Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

The Journal of Molecular Diagnostics, Vol. 23, No. 12, December 2021

ELSEVIER

the Journal of Molecular Diagnostics
jmdjournal.org

A Magnetic Modulation Biosensing-Based Molecular Assay for Rapid and Highly Sensitive Clinical Diagnosis of Coronavirus Disease 2019 (COVID-19)

Michael Margulis,* Oran Erster,† Shira Roth,* Michal Mandelboim,† and Amos Danielli*

From the Faculty of Engineering, The Institute of Nanotechnology and Advanced Materials, Bar-Ilan University, Ramat Gan; and the Central Virology Laboratory,† Israel Ministry of Health, Chaim Sheba Medical Centre, Ramat Gan, Israel*

Accepted for publication August 27, 2021.

Address correspondence to Amos Danielli, Ph.D., Faculty of Engineering, Bar Ilan University, Max and Anna Webb St., Ramat Gan, 5290002, Israel; or Michal Mandelboim, Ph.D., Central Virology Laboratory, Israel Ministry of Health, Chaim Sheba Medical Centre, Tel-haShomer, 5262000, Ramat Gan, Israel. E-mail: amos.danielli@biu.ac.il or michal.mandelboim@sheba.health.gov.il.

Rapid and sensitive detection of human pathogens, such as the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), is an urgent and challenging task for clinical laboratories. Currently, the gold standard for SARS-CoV-2–specific RNA is based on quantitative RT-PCR (RT-qPCR), which relies on target amplification by Taq polymerase and uses a fluorescent resonance energy transfer–based hydrolysis probe. Although this method is accurate and specific, it is also time consuming. Here, a new molecular assay is described that combines a highly sensitive magnetic modulation biosensing (MMB) system, rapid thermal cycling, and a modified double-quenched hydrolysis probe. *In vitro* transcribed SARS-CoV-2 RNA targets spiked in PCR-grade water, were used to show that the calculated limit of detection of the MMB-based molecular assay was 1.6 copies per reaction. Testing 309 RNA extracts from 170 confirmed RT-qPCR SARS-CoV-2–negative individuals (30 of whom were positive for other respiratory viruses) and 139 RT-qPCR SARS-CoV-2–positive patients ($C_T \leq 42$) resulted in 97.8% sensitivity, 100% specificity, and 0% cross-reactivity. The total turnaround time of the MMB-based assay is 30 minutes, which is three to four times faster than a standard RT-qPCR. By adjusting the primers and the probe set, the platform can be easily adapted to detect most of the pathogens that are currently being diagnosed by RT-qPCR. *(J Mol Diagn 2021, 23: 1680–1690; https://doi.org/10.1016/j.jmoldx.2021.08.012)*

The outbreak of the coronavirus disease 2019 (COVID-19) emphasized the need for fast, sensitive, and specific diagnostic tools for virus surveillance.[1] Current diagnosis of the acute phase of the COVID-19 is based on direct detection of either viral antigens or viral RNAs in nasopharyngeal, oropharyngeal, or midturbinate swab samples. Antigen-targeting tests are simple to use, have fast turnaround times, and allow rapid testing for point-of-care applications.[2] However, compared with viral RNA-targeting tests, their sensitivity is low, especially during the initial stages of the disease, which limits their adoption and implementation.[3,4]

Direct detection of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) RNA is based on amplification of the specific viral RNA sequences by quantitative RT-PCR (RT-qPCR), which theoretically allows for the detection of as little as a single copy of a target RNA.[5,6] Overall, the RT phase, real-time monitoring, and the high number of amplification cycles (45) are time consuming, and therefore the turnaround time of a standard RT-qPCR system is 90 to 120 minutes, which hinders its use for rapid screening.

Currently, rapid screening can use several devices that are characterized by their small footprint, short sample handling time, fast turnaround time, and simple operational procedures. These devices are based on reverse transcription loop-mediated isothermal amplification,[7,8] isothermal nicking and extension amplification reaction,[9] or conventional RT-PCR amplification (GeneXpert, Cepheid, Sunnyvale, CA; Visby Medical, San Jose, CA; Accula, Mesa Biotech, San Diego,

Supported by the Israel Ministry of Science and Technology grant 3-16908 and the Bar-Ilan Dangoor Centre for Personalized Medicine grant 101790.

M.M. and O.E. contributed equally to this work.

Disclosures: A.D. has a financial interest in MagBiosense, Inc.

https://doi.org/10.1016/j.jmoldx.2021.08.012

CA). Nicking and extension amplification reaction–based assays are fast (eg, 13 minutes) and convenient, but recently, concerns have been raised regarding the performance of these assays, suggesting a high rate of false-negative results, especially in samples with lower viral load.[10,11] The sensitivity of RT-PCR–based assays is on par with that of the gold standard RT-qPCR assays, but the turnaround time is longer (eg, 45 minutes).

In reverse transcription loop-mediated isothermal amplification–, nicking and extension amplification reaction and RT-PCR–based assays, the products of the reaction are visualized by either detection of increased fluorescence or a simple color change.[12,13] To reach a detectable fluorescent signal or a color change and retain high sensitivity, long reaction times are used. To minimize the turnaround time while maintaining high sensitivity and specificity, new optical detection methods must be explored.

Recently, to reduce the background fluorescence in RT-qPCR assays and facilitate the detection of target DNA sequences using one to two fewer amplification cycles, Wilson et al[14] employed a novel hydrolysis probe. Termed a double-quenched ZEN probe,[15] this probe uses two quenchers instead of a single quencher, thereby reducing the distance between the fluorophore and the quencher to only nine bases (Figure 1A). The method presented herein combined a modified double-quenched hydrolysis probe (Figure 1B) with a highly sensitive optical detection system, termed magnetic modulation biosensing (MMB),[16–18] to develop a novel SARS-CoV-2 molecular assay that overcomes the limitations of existing small footprint devices while maintaining the sensitivity and specificity of RT-qPCR–based assays. The basic principles of the MMB-based SARS-CoV-2 molecular assay are presented in Figure 2.

The modified double-quenched hydrolysis probe has a biotin on the same 5′ nucleotide as the fluorescent molecule (Figure 1B).[19] Thus, following separation of the fluorescent molecule from the quenchers by Taq polymerase activity, the fluorescent molecule remains attached to the biotin molecule. On completion of a conventional RT-PCR amplification step, streptavidin-coupled magnetic beads are added to the sample and capture the biotinylated fluorescent molecules (Figure 2). In MMB, to increase the sensitivity of fluorescence detection, an oscillating external magnetic field gradient is applied to the sample, attracting the beads from the entire solution volume and concentrating them in a small detection area (Supplemental Figure S1A). The oscillating magnetic field gradient moves the bead aggregate from side to side, in and out of a laser beam, thereby separating the fluorescent signal from the background noise of unbound fluorescent molecules (Supplemental Figure S1B). The amplitude of the oscillating signal is proportional to the concentration of the biotinylated fluorescent molecules, which, in turn, depends on the initial concentration of the target and the number of amplification cycles (Supplemental Figure S1C). Compared with direct optical detection of the fluorescent molecules, as performed by RT-qPCR, MMB-based detection is approximately 150 times more sensitive.[20]

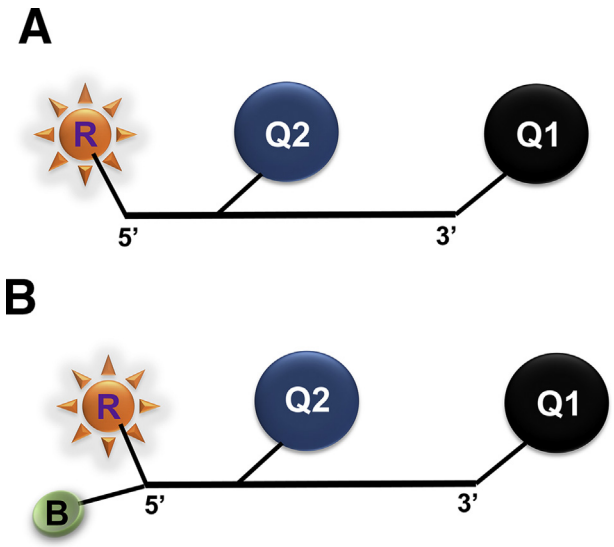

Figure 1 Standard and modified double-quenched hydrolysis probes. **A:** A standard double-quenched hydrolysis probe has a second quencher (Q2) between the fluorescent reporter dye (R), which is located at the 5′, and the first quencher (Q1), which is located at the 3′. **B:** A modified double-quenched hydrolysis probe has two quenchers (Q1 and Q2) and a biotin molecule (B) on the same 5′ nucleotide as the fluorescent reporter dye (R).

Shorter and fewer amplification cycles were used to minimize the total assay time, which may have compromised the efficiency of the PCR, releasing fewer fluorescent molecules to the solution. However, the weaker fluorescence signal was compensated for by the superior optical detection of the MMB system and by the reduced background noise of the modified double-quenched hydrolysis probe. Overall, the total turnaround time of the MMB-based assay was 30 minutes from RNA sample to result, which is approximately three to four times faster than a standard RT-qPCR protocol.

The rapid turnaround time and the use of commercially available reagents, accompanied by the high clinical sensitivity and specificity, facilitate the adoption of the MMB-based assay for rapid SARS-CoV-2 screening. Moreover, by adjusting the primers and the probe set, the assay can be easily adapted to detect a wide variety of pathogens. Although the general principles of the MMB platform have previously been described and implemented for detecting the Ibaraki virus (an agent of epizootic disease),[21] and for rapidly determining chicken sex *in ovo*,[19] this report is the first to describe its implementation for molecular detection of human pathogens.

Materials and Methods

Ethical Statement

All experiments involving collection and testing of biological materials (swab samples) from human subjects were performed according to guidelines and protocols approved

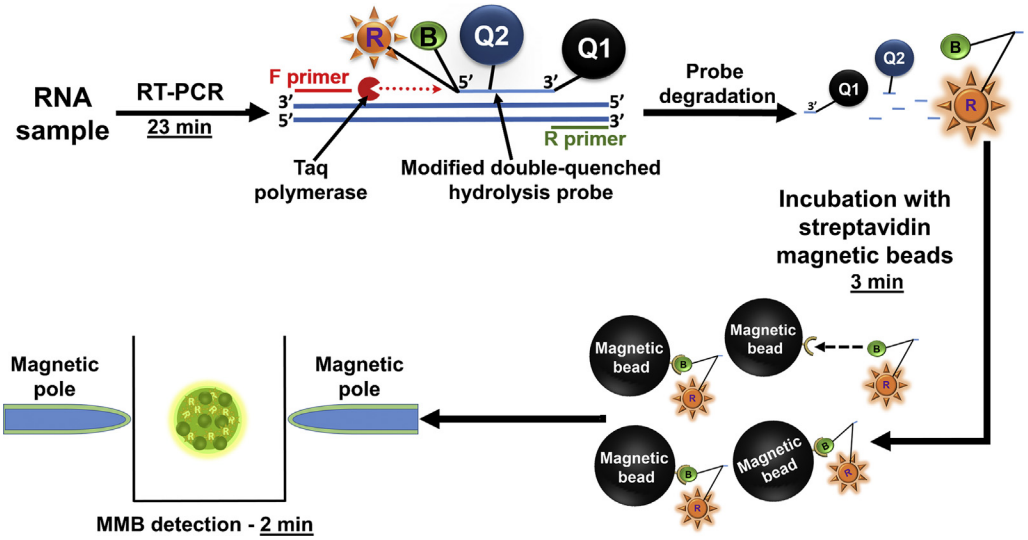

Figure 2 Workflow scheme of the magnetic modulation biosensing (MMB)–based assay. The MMB-based assay includes an RT-PCR process, performed using rapid thermal cycling (eg, 40 amplification cycles, 23 minutes). Following probe degradation, the reaction products are incubated with streptavidin-coupled magnetic beads (3 minutes) that capture the biotinylated fluorescent molecules, and transferred to the MMB system for optical detection (2 minutes). The total turnaround time of an MMB-based assay with 40 amplification cycles is approximately 30 minutes (including handling time). B, biotin molecule; Q1, first quencher; Q2, second quencher; R, reporter dye.

by the institutional review board of Sheba Medical Center in Israel.

Sample Collection and RNA Extraction

Nasopharyngeal swabs from hospitalized COVID-19–positive patients were collected by trained personnel, placed into a standard viral transport medium, and transported at 4°C for RNA extraction and RT-qPCR testing at the Central Virology Laboratory of the Israeli Ministry of Health. The RNA extraction was performed by either the PSS magLEAD instrument (Precision System Science, Chiba, Japan) or the Roche MagNA Pure 96 instrument (Roche Applied Science, Manheim, Germany), in accordance with the specific instrument's protocol and manufacturer's instructions. Immediately following the extraction, the samples were tested for SARS-CoV-2 by RT-qPCR, and the remaining materials were aliquoted and stored at −80°C. One aliquot of each tested sample was delivered to Bar-Ilan University on dry ice and thawed once for the MMB-based testing. The MMB-based molecular assay of the positive samples was performed no later than 3 weeks after extraction.

To ensure unambiguous results, as our SARS-CoV-2–negative samples, including samples positive for other respiratory viruses, RNA extracts from late 2019 (ie, before the coronavirus outbreak in Israel), and stored at −80°C, were used. One aliquot of each sample was delivered to Bar-Ilan University on dry ice and thawed once for the MMB-based testing. The samples were confirmed as negative for SARS-CoV-2 by RT-qPCR before testing them using the MMB-based molecular assay.

Oligonucleotides

Synthetic oligonucleotides (Table 1) were purchased from Integrated DNA Technologies, Inc. (IDT; Leuven, Belgium). Lyophilized oligonucleotides were reconstituted in an IDT formulated buffer (IDTE buffer; IDT, Coralville, IA) to a stock concentration of 100 μmol/L. The resulting solutions were aliquoted and frozen at −20°C until further use. Before use, the stock aliquots were thawed on ice and diluted in DNase/RNase-free molecular-grade water (Biological Industries, Beit haEmek, Israel) to a final working concentration of 10 μmol/L.

The synthetic SARS-CoV-2–specific primers and probes used in this research were introduced and validated by Corman et al[22] and by the US CDC (*https://www.fda.gov/media/134922/download*, last accessed October 14, 2021). Following protocols approved by the World Health Organization and by the US CDC (*https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html*, last accessed October 14, 2021), to ensure the quality of the sampling and extraction procedures, an additional set of oligonucleotides targeting the human *RNase P* gene (Table 1) was used by the Central Virology Laboratory.

The double-quenched (ZEN) hydrolysis probes are a catalog item in the IDT portfolio. They are mass produced and are offered for the same price as the conventional RT-qPCR hydrolysis probes. The cost increase due to the biotin addition at the 5′ of the double-quenched hydrolysis probe is negligible. Moreover, the working and handling protocols of the tests involving the double-quenched hydrolysis probes and MMB-specific modified double-quenched hydrolysis probes are identical to the protocols for regular probe-based

Table 1 Probes and Primers Used for the Development of the Assay

Oligonucleotide	Sequence
*E*_Sarbeco_F1 primer	5′-ACAGGTACGTTAATAGTTAATAGCGT-3′
*E*_Sarbeco_R2 primer	5′-ATATTGCAGCAGTACGCACACA-3′
Zen *E*_Sarbeco_P1 probe*	5′-FAM-ACACTAGCC/**ZEN**/ATCCTTACTGCGCTTCG-BBQ-3′
Modified *E*_Sarbeco _P1 probe†	5′-ATTO532N//iBiodUK-ACACTAGCC/**ZEN**/ATCCTTACTGCGCTTCG/3IABkFQ-3′
CDC 2019-nCoV_Forward primer	5′-GACCCCAAAATCAGCGAAAT-3′
CDC 2019-nCoV_*N1* reverse primer	5′-TCTGGTTACTGCCAGTTGAATCTG-3′
Zen 2019-nCoV_*N1* probe*	5′-FAM-ACCCCGCAT/**ZEN**/TACGTTTGGTGGACC 3IABkFQ-3′
Modified 2019-nCoV_*N1* probe‡	5′-ATTO532N//iBiodUK-ACCCCGCAT/**ZEN**/TACGTTTGGTGGACC -3IABkFQ-3′
RNase P-F primer	5′-AGATTTGGACCTGCGAGCG-3′
RNase P-R primer	5′-GAGCGGCTGTCTCCACAAGT-3′
RNase P-probe	5′-Cy5-TTCTGACCTGAAGGCTCTGCGCG-BHQ2-3′

*A double-quenched hydrolysis probe includes two black quenchers: a Zen quencher (in bold, proprietary to Integrated DNA Technologies, Inc.) and a BBQ at the ninth nucleotide and the 3′ end of the probe, respectively.

†A modified double-quenched *E*-gene targeting hydrolysis probe includes two black quenchers at the ninth nucleotide and the 3′ end of the probe, and a biotin molecule on the same 5′ nucleotide as the fluorophore. To match the optical characteristics of the magnetic modulation biosensing system, the fluorophore was ATTO532. To efficiently quench the ATTO532, the black quencher at the 3′ end of the probe was 3IABkFQ.

‡A modified double-quenched *N1*-gene targeting hydrolysis probe includes two black quenchers at the ninth nucleotide and the 3′ end of the probe, and a biotin molecule on the same 5′ nucleotide as the fluorophore. To match the optical characteristics of the magnetic modulation biosensing system, the fluorophore was ATTO532. To efficiently quench the ATTO532, the black quencher at the 3′ end of the probe was 3IABkFQ.

3IABkFQ, Iowa black quencher; BBQ, BlackBerry quencher.

RT-qPCR tests. Therefore, no direct or indirect technical difficulties were expected in implementing the modified double-quenched probes, and none was encountered.

Synthetic Oligonucleotide Targets

On the basis of the SARS-CoV-2 genome sequence MN908947 (available from *https://www.ncbi.nlm.nih.gov*), synthetic DNA segments were used to generate RNA-positive controls, corresponding to the target region of the Envelope (*E*) gene. The region between positions 26,067 and 26,470 was used for the *E*-gene targeting assay. To transcribe the DNA segments into RNA, PCR with 5′p primer containing the promoter sequence was used to add minimal T7 promoter sequence to the 5′ of each target DNA segment. The amplified DNA products were purified and transcribed *in vitro* using the T7 Megascript kit (catalog number AMB13345; ThermoFisher Scientific, Waltham, MA), according to the manufacturer's instructions. The RNA products were purified and quantified using a Nano-Drop spectrophotometer and a Q-Bit fluorometer (Thermo-Fisher Scientific). They were then aliquoted and stored at −80°C for future use.

Commercially available positive controls were used (2019-nCoV_N_Positive Control; catalog number 10006625; IDT, Leuven, Belgium) for the *N1*-gene targeting assay.

RT-qPCR—Based Assay

RT-qPCR tests were performed at the Central Virology Laboratory using either the Applied Biosystems 7500 RT-PCR (ThermoFisher Scientific) or the Bio-Rad CFX96 (Bio-Rad, Hercules, CA) thermal cyclers. The tests were performed according to validated clinical protocols routinely employed by the Israeli Ministry of Health for the detection of SARS-Cov-2 infection in clinical samples. A reaction mix for the RT-qPCR—based *E*-gene and *N1*-gene assays contained 10 μL of ×2 SensiFast Probe Lo-Rox One-Step mix, 0.4 μL of RiboSafe RNase inhibitor, and 0.2 μL of the reverse transcriptase enzyme, which were provided as parts of the SensiFast Probe Lo-Rox One-Step kit (catalog number BIO-780050; BioLine, London, UK). In addition, the mixture contained 0.8 μL (400 nmol/L) of each primer (forward and reverse), 0.4 μL (200 nmol/L) of the standard double-quenched hydrolysis probe (Figure 1A), 5 μL of the sample (RNA extract), and 2.4 μL of PCR-grade water, for a total reaction volume of 20 μL.

The RT-qPCR mixtures were incubated for 10 minutes 10 seconds at 45°C for reverse transcription, followed by 2 minutes 20 seconds at 95°C for inactivation of the reverse transcriptase enzyme and activation of the Taq polymerase enzyme. Subsequently, 45 cycles of 10 seconds at 95°C, 30 seconds at 58°C, and 30 seconds at 60°C were employed. The fluorescent signal was recorded at the end of each cycle. The C_T was determined on completion of the final cycle. The overall turnaround time of the RT-qPCR assay was approximately 90 minutes.[22]

MMB-Based Molecular Assay

The workflow for the MMB-based molecular assay is shown in Figure 2. The RT-PCRs for the MMB-based detection were performed using a MasterCycler ×50 PCR system (Eppendorf, Hamburg, Germany), which has a substantially

faster temperature ramping rate than a standard RT-qPCR system.[23,24] The reaction mixtures for the MMB-based *E*-gene and *N1*-gene assays contained 10 μL of ×2 One Step PrimeScript III RT-PCR mix (RR600A; TaKaRa, Shiga, Japan), 0.8 μL (400 nmol/L) of each primer (forward and reverse) from the appropriate primer set (*E* or *N1*), 0.4 μL (200 nmol/L) of either *E* or *N1* modified double-quenched hydrolysis probe (Figure 1B), 3 μL of PCR-grade water, and 5 μL of the sample, for a total reaction volume of 20 μL. Two types of samples were tested: clinical RNA samples or *in vitro* transcribed RNA targets diluted in PCR-grade water.

The MMB reaction mixtures were incubated for 5 minutes at 55°C for reverse transcription, followed by 10 seconds at 95°C for inactivation of the reverse transcriptase enzyme and activation of the Taq polymerase enzyme. Amplification was tested using 30, 35, or 40 PCR amplification cycles, and each cycle had three steps: 5 seconds at 95°C, 5 seconds at 58°C, and 5 seconds at 60°C. The total duration of each amplification cycle (including temperature adjustment times) in the MasterCycler ×50 PCR system was 27 seconds.

On completion of the final amplification step, the reaction products (20 μL) were transferred to a 96-well plate preloaded with approximately 25,000 streptavidin-coupled magnetic beads/well in 80 μL of ×1 phosphate-buffered saline buffer (Biological Industries, Beit HaEmek, Israel) with 0.05% (v/v) of Tween 20 (P9416; Sigma-Aldrich, St. Louis, MO). Before their use in the assay, the magnetic beads were photobleached for 18 hours.[25] The total volume of 100 μL in each well was mixed by pipetting, and the plate was incubated under constant shaking (RH-24 3D Gyratory Rocker; MIULAB, Hangzhou, China) for 3 minutes at room temperature. Subsequently, the beads were collected by placing the plate on the MagJET separation rack (Thermo-Fisher Scientific) for 2 minutes. The liquid was discarded, and the beads were resuspended in 100 μL of ×1 phosphate-buffered saline buffer with 0.05% Tween 20 (v/v). Then, the solution was transferred to a borosilicate cuvette (W2540; Vitrocom, Mountain Lakes, NJ) and analyzed using the MMB system. The overall turnaround time of the MMB-based assay using 40 amplification cycles was approximately 30 minutes.

The borosilicate cuvettes used in this research were cleaned after each measurement by incubation in 2 mol/L HCl solution for 30 minutes, followed by replacement of the HCl solution with deionized water and sonication (5 minutes), boiling (5 minutes), and drying in oven at 150°C (1 hour). The cleaning procedure removes any residual fluorescent materials from previous tests and allows reuse of the same cuvette without a measurable increase of background fluorescence.

MMB System Setup

The principles of the MMB system have been described previously.[16–18,21] Schematic representation of the MMB testing process and fully automated image analysis are depicted in Supplemental Figure S1. Briefly, in the MMB system, two electromagnets, one located on each side of the glass sample cell, aggregate the magnetic beads with their attached fluorescent molecules (Supplemental Figure S1A). Alternating magnetic field gradients transport the compact aggregate mass in a periodic lateral motion in and out of the orthogonal laser beam (Supplemental Figure S1B). As the aggregate mass passes in front of the laser beam, the fluorescence emitted from the fluorescent molecules that are attached to the beads generates a flashing signal (Supplemental Figure S1C) that is easily distinguished from the constant background of the sample matrix or from unbound fluorescent molecules. A sequence of 600 images is acquired over a period of 12 seconds, and the mean gray value of the laser beam area in each image (ie, the sum of the gray values of all the pixels in the selected area, divided by the number of pixels) is recorded (Supplemental Figure S1C). The peak-to-peak intensity of the modulated signal is proportional to the number of fluorescent molecules that are released to the solution following the PCR process.

Calibrating the MMB-Based *E*-Gene Assay Using *in Vitro* Transcribed RNA Targets

To determine the number of PCR amplification cycles required by the MMB-based *E*-gene assay to reliably detect 10 copies per sample, *in vitro* transcribed RNA targets diluted in PCR-grade water were used. In the reaction mixture for the MMB-based *E*-gene assay, 5 μL of a clinical RNA sample was replaced with 5 μL of PCR-grade water, containing approximately 10 copies of the *E*-gene synthetic target (ie, approximately 2 copies/μL). Five μL of the PCR-grade water without RNA targets was used as a negative control (Figure 3). The reaction mixtures were subjected to 5 minutes at 55°C, followed by 10 seconds at 95°C, and then 30, 35, or 40 amplification cycles of 5 seconds at 95°C, 5 seconds at 58°C, and 5 seconds at 60°C. Following the amplification, the mixtures were incubated with magnetic beads for 3 minutes and tested using the MMB system. For each experiment, a total of four ($n = 4$) blank reactions (negative control) and seven independent reactions ($n = 7$) with 10 copies of the *in vitro* transcribed RNA targets were tested.

Determining the Analytical Sensitivity of the MMB-Based *E*-Gene Assay

To evaluate the analytical sensitivity of the MMB-based *E*-gene assay, *in vitro* transcribed RNA targets diluted in PCR-grade water to concentrations of 0.3, 0.6, 1.2, 2.4, 5, and 10 copies/μL were used. For a sample volume of 5 μL, these concentrations correspond to 1.5, 3, 6, 12, 25, and 50 copies/reaction. A total of eight reactions ($n = 8$) were tested for each dilution. Eight blank reactions ($n = 8$) containing 5 μL of PCR-grade water were used as a negative

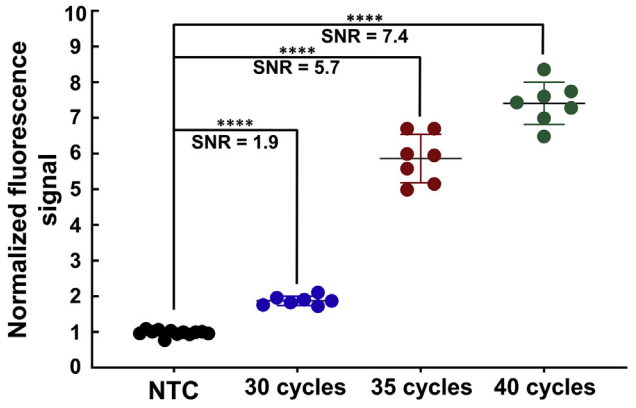

Figure 3 Normalized magnetic modulation biosensing fluorescence signals from RT-PCRs containing either 0 [negative control (NTC)] or 10 copies of a synthetic SARS-CoV-2 RNA *E*-gene target, following 30 (30 cycles), 35 (35 cycles), and 40 (40 cycles) amplification cycles. The ratios between the signal from the samples with 10 copies and the samples with no target are 1.9, 5.7, and 7.4, respectively. Error bars represent the SD. $n = 12$ independent experiments for the blank experiment (NTC); $n = 7$ independent experiments for the spiked samples. ****$P < 0.0001$ (calculated using *t*-test). SNR, signal/noise ratio.

control. The reaction mixtures were subjected to 5 minutes at 55°C, followed by 10 seconds at 95°C, and then 40 amplification cycles of 5 seconds at 95°C, 5 seconds at 58°C, and 5 seconds at 60°C. Following the amplification, the mixtures were incubated with magnetic beads for 3 minutes and tested using the MMB system.

Given a gaussian distribution of blank values, the limit of blank (LoB) was calculated as follows[26]: $LoB = \mu_B + 1.645 \bullet \sigma_B$

where μ_B and σ_B are the mean and SD of the blank measurements, respectively. The limit of detection (LoD) was calculated as follows[26]: $LoB = \mu_B + 1.645 \bullet \sigma_B + 1.645 \bullet \sigma_s$

where σ_s is the SD of the population of the low sample measurements (ie, at a concentration of three target copies/reaction). The results were also analyzed using probit regression analysis (MedCalc version 19.6.1; MedCalc Software Ltd., Ostend, Belgium).

Clinical Sensitivity and Specificity of the MMB-Based *E*-Gene and *N1*-Gene Assays

To determine the clinical performance of the MMB-based *E*-gene assay, a total of 279 RNA extract samples were used that were confirmed positive or negative to SARS-CoV-2 by a standard SARS-CoV-2 RT-qPCR analysis. To determine the receiver operating characteristic cutoff for the MMB-based *E*-gene assay, 30 SARS-CoV-2−negative samples and 30 SARS-CoV-2−positive samples with C_T reference values ranging from 15 to 42 were tested. The receiver operating characteristic was calculated with 95% CI (Wilson/Brown model) using GraphPad Prism version 8.4.2 software (GraphPad Software, Inc., San Diego, CA). The calculated receiver operating characteristic was later applied to the entire data set of 279 samples (including the first batch of 60 samples). The MMB-based *E*-gene clinical assays were performed using 40 amplification cycles. A total of 30 nasopharyngeal swab samples collected in 2019 from patients with different viral respiratory diseases, such as influenza A (13 samples), influenza B (10 samples), and respiratory syncytial virus (7 samples), were tested to further evaluate the specificity of the assay. These samples were confirmed negative for SARS-CoV-2 using RT-qPCR.

To improve diagnostic accuracy, many approved SARS-CoV-2 RT-qPCR assays rely on the detection of two or more SARS-CoV-2 genes. To show that the MMB-based assay can successfully detect other viral genetic targets, an MMB-based *N1*-gene assay was used to test a limited subset of the original 309 samples, composed of 40 SARS-CoV-2−negative samples (30 of which were positive for other respiratory viruses) and 30 SARS-CoV-2−positive samples ($18 \leq C_T \leq 38$).

The clinical sensitivity of the MMB-based assays was calculated as the percentage of SARS-CoV-2−positive patients who were identified as positive by each assay. Specificity was calculated as the percentage of SARS-CoV-2−negative patients who were identified as negative by the assay.

Results

Calibrating the MMB-Based *E*-Gene Assay Using *in Vitro* Transcribed RNA Targets

The calibration experiment depicted in Figure 3 was performed to determine the number of amplification cycles required by the MMB-based *E*-gene assay to reliably detect 10 copies of the *in vitro* transcribed RNA targets. The signal/noise ratios (SNRs) between the experimental samples and the negative controls after 30, 35, and 40 amplification cycles were 1.9, 5.7, and 7.4, respectively. Regardless of the number of amplification cycles, no significant difference in the normalized fluorescence signal was observed for the negative controls (four for each experiment) (Figure 3). In all cases, the *P* values, calculated using *t*-test, were <0.0001.

Determining the Analytical Sensitivity of the MMB-Based *E*-Gene Assay

To evaluate the analytical performance of the MMB-based *E*-gene assay, samples containing increasing numbers of *in vitro* transcribed RNA targets, ranging from 0 to 50 copies/reaction were tested (Figure 4A). Following 40 amplification cycles, the dynamic range of the assay, in which the signal is proportional to the number of copies, was narrow (approximately 1 to 10 copies/reaction). The calculated LoD of the MMB-based *E*-gene assay was 1.6 copies/reaction. Considering that each reaction contained 5 μL of the original sample, this is equivalent to 0.32 copies

per 1 μL of the original sample. Using the probit regression analysis of the dose-response curve and the predetermined receiver operating characteristic cutoff value (Figure 4B), the calculated LoD was 3.3 copies/reaction, which is equivalent to 0.66 copies per 1 μL of the original sample (95% CI, 2.7−5.7).

Clinical Sensitivity and Specificity of the MMB-Based *E*-Gene and *N1*-Gene Assays

The clinical sensitivity and specificity of the MMB-based *E*-gene assay are presented in Figure 5A. The assay successfully detected 136 of 139 SARS-CoV-2−positive samples (97.8% sensitivity). In addition, all 140 SARS-CoV-2−negative samples and all 30 SARS-CoV-2−negative samples that were positive for other respiratory viruses were identified as negative (100% specificity). The correlation between the MMB fluorescence signal and the C_T value is depicted in Figure 5B. In general, the MMB fluorescence signal had an inverse correlation with the C_T values of the corresponding samples. Samples with low C_T (ie, high viral load) yielded a high fluorescence signal, whereas samples with high C_T (ie, low viral load) yielded a low fluorescence signal. The three samples that were misidentified as negative had C_T values of 38, 38, and 40. However, a few other samples with similar or higher C_T values (eg, 38 and 41) were successfully identified as positive. Overall, for samples with $C_T \leq 37$, the sensitivity of the MMB-based *E*-gene assay was 100%.

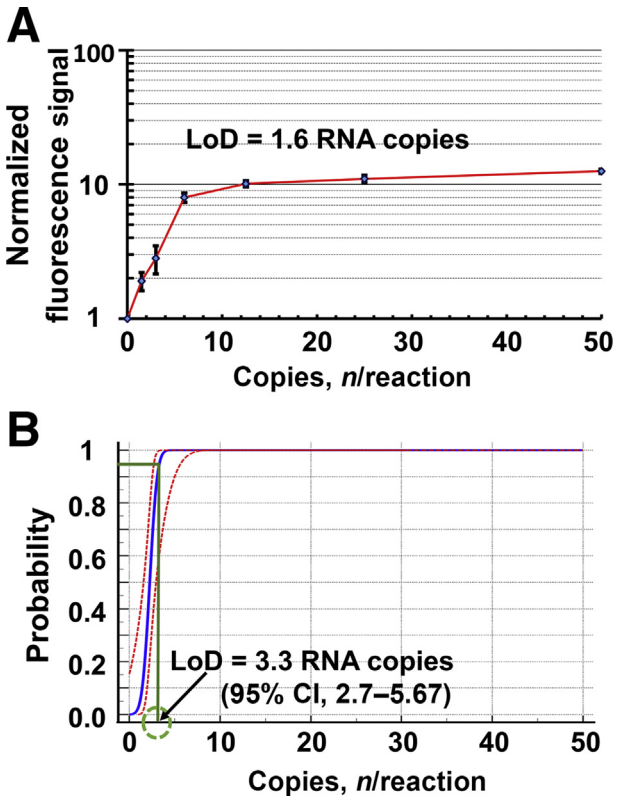

Figure 4 Analytical sensitivity of the magnetic modulation biosensing (MMB)−based *E*-gene assay. **A:** Normalized MMB fluorescence signals from samples containing 0, 1.5, 3, 6, 12, 25, and 50 copies/reaction of *in vitro* transcribed *E*-gene targets in PCR-grade water. All samples were subjected to 40 amplification cycles. The calculated limit of detection (LoD) is 1.6 copies/reaction. **B:** Probit regression analysis of the dose-response experiment. The calculated LoD is 3.3 (95% CI, 2.7−5.7). Error bars represent the SD (**A**). $n = 8$ independent measurements (**A**).

The clinical sensitivity and specificity of the MMB-based *N1*-gene assay are presented in Figure 6A. The assay successfully detected 30 of 30 SARS-CoV-2−positive samples (100% sensitivity). In addition, the assay successfully identified all 40 SARS-CoV-2−negative samples (30 of which were positive for other respiratory viruses) as negative (100% specificity). The correlation between the MMB fluorescence signal and the C_T value is depicted in Figure 6B. In general, the results were similar to the results obtained using the *E*-gene assay: the MMB fluorescence signal had an inverse correlation with the C_T values of the corresponding samples. However, in case of the *N1*-gene MMB-based assay, the signal reduction tendency in samples with high C_T values was much less pronounced than in the *E*-gene assay.

Discussion

The current gold standard for COVID-19 diagnosis is based on direct detection of SARS-CoV-2 by RT-qPCR. This method is well established and highly sensitive, but requires continuous monitoring of the fluorescence signal and maintaining optimal enzymatic conditions. This results in a turnaround time of 90 to 120 minutes,[22] which limits its potential throughput. This study describes an alternative approach that significantly shortens the turnaround time while maintaining the same levels of sensitivity and specificity. The faster turnaround time of the MMB-based assay is achieved by detecting the fluorescence signal at the end of the amplification step (ie, end point detection) rather than monitoring it in real time after each amplification cycle, and by using a much more sensitive optical detection system. The MMB detection system is approximately 150 times more sensitive than the direct optical detection system used in a standard RT-qPCR device.[20] Therefore, fewer and shorter amplification cycles are required to reach a detectable level of fluorescence. These changes, combined with the use of the Eppendorf X50 PCR MasterCycler, which has much higher temperature adjustment rates than a standard RT-qPCR,[23,24] allow for the reduction of the overall assay time to 30 minutes, which is three to four times faster than the current gold standard RT-qPCR.

Following 30 amplification cycles (Figure 3), the MMB-based *E*-gene assay detected as few as 10 copies of *in vitro* transcribed RNA *E*-gene targets, with an SNR of 1.9. Additional five rapid amplification cycles extended the assay time by approximately 2 minutes, but provided a 300% improvement in SNR (5.7 versus 1.9). Further increasing the number of amplification cycles to 40 improved the SNR by only an additional 30% (7.4 versus

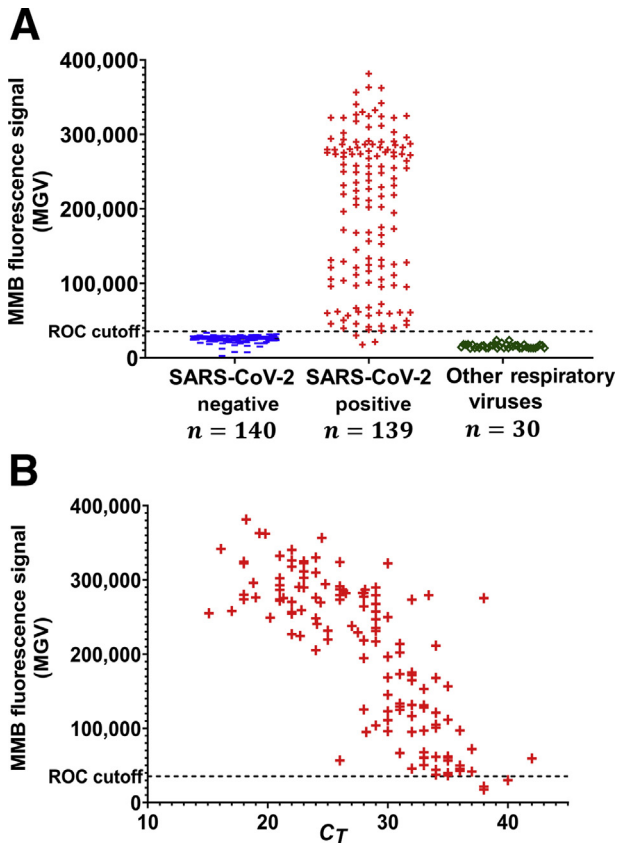

Figure 5 Clinical sensitivity and specificity of the magnetic modulation biosensing (MMB)—based *E*-gene assay following 40 amplification cycles. **A:** MMB fluorescence signals for 309 samples, including 170 SARS-CoV-2—negative samples (30 of which are positive to other respiratory viruses) and 139 RT-qPCR SARS-CoV-2—positive samples. All SARS-CoV-2—negative samples were collected in 2019 (before the outbreak of COVID-19 in Israel). The receiver operating characteristic (ROC) cutoff was calculated with 95% CIs (Wilson/Brown model), using GraphPad Prism software version 8.4.2. All negative samples were also identified as negative (100% specificity) by the MMB-based assay. For samples with $C_T \leq 42$, the clinical sensitivity was 97.8%. For samples with $\mathbf{C_T \leq 37}$, the clinical sensitivity was 100%. **B:** Correlation between MMB fluorescence signal and the $\mathbf{C_T}$ value of each sample. MGV, mean gray value.

5.7), suggesting that the assay had approached or reached its saturation level and that a higher number of amplification cycles would not further improve the SNR. Although the turnaround time of the assay is important, achieving a high SNR is critical, especially for clinical samples that might contain fewer viral genome copies and involve a more challenging sample matrix than the PCR-grade water. Therefore, for analytical and clinical sensitivity analysis, the MMB-based *E*-gene assay was performed using 40 amplification cycles.

In general, the MMB-based assays rely on the same biochemical principle as the probe-based RT-qPCR. For example, in a SARS-CoV-2—positive sample, fluorescent molecules are released into the solution as a result of a fluorescent resonance energy transfer—based probe hydrolysis by Taq polymerase. The total number of free fluorescent molecules at the end of the process is directly proportional to the initial viral load in the sample and to the number of amplification cycles. However, in an MMB-based molecular assay, the number of magnetic beads in the assay and the number of streptavidin-binding sites on each bead are constant. Therefore, once the beads are fully coated with fluorescent molecules, the signal saturates, and a further increase in the number of free fluorescent molecules does not result in an increase in the MMB fluorescent signal (Figure 4A). As a result, the ability to estimate the initial number of copies in the sample is limited to low numbers of copies in the sample.

The LoD—calculated according to the Clinical and Laboratory Standard Institute guidelines[26]—is 1.6 copies/reaction (0.32 copies/μL of the sample), which is on par with the reported LoD of commercially available RT-qPCR—based SARS-CoV-2 detection kits.[27,28] Using probit regression analysis, the LoD was calculated to be 3.3 copies/reaction (95% CI, 2.7—5.67 copies/reaction). The discrepancy between the LoD values can be explained by the inherent differences in the two calculation methods and by the limited number of tested samples.

Overall, the MMB-based *E*-gene assay had 100% specificity and 97.8% clinical sensitivity for samples with $C_T \leq 42$ (Figure 5). For samples with $C_T \leq 37$, the clinical sensitivity was 100%. The MMB-based *E*-gene assay missed three borderline SARS-CoV-2—positive samples ($38 \leq C_T \leq 42$). At a high reference C_T value (eg, $C_T \geq 38$), the viral load in the sample was extremely low, as little as 10 copies/mL. Thus, the number of copies in 5 μL of clinical sample may statistically vary between 0 and 1. In such cases, the test outcome is affected primarily by the statistical probability of having a viral copy in the tested volume, rather than by the actual analytical sensitivity of the detection method.

A correlation study between the MMB signal and the C_T values (Figure 5B) showed that for low C_T values ($15 \leq C_T \leq 30$), the MMB fluorescence signal was high and relatively constant, and for high C_T values ($C_T > 30$), the MMB fluorescence signal diminished gradually. Thus, the MMB-based molecular assay can provide semiquantitative results, such as negative, borderline positive, and positive. The primary purpose of the MMB-based SARS-CoV-2 assay is to rapidly provide an accurate qualitative result (ie, positive/negative) rather than an accurate estimate of the initial viral load.

In this small-scale clinical study, neither the MMB-based *E*-gene nor the MMB-based *N1*-gene SARS-CoV-2 assay exhibited cross-reactivity with other tested respiratory viruses, providing additional evidence of the high specificity of the assays. The primers and the probe combinations used in the article were validated for specificity and sensitivity by their respective developers. In particular, Corman et al[22] validated the *E*-gene assay and the US CDC (*https://www.fda.gov/media/134922/download*, last accessed October 14, 2021) validated the *N1*-gene assay. In both cases, the specificity and lack of the cross-reactivity of the assays

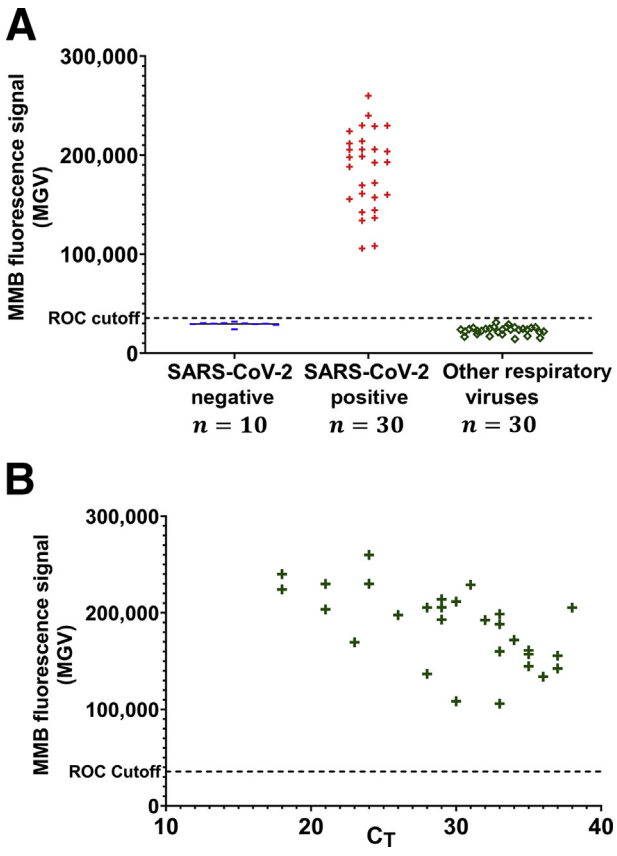

Figure 6 Clinical sensitivity and specificity of the magnetic modulation biosensing (MMB)—based *N1*-gene assay following 40 amplification cycles. **A:** MMB fluorescence signals for 70 samples, including 40 SARS-CoV-2—negative samples (30 of which are positive to other respiratory viruses) and 30 RT-qPCR SARS-CoV-2—positive samples. All SARS-CoV-2—negative samples were collected in 2019 (before the outbreak of COVID-19 in Israel). Using the same receiver operating characteristic (ROC) cutoff that was calculated for the MMB-based *E*-gene assay, all negative samples were also identified as negative (100% specificity) by the *N1*-gene MMB-based assay. For samples with $C_T \leq 38$, the clinical sensitivity was 100%. **B:** Correlation between MMB fluorescence signal and the C_T value of each sample.

were demonstrated on a wide panel of the respiratory pathogens (eg, influenza A, influenza B, respiratory syncytial virus, human adenovirus, and multiple others). Herein, other than attaching a biotin and adding another quencher, no modifications were made to the oligonucleotide sequences of the probes or to the sequences of the respective primers. Hence, no changes in the specificity of the assay are expected.

Increasing the number of amplification cycles to 45 might have increased the MMB fluorescent signal of borderline cases. However, because of possible presence of nonspecific contaminants, a higher number of amplification cycles may also result in a higher probability of false-positive results. Moreover, the SNR of samples with 10 copies of the *in vitro* transcribed RNA target reached its maximum following 40 amplification cycles (Figures 3 and 4). Thus, 45 amplification cycles probably will not contribute to improved clinical sensitivity.

A challenging bottleneck of RT-qPCR—based testing methods is the nucleic acid extraction and purification step, which extends the total assay time. Recently, significant efforts were made to eliminate the need for RNA extraction and purification, making a shift from swab-based sampling to saliva-based sampling. Collecting saliva samples is noninvasive and easy, even when done by the patients themselves. Some saliva-based SARS-CoV-2 extraction-free detection methods (eg, the SalivaDirect protocol by the Yale School of Public Health) have already received emergency use authorization from the US Food and Drug Administration.[29] Combining saliva-based extraction-free sample collection with an MMB-based molecular assay could reduce the total turnaround time from sample collection to result to <40 minutes. Moreover, because of the improved optical sensitivity of the MMB system, it could be beneficially combined with isothermal amplification, further shortening the turnaround time while maintaining high clinical sensitivity and specificity.

Contamination, primarily of the stock reagents and equipment, poses a significant concern for molecular laboratories. Herein, to avoid such contamination while handling the open amplicons during the preparation of the samples for the MMB testing, the pre-PCR and post-PCR steps were performed in separate rooms. Moreover, to actively remove the contaminating amplification products before testing, uracil N-glycosylase—containing RT-qPCR kits can be used. If the assay is used for point-of-care testing, a microfluidic disposable cartridge containing all the reagents needed for the assay can eliminate the need to manually manipulate the open amplicons and can prevent contamination of the reagents and equipment.

Compared with the standard RT-qPCR assay, the MMB-based molecular assay includes three additional steps that currently require manual manipulation. First, beads are added to the reaction products following PCR amplification. Second, the buffer solution is replaced at the end of the incubation with the beads. Third, the final solution is transferred to the cuvette for MMB testing. Combined, the first two steps add about 4 minutes of handling time for a full 96-well plate (<30 seconds if a single sample is tested). The third step requires a few seconds per sample. Although these steps are currently performed manually, the entire MMB-based molecular assay can be automated and incorporated in a microfluidic-based disposable cartridge.

The costs associated with the MMB-based molecular assay are different from those of a standard RT-qPCR assay in two aspects: equipment and reagents. The equipment used for the MMB-based molecular assays comprises a dedicated optical detection system (referred to as the MMB system)[30] and a rapid PCR cycler, whose combined cost is expected to be cheaper than the cost of a standard RT-qPCR system, which ranges between $30,000 and $50,000. The reagents used in the MMB-based molecular assay are similar to the ones used in a standard RT-qPCR assay, excluding the magnetic beads and the modified double-

quenched hydrolysis probe, whose combined cost (approximately $0.2/reaction) is negligible compared with the overall cost of the other RT-qPCR reagents.

Finally, a limiting factor of the current single-channel MMB system is its relatively low throughput (approximately 30 samples/hour). Ongoing modification to the MMB system will enable detection of 96 samples in a 96-well plate (rather than a single cuvette at a time). Combining preprocessing and post-processing robots with a 96-well plate MMB system will eliminate the need for manual manipulation of the samples and enable high-throughput sample testing.

Conclusion

This study presents a rapid molecular assay to detect SARS-CoV-2−specific RNA sequences on the basis of the established MMB technology. The MMB-based *E*-gene molecular assay demonstrated an LoD of 1.6 copies/reaction. In tests using 309 clinical samples from SARS-CoV-2−positive and SARS-CoV-2−negative patients with a wide range of initial viral loads ($C_T \leq 42$), the clinical sensitivity and specificity were 97.8% and 100%, respectively. In addition, the signal of the MMB-based molecular assays was correlated to the initial viral load, and the total turnaround time (30 minutes) was much shorter than the average RT-qPCR test (90 to 120 minutes).

The proposed SARS-CoV-2 MMB-based assay can be adjusted to detect a wide variety of pathogens, viral or others. Implementation of the current assay with a high-throughput detection device will enable rapid screening of large groups of people in the community and improve the management of current SARS-CoV-2 pandemic.

Acknowledgments

We thank Dr. Meir Cohen for technical assistance, Dr. Paul Olivo for critical review of the manuscript, James Ballard for providing an editorial review of the manuscript, and Eppendorf AG and Lumitron Inc. for providing the Mastercycler ×50 PCR system.

Supplemental Data

Supplemental material for this article can be found at *http://doi.org/10.1016/j.jmoldx.2021.08.012*.

References

1. Choi S, Ki M: Estimating the reproductive number and the outbreak size of COVID-19 in Korea. Epidemiol Health 2020, 42:e2020011
2. CDC: Interim Guidance for Rapid Antigen Testing for SARS-CoV-2. Atlanta, GA: CDC, 2020
3. Prendergast C, Papenburg J: Rapid antigen-based testing for respiratory syncytial virus: moving diagnostics from bench to bedside? Future Microbiol 2013, 8:435−444
4. Scohy A, Anantharajah A, Bodeus M, Kabamba-Mukadi B, Verroken A, Rodriguez-Villalobos H: Low performance of rapid antigen detection test as frontline testing for COVID-19 diagnosis. J Clin Virol 2020, 129:104455
5. Kubista M, Andrade JM, Bengtsson M, Forootan A, Jonak J, Lind K, Sindelka R, Sjoback R, Sjogreen B, Strombom L, Stahlberg A, Zoric N: The real-time polymerase chain reaction. Mol Aspects Med 2006, 27:95−125
6. Johnson G, Nolan T, Bustin SA: Real-time quantitative PCR, pathogen detection and MIQE. Methods Mol Biol 2013, 943:1−16
7. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T: Loop-mediated isothermal amplification of DNA. Nucleic Acids Res 2000, 28:E63
8. Mori Y, Notomi T: Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases. J Infect Chemother 2009, 15:62−69
9. Nie SP, Roth RB, Stiles J, Mikhlina A, Lu XD, Tang YW, Babady NE: Evaluation of alere i influenza A&B for rapid detection of influenza viruses A and B. J Clin Microbiol 2014, 52:3339−3344
10. Basu A, Zinger T, Inglima K, Woo KM, Atie O, Yurasits L, See B, Aguero-Rosenfeld ME: Performance of Abbott ID Now COVID-19 Rapid Nucleic Acid Amplification Test using nasopharyngeal swabs transported in viral transport media and dry nasal swabs in a New York City academic institution. J Clin Microbiol 2020, 58:e01136-20
11. Smithgall MC, Scherberkova I, Whittier S, Green DA: Comparison of Cepheid Xpert Xpress and Abbott ID Now to Roche cobas for the rapid detection of SARS-CoV-2. J Clin Virol 2020, 128:104428
12. Yu L, Wu SS, Hao XW, Dong X, Mao LL, Pelechano V, Chen WH, Yin XS: Rapid detection of COVID-19 coronavirus using a reverse transcriptional loop-mediated isothermal amplification (RT-LAMP) diagnostic platform. Clin Chem 2020, 66:975−977
13. Dao Thi VL, Herbst K, Boerner K, Meurer M, Kremer LP, Kirrmaier D, Freistaedter A, Papagiannidis D, Galmozzi C, Stanifer ML, Boulant S, Klein S, Chlanda P, Khalid D, Barreto Miranda I, Schnitzler P, Krausslich HG, Knop M, Anders S: A colorimetric RT-LAMP assay and LAMP-sequencing for detecting SARS-CoV-2 RNA in clinical samples. Sci Transl Med 2020, 12:eabc7075
14. Wilson PM, Labonte MJ, Russell J, Louie S, Ghobrial AA, Ladner RD: A novel fluorescence-based assay for the rapid detection and quantification of cellular deoxyribonucleoside triphosphates. Nucleic Acids Res 2011, 39:e112
15. Pilotte N, Papaiakovou M, Grant JR, Bierwert LA, Llewellyn S, McCarthy JS, Williams SA: Improved PCR-based detection of soil transmitted helminth infections using a next-generation sequencing approach to assay design. PLoS Negl Trop Dis 2016, 10:e0004578
16. Danielli A, Arie A, Porat N, Ehrlich M: Detection of fluorescent-labeled probes at subpicomolar concentrations by magnetic modulation. OPT Express 2008, 16:19253−19259
17. Danielli A, Porat N, Ehrlich M, Arie A: Magnetic modulation biosensing for rapid and homogeneous detection of biological targets at low concentrations. Curr Pharm Biotechnol 2010, 11:128−137
18. Michelson Y, Lustig Y, Avivi S, Schwartz E, Danielli A: Highly sensitive and specific Zika virus serological assays using a magnetic modulation biosensing system. J Infect Dis 2019, 219:1035−1043
19. Margulis M, Danielli A: Rapid and sensitive detection of repetitive nucleic acid sequences using magnetically modulated biosensors. ACS Omega 2019, 4:11749−11755
20. Margulis M, Ashri S, Cohen M, Danielli A: Detecting nucleic acid fragments in serum using a magnetically modulated sandwich assay. J Biophotonics 2019, 12:e201900104
21. Danielli A, Porat N, Arie A, Ehrlich M: Rapid homogenous detection of the Ibaraki virus NS3 cDNA at picomolar concentrations by magnetic modulation. Biosens Bioelectron 2009, 25:858−863
22. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, Bleicker T, Brunink S, Schneider J, Schmidt ML, Mulders DG, Haagmans BL, van der Veer B, van den Brink S, Wijsman L, Goderski G, Romette JL, Ellis J, Zambon M, Peiris M, Goossens H,

Reusken C, Koopmans MP, Drosten C: Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 2020, 25:2000045
23. Bio-Rad: CFX96 TouchTM Real-Time PCR Detection System Specifications. Hercules, CA: Bio-Rad Laboratories, Inc., 2020
24. Eppendorf: Eppendorf Mastercycler ×50 Specifications. Hamburg, Germany: Eppendorf AG, 2020
25. Roth S, Hadass O, Cohen M, Verbarg J, Wilsey J, Danielli A: Improving the sensitivity of fluorescence-based immunoassays by photobleaching the autofluorescence of magnetic beads. Small 2019, 15:1803751
26. Tholen D, Linnet K, Kondratovich M, Armbruster D, Garrett P, Jones R, Kroll MH, Lequin R, Pankratz T, Scassellati GA, Schimmel H, Tsai J: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guidelines. Wayne, PA: Clinical Laboratory Standards Institute (NCCLS), 2004
27. BGI: InstructionsFor Use: Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV. Shenzhen, China: BGI Inc., 2020
28. SeeGene: Allplex 2019-nCov Assay. Seoul, South Korea: Seegene Inc., 2020
29. Vogels CBF, Watkins AE, Harden CA, Brackney D, Shafer J, Wang J, Caraballo C, Kalinich CC, Ott I, Fauver JR, Kudo E, Lu P, Venkataraman A, Tokuyama M, Moore AJ, Muenker MC, Casanovas-Massana A, Fournier J, Bermejo S, Campbell M, Datta R, Nelson A, Dela Cruz C, Ko A, Iwasaki A, Krumholz HM, Matheus J, Hui P, Liu C, Farhadian S, Sikka R, Wyllie AL, Grubaugh N: SalivaDirect: a simplified and flexible platform to enhance SARS-CoV-2 testing capacity. Med (N Y) 2021, 2:263−280.e6
30. Roth S, Zander I, Michelson Y, Ben-David Y, Banin E, Danielli A: Identification of protein-protein interactions using a magnetic modulation biosensing system. Sensor Actuat B-Chem 2020, 303: 127228