sample of election is extracted viral RNA. Moreover, when directly assessing RNA samples, the isothermal lab-on-phone COVID-19 assay performance in the EQA exercise, which was promoted by National Health Institute Doutor Ricardo Jorge (INSA), showed to be 100% efficient in the diagnosis of the provided blind samples. In particular, the assay even allowed correct detection of the presence of SARS-CoV-2 RNA in a sample with a high Ct value, for which only 68.1% of all EQA participants managed to correctly diagnose (see Sample ID2421 in Table 4).

SARS-CoV-2 variants Beta (B.1.351), Delta (B.1.617.2), and Omicron (B.1.1.529) samples were also successfully detected by the isothermal lab-on-phone COVID-19 assay (Figure 2). In particular, in the case of Delta and Omicron variants, which are currently the most predominant variants, the isothermal lab-on-phone COVID-19 assay proved to be useful as a daily tool for monitoring the evolution of the viral infection until it no longer presents a risk for viral transmission. Overall, the Ct values of the rRT-PCR method seem to correlate with the time-to-positive values of the isothermal lab-on-phone COVID-19 assay, which eventually could allow it to be a quantitative or, at least, a semiquantitative assay. Nonetheless, some deviations, especially at later stages of the disease, were observed. This could be due to the fact that the isothermal lab-on-phone COVID-19 assay uses crude samples that may include inhibitors, which could eventually affect the assay performance and thus affect such correlation. Therefore, it is advisable to consider this assay only as a qualitative assay. Still, the isothermal lab-on-phone COVID-19 assay could help to better manage the quarantine of infected individuals, especially nonsymptomatic individuals, without risking further viral transmission due to the early release of nontested infective individuals (currently, CDC guidelines recommend only 5 days of quarantine, whereas WHO guidelines recommend 10 days [+3 days without symptoms]). Moreover, the isothermal lab-on-phone COVID-19 assay enables PoC testing for COVID-19 without sending samples to a central laboratory, further minimizing contamination risks.
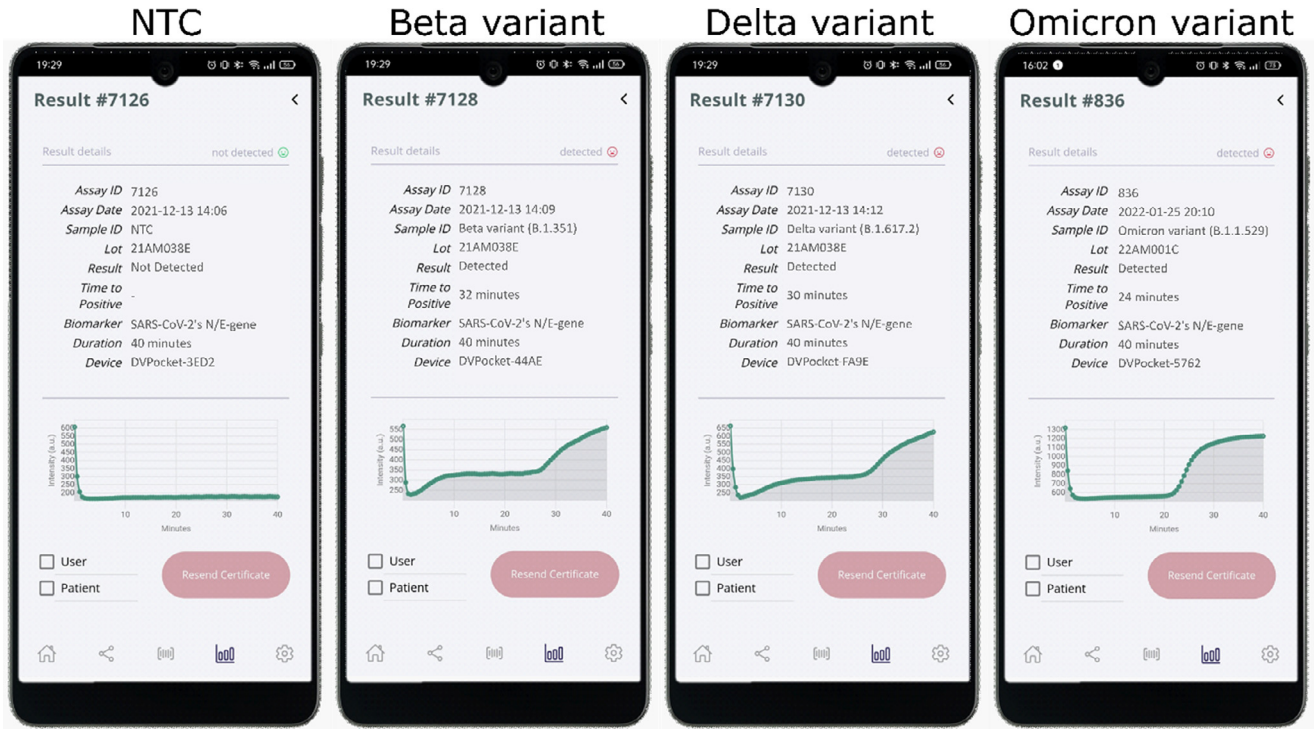
Figure 2. Results of the isothermal lab-on-phone – COVID-19 assay, as assessed through its mobile app, using direct crude samples from Beta (B.1.351), Delta (B.1.617.2) and Omicron (B.1.1.529) variants. NTC: nontemplate control.
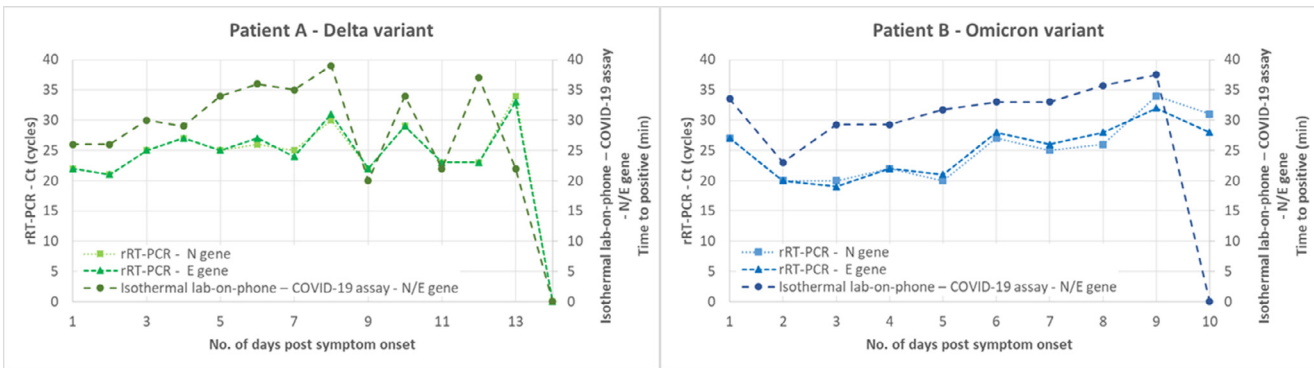
Figure 3. Daily evolution of two different variants of SARS-CoV-2 diagnosed by rRT-PCR (using extracted RNA samples) versus the isothermal lab-on-phone COVID-19 assay (using direct crude nasopharyngeal swab samples) rRT-PCR: real-time reverse transcription–PCR.

Figure 4. COVID-19 testing at point of care (downtown of Lisbon, Portugal) using the pocket-sized isothermal lab-on-phone COVID-19 assay (A), which can display results in real-time through a mobile app for up to four devices simultaneously (B).

Using direct mouthwash samples, which are less invasive than nasopharyngeal swabs, the overall performance of the assay decayed to a relative sensitivity of 78.95% (95% CI 54.43-93.95%); whereas the relative specificity remained at 100% (95% CI 47.82-100.00%). Compared with rapid antigen-based assays, which are frequently used for auto testing of COVID-19, the mouthwash performance still outperformed most rapid antigen tests, which have been demonstrated to have a much lower performance in the real world, especially for samples with a Ct value ≥ 25, in which most rapid antigen tests fail (Kahn *et al.*, 2021; Yamayoshi *et al.*, 2020). Nonetheless, the use of mouthwash samples with the isothermal lab-on-phone COVID-19 assay should be avoided, and the use of nasopharyngeal swab samples should be preferred.

Conclusion

In conclusion, the isothermal lab-on-phone COVID-19 assay proved to be a reliable pocket-sized portable system for a PoC diagnostic test, which can be easily used in the field, over-the-counter, or at-home for COVID-19 diagnosis (Figure 4). The assay provides rapid real-time results to identify patients infected with SARS-CoV-2 viruses, covering all current variants of concern, which can be a very important factor for effective control, proper treatment choice, and the prevention of widespread and local community outbreaks, especially in low-resources environments.

Conflict of interest declaration

G. Doria, C. Clemente, E. Coelho, J. Colaço, R. Crespo, and O. Flores are employed by STAB VIDA Lda, the developer and manufacturer of the Doctor Vida® pocket CE-IVD system described in this manuscript.

Funding

Ethical approval statement

Ethical approvals were assessed and granted by relevant ethics committees of each institute: Garcia de Orta Hospital, Portugal; King Saud Bin Abdulaziz's University for Health Sciences, Saudi Arabia; and STAB VIDA Lda, Portugal. Nasopharyngeal swab and mouthwash samples were collected from patients who volunteered and were visiting the previously mentioned clinical services for SARS-CoV-2 testing, after a signed written informed consent was obtained. All samples were anonymized after being collected.

Acknowledgments

Author contributions

G. Doria idealized and managed the development of Doctor Vida® pocket CE-IVD system, performed experiments, reviewed the data and wrote the manuscript.

C. Clemente supervised the clinical validation and CE-IVD certification of Doctor Vida® pocket system, managed manufacturing, performed experiments and analysed the data.

E. Coelho performed experiments, handled production and clinical validation logistics.

J. Colaço performed experiments, supported clinical validation logistics and analysed data.

R. Crespo characterized samples at STAB VIDA using reference method rRT-PCR.

A. Semikhodskii provided data from clinical validation at KSAU-HS.

H. Mansinho helped with reviewing protocols and approved internally the application of the experimental protocol in the Oncology Service, facilitating the articulation among all the HGO stake-

holders in his team, to guarantee proper execution of the work in the hospital.

M. Dinis helped reviewing HGO protocols.

M.F. Carvalho performed tests in selected patients.

M. Casmarrinha performed tests in selected patients.

C. Samina performed tests in selected patients.

A.C. Vidal evaluated the proposal presented by STABVIDA to present to the President of Garcia de Orta Center, in order to obtain the approval from the Board of HGO during the pandemic crisis in record time. Coordinated the teams from HGO, acting as the contact person between HGO and STABVIDA.

F. Delarue coordinated all the Internal Medicine team of HGO.

S. Graúdo helped with sample collection and testing in the Internal Medicine and COVID-19 areas of HGO.

A.C. Santos helped with sample collection and testing in the Internal Medicine and COVID-19 areas of HGO.

D. Peças helped with sample collection and testing in the Internal Medicine and COVID-19 areas of HGO.

O. Carreira approved internally the application of the experimental protocol in the Clinical Pathology Service, allowing the access to the Gold Standard Test already in use. Also implemented and validated SARS-CoV-2 analysis at Clinical Pathology Service of HGO.

R. Marques implemented and validated SARS-CoV-2 analysis at Clinical Pathology Service of HGO, as well as support STAB VIDA tests.

C. Gaspar helped with sample collection and characterization at HGO, using HGO routine rRT-PCR methods.

O. Flores provided financial support, supervised experiments, analyzed data, reviewed and approved submission of the manuscript for publication.

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