

The One Hour COVID Test: A Rapid Colorimetric Reverse-Transcription LAMP-Based COVID-19 Test Requiring Minimal Equipment

Christopher M. Monaco, Ellen Jorgensen and Sarah Ware

BioBlaze Community Bio Lab, Aanika Biosciences, Inc.

At this writing, over 100 million people have tested positive for Corona Virus Disease-19 (COVID-19), and the global death toll from this disease has reached nearly 3 million. Despite the many tests currently available, we have not yet achieved the testing capacity needed to limit the spread of the virus and mitigate suffering worldwide. We have developed the One Hour COVID Test to address this challenge. Our test leverages an easy-to-use, commercially available oral swab kit for sample collection paired with a novel RNA processing protocol and a simple colorimetric assay that requires minimal equipment. The test can be easily scaled via automation and takes 1 h from sample collection to result.

KEY WORDS: SARS-CoV-2 · point-of-care · NAAT

INTRODUCTION

SARS Coronavirus 2 (SARS-CoV-2) has, to date, infected over 100 million people worldwide and tragically ended the lives of nearly 3 million.¹ Public health experts agree that we must rely on accessible, inexpensive, and frequent testing for the foreseeable future in order to limit the spread of the virus and mitigate suffering worldwide.² Isothermal amplification has gained popularity as an alternative to RT-qPCR for the detection of viral infections because of its relative simplicity.^{3–5} In particular, reverse-transcription loop-mediated isothermal amplification (LAMP) with a pH-based colorimetric readout has become an attractive approach to COVID-19 testing, as the results are easily interpreted with the naked eye without the need for any specialized equipment.⁶ One of the main challenges of this assay technique is the sensitivity of pH indicators, necessitating purification of the viral RNA as part of the upstream sample preparation. Standard RNA purification methods are time-consuming and often represent bottlenecks because RNA purification kits are a vital part of many other nucleic acid-based testing assays. Our One Hour COVID Test detects SARS-CoV-2 by leveraging the combination of a commercially available oral swab RNA collection kit, 2 simple RNA processing reagents, and a pH-based colorimetric LAMP assay (**Fig. 1**). The advantage of this test is that it can be performed in 1

h without lengthy RNA extraction and with only a set of pipettors, a heat source, and an individual with minimal training.

MATERIALS AND METHODS

Saliva samples were collected using the ORAcCollect-RNA swab kit (DNA Genotek, OR-100) according to the manufacturer's instructions. The kit contains a sponge-tipped swab that is immediately placed into a collection tube containing RNA stabilization buffer after use. For the test procedure, 100- μ l aliquots of stabilization buffer were removed from each kit postcollection and transferred to 0.2-ml PCR tubes. To produce contrived samples, 100- μ l aliquots of buffer from swab kit samples collected from an uninfected person were spiked with inactivated SARS-CoV-2 virus particles (American Type Culture Collection NR-52286 SARS-CoV-2, Isolate USA-WA1/2020, Heat Inactivated obtained from BEI Resources). RNA isolation was achieved by adding the 2 reagents comprising the prepIT-Q2A kit (PT-Q2A-96; DNA Genotek) to each sample (10 μ l reagent AG followed by 20 μ l reagent ST) and then mixing by either inversion or pipetting up and down. The tubes were incubated upright at room temperature for 15 min to allow a phase separation to occur. An aliquot of the top phase was carefully collected and diluted 20 \times with molecular biology-grade water (W4502-1L; Millipore-Sigma). In total, 1 μ l of the diluted sample was used as the substrate for the colorimetric LAMP assay. All primers were synthesized by International DNA Technologies Inc. LAMP assays were performed using the N2/E1 primer set as described previously⁷ with a human β -actin internal control.⁸ Briefly, 20- μ l reactions were prepared containing 10 μ l of WarmStart Colorimetric LAMP 2 \times Master Mix

ADDRESS CORRESPONDENCE TO: Sarah Ware, BioBlaze Community Bio Lab, 1800 W. Hawthorne Ln., Suite J-1, West Chicago, IL 60185, USA (Phone: 479-871-7151; E-mail: sarah.ware@bioblaze.org).

This study has been approved via BioBlaze Community Bio Lab, and all anonymous human subjects participating in the study have given the requisite informed consent.

<https://doi.org/10.7171/jbt.21-3203-008>

Overview of the One Hour COVID Test

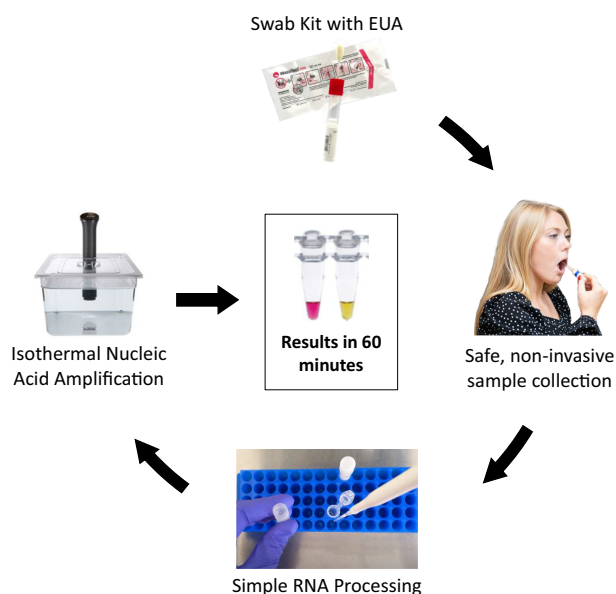


FIGURE 1

One Hour COVID Test overview. Oral swab samples are collected from the patient using a commercially available swab kit that inactivates the virus and stabilizes RNA. The RNA is then concentrated and isolated through a novel processing step that requires minimal equipment. The colorimetric isothermal amplification assay further removes the need for sophisticated equipment while still providing a sensitive and specific test. Results can be read by the naked eye in as little as 20 min, with the entire test taking only 1 h from sample collection to result. EUA, emergency use authorization.

(M1800; New England Biolabs), 2 μ l of 10 \times primer mix consisting of 16 μ M each Forward Inner Primer (FIP) and Backward Inner Primer (BIP), 2 μ M each Forward Primer 3 (F3) and Backward Primer 3, and 8 μ M each Loop Forward Primer (LF) and Loop Backward Primer (LB) along with 40 mM guanidine HCl (G3572; Millipore-Sigma) and molecular biology-grade water. LAMP reactions were incubated at 68°C for 30 min using an Anova Culinary Sous Vide Precision Cooker Nano (available at Amazon.com) immersed in a covered plastic container full of water.

RESULTS

Sensitivity using contrived samples

In experiments with 20 replicates, we can consistently detect 20 out of 20 samples down to a concentration of 50 SARS-CoV-2 genome copies per microliter in the contrived sample (data not shown). Control samples (saliva collected with the DNA Genotek OR-100 swab but not spiked with viral RNA) were consistently negative.

Clinical samples

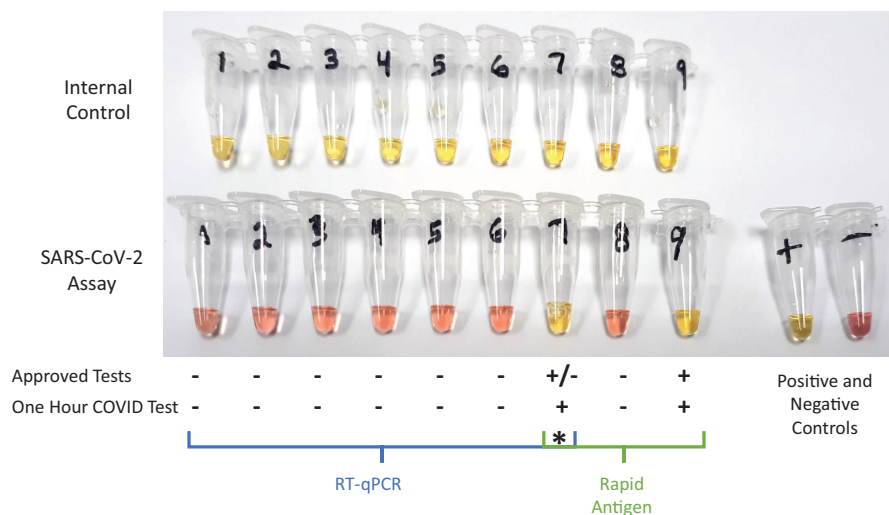
A comparison of results using Food and Drug Administration (FDA)-approved tests and the One Hour COVID Test was made using clinical samples obtained from 9 volunteers. The results in **Fig. 2** show agreement of the One Hour COVID Test with results from both RT-qPCR and rapid antigen tests. Although only one test result is shown here, the One Hour COVID Test showed reproducibility over 4 replicates.

DISCUSSION

We present a promising new testing method for SARS-CoV-2 that has several advantages over many current testing protocols. First, the self-contained collection kit from DNA Genotek minimizes the risk of exposure to potentially infectious material. Samples can be collected at home by the individual being tested without visiting a healthcare provider. The ORAcCollect-RNA swab kit is currently approved for sample collection under FDA emergency use authorization and has been shown to inactivate SARS-CoV-2 while stabilizing RNA for room temperature storage and transport according to the manufacturer.⁹ In addition, the sponge-tipped swabs can be used by individuals who struggle to produce the volume of saliva typically required for other saliva-based tests including those who may have been intubated or are incapacitated.

Second, an equipment-free RNA processing step helps improve the sensitivity of the assay without the need for additional laboratory equipment. We have shown that the prepIT-Q2A reagents, normally used for DNA isolation, can be used to concentrate RNA using a simple, fast protocol that results in a sample with minimal inhibitors or contaminants that might interfere with the LAMP reaction or pH-based readout. Although originally designed to remove inhibitors from samples for PCR, we have not encountered any instances in which samples prepared with the prepIT-Q2A kit have shown issues when run through our LAMP assay. We have adapted the DNA Genotek protocol for compatibility with our colorimetric LAMP assay and, in doing, so have also shown that the prepIT reagent works well for RNA isolation as well as DNA.

We deliberately employed several strategies that had proved successful in the hands of others, including New England Biolabs' colorimetric LAMP platform for the downstream component of the assay. Although we also evaluated other readout methods for LAMP, such as fluorometric, a colorimetric readout was the best choice, as it can easily be read with the naked eye, further eliminating the need for extra equipment such as a fluorometer or transilluminator. During test development, we compared several different primer sets. The primer set used in our test has


FIGURE 2

Comparison of One Hour COVID Test to results of FDA-approved tests. Representative test results from 1 of 4 replicate tests from 9 different individuals. The One Hour COVID Test was performed on clinical samples obtained from volunteers who had received a current FDA-approved test for SARS-CoV-2 within 1 to 2 d of symptom onset. Tubes 1–6 are samples from individuals who tested negative by both RT-qPCR and the One Hour COVID Test. Tubes 8 and 9 are samples from individuals who tested negative and positive, respectively, by both rapid antigen test and One Hour COVID Test. Tube 7, indicated by *, shows an individual who tested negative by rapid antigen test but positive by both RT-qPCR and the One Hour COVID Test. The top row of tubes is the results of our internal control assay showing that a viable sample was obtained from each volunteer. Positive and negative controls are also shown in tubes labeled with a plus and a minus.

been successfully used in other tests,⁷ although we chose to verify specificity against SARS-CoV-1 and influenza A and found no cross-reactivity (data not shown). For all primer sets used, we found that doubling the concentration of the loop primers to 8 μ M each from the 4 μ M each typically used in the LAMP reaction increased the sensitivity and speed of our assay. We also found that incubating the reaction at 68°C increased sensitivity while decreasing the number of false positives (data not shown.) We consistently find that our assay is sensitive down to 50 copies of the viral genome per microliter in contrived samples, and preliminary results suggest that the One Hour COVID Test is more sensitive than some currently available rapid antigen tests.

ACKNOWLEDGMENTS

The authors thank Just One Giant Lab (JOGL) for providing their innovative collaborative research platform, with special thanks to the Nucleic Acid Amplification Group for scientific discussions and suggestions and to Thomas Landrain and other JOGL administrators for their commitment to open science. Financial support for this work was provided by JOGL, the AXA Research Fund, and Aanika Biosciences Inc. The authors declare no conflicts of interest.

REFERENCES

1. Johns Hopkins Coronavirus Resource Center. COVID-19 Map. Available at: <https://coronavirus.jhu.edu/map.html>. Accessed January 26, 2021.

2. Mina MJ, Parker R, Larremore DB. Rethinking Covid-19 test sensitivity - a strategy for containment. *N Engl J Med* 2020;383:e120 <https://doi.org/10.1056/NEJMp2025631>.
3. Poon LL, Leung CS, Chan KH, Lee JH, Yuen KY, Guan Y, Peiris JS. Detection of human influenza A viruses by loop-mediated isothermal amplification. *J Clin Microbiol* 2005;43:427–430 <https://doi.org/10.1128/JCM.43.1.427-430.2005>.
4. Pham HM, Nakajima C, Ohashi K, Onuma M. Loop-mediated isothermal amplification for rapid detection of Newcastle disease virus. *J Clin Microbiol* 2005;43:1646–1650 <https://doi.org/10.1128/JCM.43.4.1646-1650.2005>.
5. Silva SJRD, Paiva MHS, Guedes DRD, Krokovsky L, Melo FL, Silva MALD, Silva AD, Ayres CFJ, Pena LJ. Development and validation of reverse transcription loop-mediated isothermal amplification (RT-LAMP) for rapid detection of ZIKV in mosquito samples from Brazil. *Sci Rep* 2019;9:4494 <https://doi.org/10.1038/s41598-019-40960-5>.
6. Zhang Y, et al. (2020). Rapid molecular detection of SARS-CoV-2 (COVID-19) virus RNA using colorimetric LAMP. *MedRxiv*. 2020.2002.2026.20028373. <https://doi.org/10.1101/2020.02.26.20028373>
7. Zhang Y, et al. (2020). Enhancing colorimetric LAMP amplification speed and sensitivity with guanidine chloride. *BioRxiv*. 2020.2006.2003.132894.
8. Butler D, Mozsary C, Meydan C, et al. Shotgun transcriptome, spatial omics, and isothermal profiling of SARS-CoV-2 infection reveals unique host responses, viral diversification, and drug interactions. *Nat Commun* 2021;12:1660 <https://doi.org/10.1038/s41467-021-21361-7>.
9. DNA Genotek. Inactivation of SARS-CoV-2 in samples collected using Oragene, ORAcollect, OMNIgene products from DNA Genotek. 2020.