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

TO THE EDITOR: CRISPR (clustered regularly interspaced short palindromic repeats)–based diagnostic tests[1,2] 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),[1,2] have been used to detect SARS-CoV-2.[3] 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.
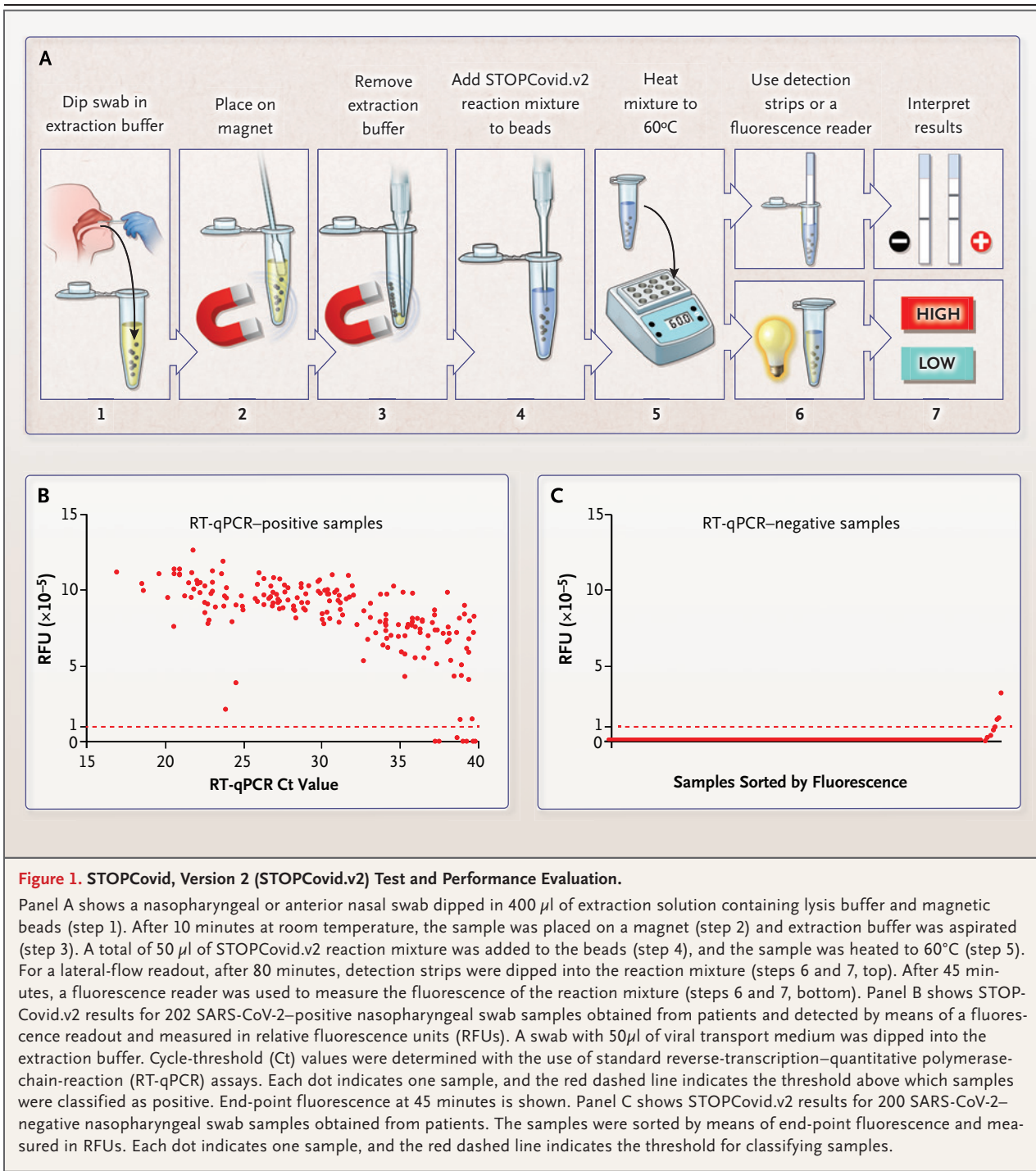
The integration of isothermal amplification with CRISPR-mediated detection required the development of a common reaction buffer that could accommodate both steps. To amplify viral RNA, we chose reverse transcription followed by loop-mediated isothermal amplification (LAMP)[4] because LAMP reagents are 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).[5] 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 μl of swab specimens obtained from patients with Covid-19 to a clean swab, in accordance with

Figure 1. STOPCovid, Version 2 (STOPCovid.v2) Test and Performance Evaluation.

Panel A shows a nasopharyngeal or anterior nasal swab dipped in 400 μl of extraction solution containing lysis buffer and magnetic beads (step 1). After 10 minutes at room temperature, the sample was placed on a magnet (step 2) and extraction buffer was aspirated (step 3). A total of 50 μl of STOPCovid.v2 reaction mixture was added to the beads (step 4), and the sample was heated to 60°C (step 5). For a lateral-flow readout, after 80 minutes, detection strips were dipped into the reaction mixture (steps 6 and 7, top). After 45 minutes, a fluorescence reader was used to measure the fluorescence of the reaction mixture (steps 6 and 7, bottom). Panel B shows STOP-Covid.v2 results for 202 SARS-CoV-2–positive nasopharyngeal swab samples obtained from patients and detected by means of a fluorescence readout and measured in relative fluorescence units (RFUs). A swab with 50μl of viral transport medium was dipped into the extraction buffer. Cycle-threshold (Ct) values were determined with the use of standard reverse-transcription–quantitative polymerase-chain-reaction (RT-qPCR) assays. Each dot indicates one sample, and the red dashed line indicates the threshold above which samples were classified as positive. End-point fluorescence at 45 minutes is shown. Panel C shows STOPCovid.v2 results for 200 SARS-CoV-2–negative nasopharyngeal swab samples obtained from patients. The samples were sorted by means of end-point fluorescence and measured in RFUs. Each dot indicates one sample, and the red dashed line indicates the threshold for classifying samples.

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
	number			*number/total number (percent)*			
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. Krajeski, B.S.

Massachusetts Institute of Technology
Cambridge, MA

Robert Bruneau, B.S.
Meei-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

This letter was published on September 16, 2020, at NEJM.org.

1. Gootenberg JS, Abudayyeh OO, Lee JW, et al. Nucleic acid detection with CRISPR-Cas13a/C2c2. Science 2017;356:438-42.
2. Chen JS, Ma E, Harrington LB, et al. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. Science 2018;360:436-9.
3. Broughton JP, Deng X, Yu G, et al. CRISPR-Cas12-based detection of SARS-CoV-2. Nat Biotechnol 2020;38:870-4.
4. Notomi T, Okayama H, Masubuchi H, et al. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res 2000; 28(12):E63.
5. Teng F, Cui T, Feng G, et al. Repurposing CRISPR-Cas12b for mammalian genome engineering. Cell Discov 2018;4:63.