

## CORRESPONDENCE

## Detection of SARS-CoV-2 with SHERLOCK One-Pot Testing

**TO THE EDITOR:** CRISPR (clustered regularly interspaced short palindromic repeats)–based diagnostic tests<sup>1,2</sup> collectively provide a nascent platform for the detection of viral and bacterial pathogens. Methods such as SHERLOCK (specific high-sensitivity enzymatic reporter unlocking), which typically use a two-step process (target amplification followed by CRISPR-mediated nucleic acid detection),<sup>1,2</sup> have been used to detect SARS-CoV-2.<sup>3</sup> These approaches, however, are more complex than those used in point-of-care testing because they depend on an RNA extraction step and multiple liquid-handling steps that increase the risk of cross-contamination of samples.

Here, we describe a simple test for detection of SARS-CoV-2. The sensitivity of this test is similar to that of reverse-transcription–quantitative polymerase-chain-reaction (RT-qPCR) assays. STOP (SHERLOCK testing in one pot) is a streamlined assay that combines simplified extraction of viral RNA with isothermal amplification and CRISPR-mediated detection. This test can be performed at a single temperature in less than an hour and with minimal equipment.

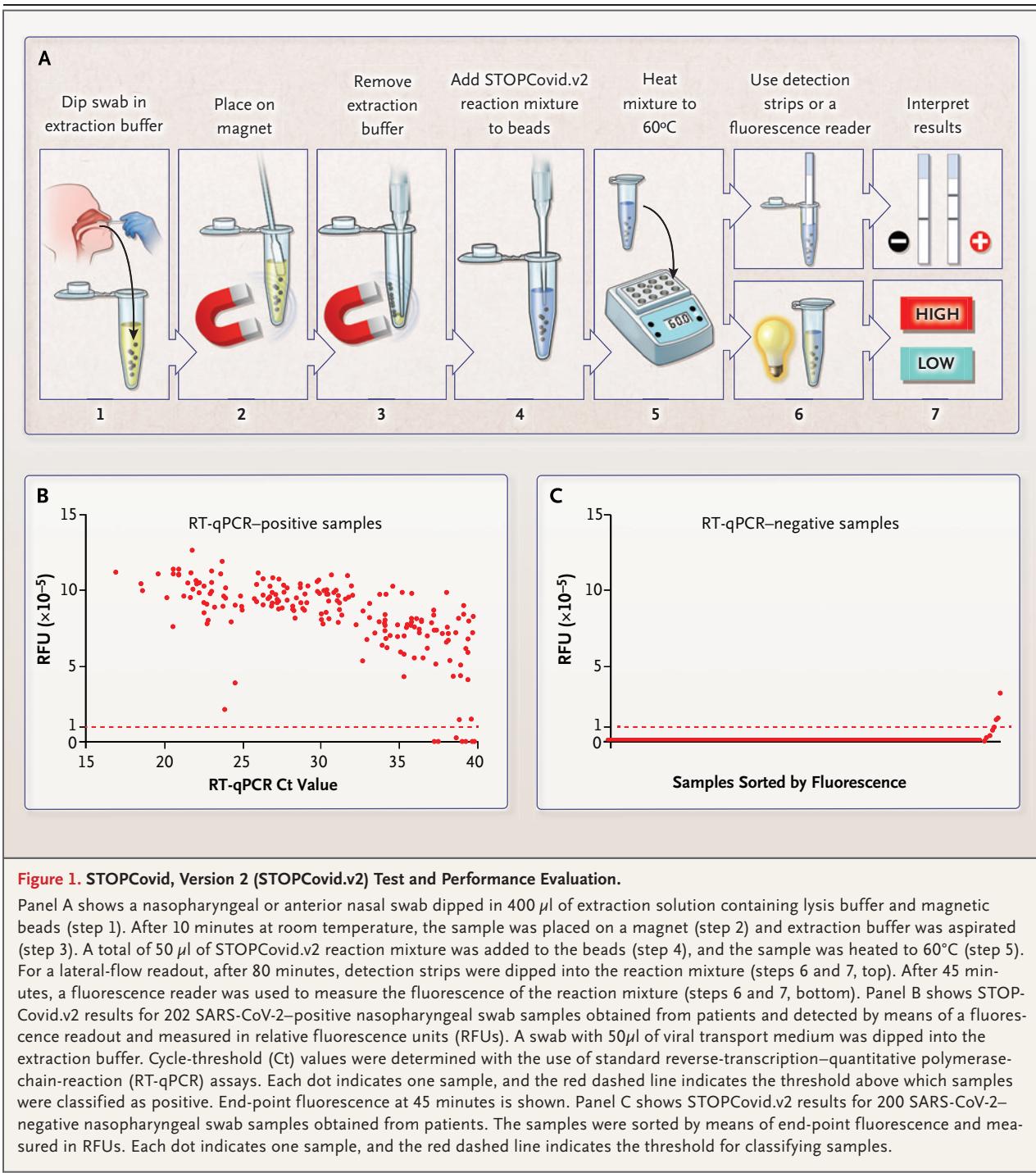
The integration of isothermal amplification with CRISPR-mediated detection required the development of a common reaction buffer that could accommodate both steps. To amplify viral RNA, we chose reverse transcription followed by loop-mediated isothermal amplification (LAMP)<sup>4</sup> because LAMP reagents are widely available and use defined buffers that are amenable to Cas enzymes. LAMP operates at 55 to 70°C and requires a thermostable Cas enzyme such as Cas12b from *Alicyclobacillus acidiphilus* (AapCas12b).<sup>5</sup> We systematically evaluated multiple LAMP primer sets and AapCas12b guide RNAs (a guide RNA helps AapCas12b recognize and cut target DNA) to identify the best combination to target gene *N*, encoding the SARS-CoV-2 nucleocapsid protein, in a one-pot reaction mixture (see Figs. S1 through S3 in the Supplementary Appendix, available with the full text of this letter at NEJM.org). We termed this assay STOPCovid, version 1 (STOPCovid.v1).

As expected, STOPCovid.v1 detection produced a signal only when the target was present, whereas LAMP alone can produce a nonspecific signal (Fig. S3E). STOPCovid.v1 is compatible with lateral-flow and fluorescence readouts and can detect an internal control with the use of a fluorescence readout (Figs. S4 through S6).

To simplify RNA extraction and to boost sensitivity, we adapted a magnetic bead purification method (Fig. S9). The magnetic beads concentrated SARS-CoV-2 RNA genomes from an entire nasopharyngeal or anterior nasal swab into one STOPCovid reaction mixture. We streamlined the test by combining the lysis and magnetic bead-binding steps and eliminating the ethanol wash and elution steps to reduce the duration of sample extraction to 15 minutes with minimal hands-on time. We refer to this streamlined test as STOPCovid, version 2 (STOPCovid.v2) (Fig. 1A).

We compared STOPCovid.v2 with the Centers for Disease Control and Prevention (CDC) standard two-step test (i.e., RNA extraction followed by RT-qPCR) (Fig. S10C). The concentration of substrate by magnetic beads in STOPCovid.v2 allowed detection of viral RNA from the entire swab sample, yielding an input (in terms of quantity of viral RNA) that was 600 times that afforded by the CDC test. As a result, STOPCovid.v2 reliably detected a viral load that was one thirtieth that detected by the CDC RT-qPCR test (100 copies per sample, or 33 copies per milliliter, as compared with 1000 copies per milliliter). Analysis of two independent dilution series from nasopharyngeal swab samples revealed that STOPCovid.v2 had a limit of detection that was similar to an RT-qPCR cycle-threshold (Ct) value of 40.3 (Fig. S10D and S10E).

In blinded testing at an external laboratory at the University of Washington, we tested 202 SARS-CoV-2–positive and 200 SARS-CoV-2–negative nasopharyngeal swab samples obtained from patients. These samples were prepared by adding 50  $\mu$ l of swab specimens obtained from patients with Covid-19 to a clean swab, in accordance with



the recommendation of the Food and Drug Administration for simulating whole swabs for regulatory applications (see the Methods section in the Supplementary Appendix). This testing showed that STOPCovid.v2 had a sensitivity of 93.1% and a specificity of 98.5% (Fig. 1B and 1C, Fig. S11A,

and Table 1). STOPCovid.v2 false negative samples had RT-qPCR Ct values greater than 37. Positive samples were detected in 15 to 45 minutes. Finally, we used fresh, dry, anterior nasal swabs (collected according to the recommendations of the CDC) to validate STOPCovid.v2, and we cor-

**Table 1. Positive and Negative Predictive Values, Sensitivity, and Specificity of STOPCovid.v2 for Detection of SARS-CoV-2 in Nasopharyngeal Samples.\***

STOPCovid.v2 Result	Positive Samples on RT-qPCR (N=202)	Negative Samples on RT-qPCR (N=200)	Total Samples (N=402)	Positive Predictive Value	Negative Predictive Value	Sensitivity	Specificity
	<i>number</i>			<i>number/total number (percent)</i>			
Positive	188	3	191	188/191 (98.4)		188/202 (93.1)	
Negative	14	197	211		197/211 (93.4)		197/200 (98.5)

\* RT-qPCR denotes reverse-transcription–quantitative polymerase chain reaction.

rectly identified 5 positive samples (Ct values, 19 to 36) and 10 negative samples (Fig. S11B through S11E). A detailed protocol for STOPCovid.v2 is provided in the Supplementary Appendix. The simplified format of STOPCovid.V2 is suited for use in low-complexity clinical laboratories.

Julia Joung, B.S.

Alim Ladha, B.S.

Massachusetts Institute of Technology  
Cambridge, MA

Makoto Saito, Ph.D.

Broad Institute of MIT and Harvard  
Cambridge, MA

Nam-Gyun Kim, Ph.D.

University of Washington  
Seattle, WA

Ann E. Woolley, M.D., M.P.H.

Brigham and Women's Hospital  
Boston, MA

Michael Segel, Ph.D.

Broad Institute of MIT and Harvard  
Cambridge, MA

Robert P.J. Barretto, Ph.D.

Kallyope  
New York, NY

Amardeep Ranu, B.S.

DynamiCare Health  
Boston, MA

Rhiannon K. Macrae, Ph.D.

Guilhem Faure, Ph.D.

Broad Institute of MIT and Harvard  
Cambridge, MA

Eleonora I. Ioannidi, B.S.

Rohan N. Krajieski, B.S.

Massachusetts Institute of Technology  
Cambridge, MA

Robert Bruneau, B.S.

Mei-Li W. Huang, Ph.D.

University of Washington  
Seattle, WA

Xu G. Yu, M.D.

Ragon Institute of MGH, MIT, and Harvard  
Cambridge, MA

Jonathan Z. Li, M.D.

Brigham and Women's Hospital  
Boston, MA

Bruce D. Walker, M.D.

Ragon Institute of MGH, MIT, and Harvard  
Cambridge, MA

Deborah T. Hung, M.D., Ph.D.

Broad Institute of MIT and Harvard  
Cambridge, MA

Alexander L. Greninger, M.D., Ph.D.

University of Washington  
Seattle, WA

Keith R. Jerome, M.D., Ph.D.

Fred Hutchinson Cancer Research Center  
Seattle, WA

Jonathan S. Gootenberg, Ph.D.

Omar O. Abudayyeh, Ph.D.

Massachusetts Institute of Technology  
Cambridge, MA  
jgoot@mit.edu  
omarabu@mit.edu

Feng Zhang, Ph.D.

Broad Institute of MIT and Harvard  
Cambridge, MA  
zhang@broadinstitute.org

Ms. Joung and Mr. Ladha and Drs. Gootenberg, Abudayyeh, and Zhang contributed equally to this letter.

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