



Portable RT-PCR System: a Rapid and Scalable Diagnostic Tool for COVID-19 Testing

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ABSTRACT Combating the ongoing coronavirus disease 2019 (COVID-19) pandemic demands accurate, rapid, and point-of-care testing with fast results to triage cases for isolation and treatment. The current testing relies on reverse transcriptase PCR (RT-PCR), which is routinely performed in well-equipped laboratories by trained professionals at specific locations. However, during busy periods, high numbers of samples queued for testing can delay the test results, impacting efforts to reduce the infection risk. Besides, the absence of well-established laboratories at remote sites and low-resourced environments can contribute to a silent spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). These reasons compel the need to accommodate point-of-care testing for COVID-19 that meets the ASSURED criteria (affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable). This study assessed the agreement and accuracy of the portable Biomeme SARS-CoV-2 system against the gold standard tests. Nasopharyngeal and nasal swabs were used. Of the 192 samples tested using the Biomeme SARS-CoV-2 system, the results from 189 samples (98.4%) were in agreement with the reference standard-of-care RT-PCR testing for SARS-CoV-2. The portable system generated simultaneous results for nine samples in 80 min with high positive and negative percent agreements of 99.0% and 97.8%, respectively. We performed separate testing in a sealed glove box, offering complete biosafety containment. Thus, the Biomeme SARS-CoV-2 system can help decentralize COVID-19 testing and offer rapid test results for patients in remote and low-resourced settings.

KEYWORDS Biomeme, COVID-19, portable, RT-PCR, point-of-care

The current pandemic, coronavirus disease-19 (COVID-19)—caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)—is rapidly spreading and challenging human lives and health care facilities worldwide. Despite the vaccine rollout, rapid testing, isolating, and treating infected patients remain very important measures to mitigate the spread of SARS-CoV-2. Hence, “test, trace, and

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isolate" is the cornerstone of breaking the transmission chain and controlling the outbreak. Rapid mass testing at a global level is critical, as stated by the Director-General of the World Health Organization (WHO), "You cannot fight a fire blindfolded, and we cannot stop this pandemic if we do not know who is infected. We have a simple message for all countries: test, test, test. Test every suspected case" (1).

However, only well-established laboratories with sophisticated infrastructure, standard biosafety, and trained professionals can currently perform the RNA extraction and molecular testing with a high throughput or at point of care for COVID-19, and the number of positive cases is greatly influenced by the availability of these tests (2, 3). Lack of infrastructure in remote or low-resourced localities and war or conflict zones impacts the overall positive cases and also increases the burden on major central laboratories testing for COVID-19, leading to delayed test results.

SARS-CoV-2 has a high infection rate and is easily transmitted among individuals, with a long incubation period. In addition, patients infected with SARS-CoV-2 can transmit the virus 2 days prior to the development of symptoms, and the virus is transmissible among many asymptomatic patients. So, people can unknowingly and rapidly transmit the infection among communities (3, 4). Rapid testing and isolating infected cases, including asymptomatic or paucisymptomatic ones, is one of the most effective strategies to control the global spread of COVID-19 (5, 6). However, the current testing structure has a lengthy turnaround time that can reach up to 48 to 72 h (7). This delay can increase viral spread by infected individuals and, more specifically, asymptomatic individuals in communities. It can also increase viral exposure to health care workers and other patients in a hospital setting (8). Results with a short turnaround time can help to immediately allocate resources that might be limited, such as health care resources, including isolation rooms and personal protective equipment (PPE) (9). Thus, an affordable testing system for COVID-19 that is rapid, portable, and easily accessible in remote and low-resourced environments can significantly mitigate the spread of the virus in many communities.

To tackle the current crisis, portable point-of-care molecular, antigen, and antibody tests are becoming available to diagnose COVID-19. For example, the Accula SARS-CoV-2 POC by Mesa Biotech uses reverse transcriptase PCR (RT-PCR) to target the nucleocapsid protein (*N*) gene and is read out via lateral flow. The performance of the Accula test was compared to that of a standard RT-PCR and demonstrated a positive percent agreement (PPA) of 68.0%, missing 16 of 50 positive samples (10). The BD Veritor system that provides a rapid detection of SARS-CoV-2 nucleocapsid antigen was compared to a RT-PCR and demonstrated PPA that ranges between 81.8% and 87.5% (11). The Panbio COVID-19 rapid antigen test by Abbott demonstrated a lower sensitivity (75.5%) than a rapid PCR assay (12). Rapid antibody tests are less specific and sensitive than the standard RT-PCR, and their test results depend on the individual's immune response to SARS-CoV-2, which can take several weeks to develop (13). Other affordable diagnostic solutions include the molecular loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA); however, limited work is described for SARS-CoV-2 testing (14), except for the ID NOW system by Abbott, where studies showed sensitivity and specificity lower than standard RT-PCR assays for testing SARS-CoV-2 (15). Given these limitations, RT-PCR, with its sophisticated benchtop thermocycler-related infrastructure, remains the gold standard for COVID-19 testing.

Our study evaluates, for the first time, a rapid, deployable, and portable RT-PCR system to diagnose COVID-19, which generates results within 80 min. This system uses a lyophilized master mix that can be transported and stored at room temperature alongside a rapid RNA extraction kit and a portable biosafety containment. This testing method meets the ASSURED criteria (16), which could revolutionize future diagnostics of infectious diseases at point-of-care testing.

MATERIALS AND METHODS

Sample collection. The study was performed at the King Faisal Specialist Hospital and Research Centre (KFSHRC), Riyadh, Saudi Arabia, and was approved by the research ethics committee (reference

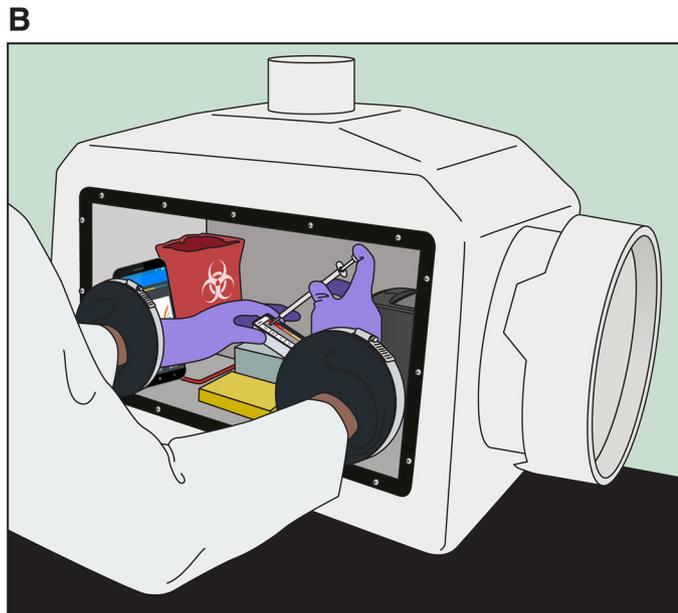
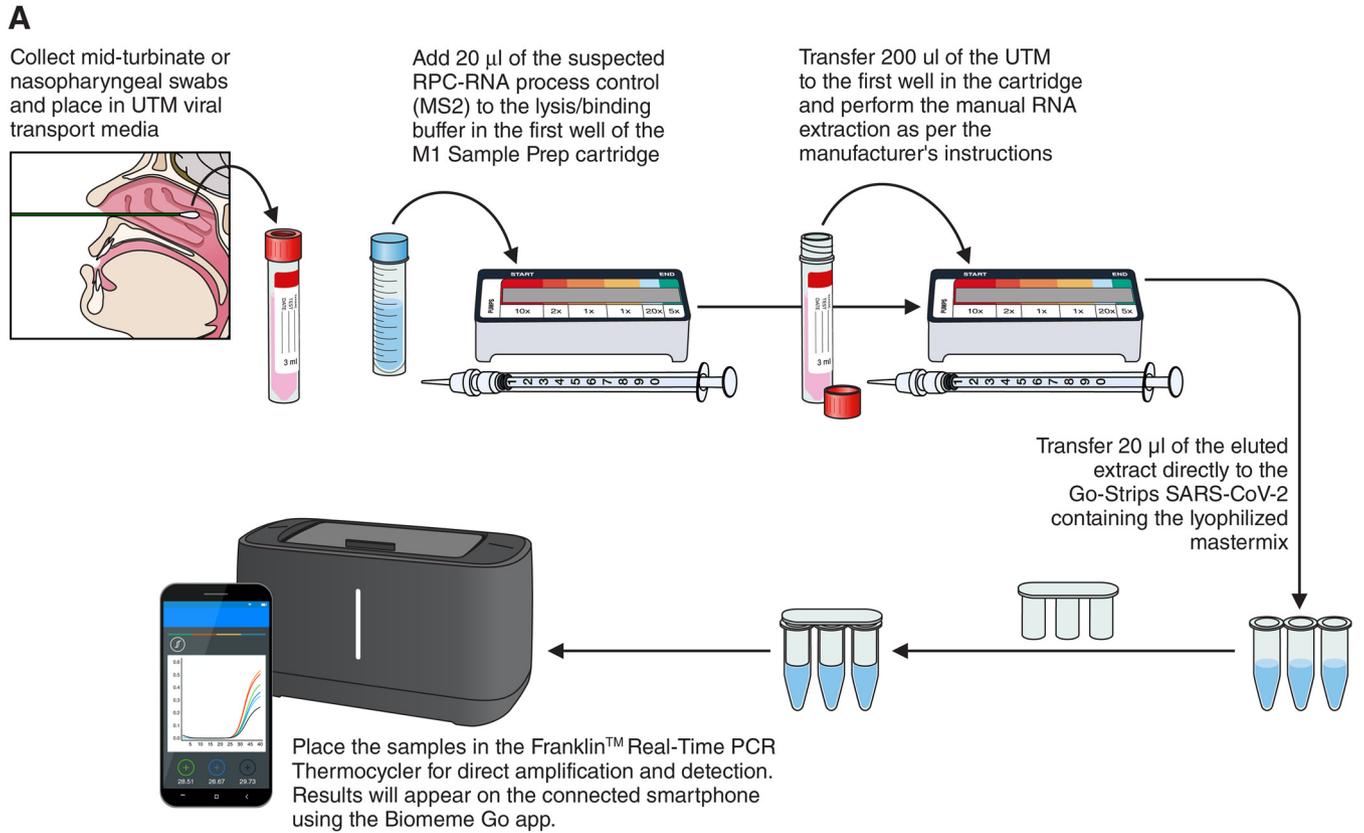


FIG 1 Workflow for the deployable Biomeme SARS-CoV-2 system (A) and the performance of the complete workflow inside the portable glove box to achieve an optimum biosafety containment (B).

C380/443/42). The portable RT-PCR system from Biomeme was assessed for its positive and negative percent agreements on a total of 192 samples to detect SARS-CoV-2 from nasopharyngeal and nasal swabs (mid-turbinate) using flocked swabs paired with 2 ml UTM virus transport medium (Mantacc, Miraclean Technology Co., Ltd.) (Fig. 1A). The nasopharyngeal samples ($n = 173$) were collected in viral transport medium and tested for SARS-CoV-2 using the standard-of-care RT-PCR diagnostic tools. The nasopharyngeal samples in viral transport medium were stored at -70°C before testing. Of the 173 nasopharyngeal samples in viral transport medium that were assessed using the portable RT-PCR system, 92 samples were positive for SARS-CoV-2, while the remaining 81 were negative. The results from the

Biomeme's portable RT-PCR system were compared with the routine standard-of-care RT-PCR for COVID-19 diagnosis.

The fresh nasal swabs (mid-turbinate) from one nostril were collected from 5 patients from the COVID-19 ward and 14 patients from the emergency ward at KFSHRC. All the samples were tested immediately for SARS-CoV-2 using the portable RT-PCR system at the point of care. Nasopharyngeal swabs were also collected from the same patients and transported on the same day in viral transport medium to the molecular virology laboratory for COVID-19 testing using the routine standard-of-care RT-PCR.

To achieve maximum biosafety while handling COVID-19 samples, two separate runs were tested inside a sealed portable glove box, which acted like a small biosafety hood (Fig. 1B). The inner surface of the portable glove box was decontaminated with 70% ethanol before and after the tests.

Biomeme SARS-CoV-2 system. The portable, ultracompact, and user-friendly system from Biomeme Inc., (PA, USA) to identify SARS-CoV-2-positive patients was granted emergency use authorization (EUA) by the Food and Drug Administration (FDA) on the 11th of August 2020. The system consists of four main components. The first component is the manual and equipment-free M1 sample prep cartridges for rapid nucleic acid extraction (Fig. 1A). RNA was extracted in 1 to 2 min/sample with 750 μ l of eluted extract. Biomeme RNA process/internal control (RPC) was introduced into each M1 sample prep cartridge at the beginning of the sample preparation process to demonstrate that the entire process was completed correctly for each specimen and control.

The second component includes Biomeme SARS-CoV-2 Go-Strips (Fig. 1A). Each Go-Strip consists of three connected PCR tubes containing lyophilized master mix, multiplexed primers, and probes for the simultaneous qualitative detection of SARS-CoV-2-specific 1ab open reading frame gene (*Orf1ab*) and SARS-CoV-2-specific spike gene (*S*). The tubes also include the RPC (MS2) in a triplex reaction mixture. The RT-PCR was prepared according to the manufacturer's instructions by adding 20 μ l of the eluted extract directly to the lyophilized master mix. The M1 sample prep cartridge and lyophilized Go-Strip pouches can be stored in a dry place, at room temperature (15 to 30°C), and they can remain stable for up to 18 months if unopened.

The third component is the Franklin three9 real-time PCR thermocycler, which runs on a chargeable battery for up to five PCR runs (Fig. 1A). A unit can detect three different fluorophores per sample simultaneously but is limited to nine samples per run. The SARS-CoV-2 *Orf1ab* gene was detected by the 6-carboxyfluorescein (FAM) fluorophore in the green channel, while the ATTO647N fluorophore detected the SARS-CoV-2 *S* gene in the red channel, and the TexasRedX fluorophore detected the RPC in the amber channel. Reactions were amplified with an initial reverse transcriptase (RT) step at 55°C for a 2-min hold, followed by an initial denaturation step at 95°C for a 1-min hold and 45 cycles at 95°C for 3 s and 60°C for 30 s.

Results were considered positive when one or both SARS-CoV-2-specific *Orf1ab* or *S* genes were detected in the same sample, regardless of the amplification or failure of the RPC. However, any negative results due to the absence of both SARS-CoV-2-specific genes would require positive amplification of the RPC to rule out false-negative results that might occur due to inactive reaction or PCR inhibitors in the sample. Thus, any sample with negative RT-PCR results on both SARS-CoV-2-specific genes as well as RPC would be considered invalid and subjective to reextraction and retesting.

The fourth and last component is a smartphone with Biomeme Go mobile app for thermocycler operation, data entry, and visualizing the test results (Fig. 1A).

Portability of the Biomeme SARS-CoV-2 system. Each M1 sample prep cartridge weighed 20 g, and the cartridges used for the patient testing in the emergency and COVID-19 wards weighed 380 g in total, which were carried in a medium-sized backpack (TUMI Inc.; height, 19 in.; width, 14 in.; depth, 6 in.) weighing 1.6 kg in total. It also included a pouch containing 96 Go-Strips with the lyophilized master mix for SARS-CoV-2 weighing 62 g, a 20- to 200- μ l adjustable-volume micropipette (Amplyus, MA) weighing 84 g, a bag with 150 1.5-ml tubes (Eppendorf, Germany) weighing 160 g, a polypropylene microcentrifuge PCR 80-well tube rack weighing 150 g, and two boxes of 300- μ l pipette tips (Eppendorf, Germany) weighing 120 g each. The entire package weighed around 3 kg, and all items fitted nicely inside the backpack.

The battery-powered Franklin three9 real-time PCR thermocycler alongside a smartphone and their chargers were carried inside a small waterproof case with inside foam (Seahorse, CA; length, 13.6 in.; width, 10.7 in.; height, 6.3 in.) weighing 3.6 kg in total. The portable glove box weighs 11.3 kg, with overall dimensions of 27-in. width, 13-in. depth, and 22-in. height. All components needed to perform a complete run using the Biomeme SARS-CoV-2 system fit inside the portable glove box (Fig. 1B).

Standard-of-care RT-PCR testing for SARS-CoV-2. Nasopharyngeal swabs transported in viral transport medium were processed at the molecular virology laboratory at KFSHRC. Four platforms were used for the routine diagnosis of SARS-CoV-2. The first platform used the Abbott *m2000* system (Abbott, IL) for high-throughput RNA extraction, and elutions were tested using the RealStar SARS-CoV-2 RT-PCR kit (Altona Diagnostics, Germany) and amplified on the Rotor-Gene Q real-time PCR cycler (Qiagen, Germany). The second platform used the Applied Biosystems MagMAX viral RNA kit on the KingFisher instrument (Thermo Fisher Scientific) for high-throughput RNA extraction, and elutions were tested using the DiaPlexQ novel coronavirus detection kit (2019-nCoV) (SolGent, South Korea) and amplified on Applied Biosystem 7500 real-time PCR system (Thermo Fisher Scientific). The third platform is the sample-to-result Abbott *m2000* RNA extraction system combined with the Abbott RealTime SARS-CoV-2 assay, and the fourth platform is the point-of-care GeneXpert using the Xpert Xpress SARS-CoV-2 test (Cepheid, CA). The samples were tested according to the manufacturer's protocols.

Statistical analysis. The sample size for this project was calculated based on the following parameters: anticipated sensitivity of 93%, precision level of 0.05, an alpha level of 0.05, and proportion of

TABLE 1 Results of head-to-head comparative evaluation of the deployable Biomeme SARS-CoV-2 system and standard-of-care RT-PCR tests for SARS-CoV-2^a

Deployable Biomeme SARS-CoV-2 result	No. of samples in standard-of-care RT-PCR testing for SARS-CoV-2 with the following result:			Overall agreement (%)	PPA (% [95% CI])	NPA (% [95% CI])	PPV (% [95% CI])	NPV (% [95% CI])
	Positive	Negative	Total					
Positive	99	2	101	98.4	99 (97.05–1.00)	97.83 (94.85–1.00)	98.02 (95.30–1.00)	98.9 (96.76–1.00)
Negative	1	90	91					
Total	100	92	192					

^aPPA, positive percent agreement; NPA, negative percent agreement; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval.

positive samples of 52%. Thus, the sample size of this project was 192 samples, of which 100 were positive by standard-of-care RT-PCR tests. We assessed the agreement and accuracy of the portable Biomeme SARS-CoV-2 system against the gold standard tests using various diagnostic test evaluation measures, including positive percent agreement, negative percent agreement, positive predictive value, and negative predictive value. Furthermore, we assessed the correlation between the two tests according to positive cycle threshold (C_T) values using the Pearson's correlation coefficient. The values used for the correlation analysis are the C_T value of the *S* gene generated by the deployable RT-PCR Biomeme SARS-CoV-2 system and the minimum C_T values generated by the standard-of-care RT-PCR systems. Diagnostic test evaluation was applied using SAS 9.4 (SAS Institute Inc., Cary, NC, USA), and correlation analysis was applied using R v3.5.

RESULTS

Samples. The majority (51%) of the tested samples originated from female patients. The average age of all patients was 39.3 ± 19 years. Only 12% of total patients were asymptomatic, but 55% of the patients who tested positive were hospitalized, of which 14 received intensive care unit (ICU) care and four patients died. One patient was reinfecting recently after reporting a full recovery. More on the clinical data, demographics, symptoms, and comorbidities are summarized in Table S1 in the supplemental material.

Of the 192 samples tested using the portable Biomeme SARS-CoV-2 system, the results from 189 samples (98.4%) were in agreement with the reference standard-of-care RT-PCR testing for COVID-19. The results are summarized in Table S1 in the supplemental material. Of these 189 concordant samples, 99 samples were confirmed positive for SARS-CoV-2, and 90 samples were confirmed negative for SARS-CoV-2 (Table 1).

Of the 173 previously tested samples using the portable Biomeme SARS-CoV-2 system, 91 samples were correctly detected as SARS-CoV-2 positive and 80 samples as SARS-CoV-2 negative, which matched the reference results from the standard-of-care RT-PCR testing. Only one sample tested negative in the portable Biomeme SARS-CoV-2 system but tested positive in the standard-of-care RT-PCR method using Abbott RealTime SARS-CoV-2 with a C_N value (Abbott readout value) of 6.5 (Table S1). One sample provided a positive result by the portable Biomeme SARS-CoV-2 system, with high C_T values of 39.55 for *Orf1ab* and 37.55 for the *S* gene, but was reported negative by the standard-of-care RT-PCR method using the Xpert Xpress SARS-CoV-2 platform (Table S1).

Of all tested samples, 10 samples (5.2%) initially provided invalid results and had to be reextracted and retested. Only one positive sample (10%) for SARS-CoV-2 and nine negative samples for SARS-CoV-2 (90%) provided invalid results. Valid results were successfully achieved after one repeat of reextraction and retesting the same samples. On the other hand, 94.8% of the samples provided valid results from the first run (Table 2).

The results of the five patients from the COVID-19 ward and 14 COVID-19 suspected patients from the emergency ward had a 95% agreement with the reference standard-of-care RT-PCR testing for SARS-CoV-2. Only one sample from an emergency department patient with a suspected case showed a discrepant result. The portable Biomeme SARS-CoV-2 system reported positive RT-PCR with a high C_T value (42.27) of the *S* gene,

TABLE 2 Validity of the deployable Biomeme SARS-CoV-2 system

Result from the standard-of-care reference method	Initial result validity from the deployable Biomeme SARS-CoV-2			Overall result validity from the first run (%)
	No. valid	No. invalid ^a (had to be repeated)	Total no.	
Positive	99	1	100	94.79
Negative	83	9	92	
Total	182	10	192	

^aInvalid results are called when negative RT-PCR results occurred on both SARS-CoV-2-specific genes as well as RPC. Samples with invalid results were subjected to reextraction and retesting.

but the reference standard-of-care RT-PCR method for SARS-CoV-2 (Abbott RealTime SARS-CoV-2) showed a negative result (Table 1).

Test performance. The portable Biomeme SARS-CoV-2 system had positive percent agreement and negative percent agreement of 99.0% and 97.8%, respectively, compared to the reference results from the standard-of-care RT-PCR system for COVID-19. The positive predictive value was 98.0%, and the negative predictive value was 98.9%. The overall agreement of the portable Biomeme SARS-CoV-2 system versus the reference tests was 98.4% (Table 1).

The portable Biomeme SARS-CoV-2 system was used at the point of care at the emergency department and COVID-19 ward versus the standard-of-care tests, including the Xpert Xpress SARS-CoV-2 platform, which had to be conducted at the molecular virology laboratory. The overall turnaround time for the portable RT-PCR system was only approximately 80 min, which included sample preparation (RNA extraction), sample loading, data entry, and assay run time for a total of nine samples (Table 3). This turnaround time helped to rapidly identify four emergency department patients who tested positive for COVID-19, and the results were later confirmed by the standard-of-care tests.

C_T values. The *S* gene was amplified by the Biomeme SARS-CoV-2 system from all positive samples ($n = 101$), and the *Orf1ab* gene was not amplified from 16 (15.4%)

TABLE 3 Basic performance characteristics of the deployable Biomeme SARS-CoV-2 system against standard-of-care RT-PCR tests for SARS-CoV-2

Characteristic	Value or description ^a				
	Biomeme SARS-CoV-2 System	Standard-of-care RT-PCR testing for SARS-CoV-2			
		Abbott m2000 RNA extraction plus RealStar SARS-CoV-2 RT-PCR	MagMAX viral RNA kit on KingFisher plus DiaPlexQ 2019-nCoV detection kit	Abbott m2000 RNA extraction plus Abbott RealTime SARS-CoV-2	Xpert Xpress SARS-CoV-2
Sample type	Direct NP, nasal (mid-turbinate) swab ± VTM	NP + VTM	NP + VTM	NP + VTM	Direct NP, nasal (mid-turbinate) swab ± VTM
Point of care	Yes	No	No	No	Yes/No
Alternative sample prep solutions	Yes	Yes (to replace Abbott m2000 RNA extraction)	Yes (to replace MagMAX viral RNA kit on KingFisher)	No	NA
Sample prep	<2 min/sample	3 h/batch	30 min/batch	4 h/batch	NA
Hands-on time	2 min/run	60 min/run	45 min/run	60 min/run	1 min/specimen
Assay run time	65 min	2 h 17 min/run	2 h	3 h	<45 min
Overall turnaround time (per ready-to-run batch)	~80 min (batch of 9 samples)	6.5 h	3.5 h	8 h	<45 min
Throughput	9 samples	72 samples	96 samples	96 sample	1 sample/module
Output unit	C _T values	CN ^b	C _T values	C _T values	C _T values
Batching	Required for 3 samples but only for the RT-PCR step	Required	Required	Required	Not required
Targets	SARS-CoV-2-specific <i>Orf1ab</i> and <i>S</i> genes	B-betacoronavirus <i>E</i> gene and SARS-CoV-2-specific <i>S</i> gene	SARS-CoV-2-specific <i>N</i> and <i>ORF1a</i> genes	SARS-CoV-2-specific <i>RdRp</i> and <i>N</i> genes	Pan-Sarbecovirus <i>E</i> gene and SARS-CoV-2-specific <i>N2</i> gene

^aThe reported times are based on the experience attained from this study and routine testing. NP, nasopharyngeal; VTM, viral transport medium; NA, not available.

^bThe CN values are specific values reported by the Abbott RealTime SARS CoV-2 assay and not directly comparable to C_T values (17).

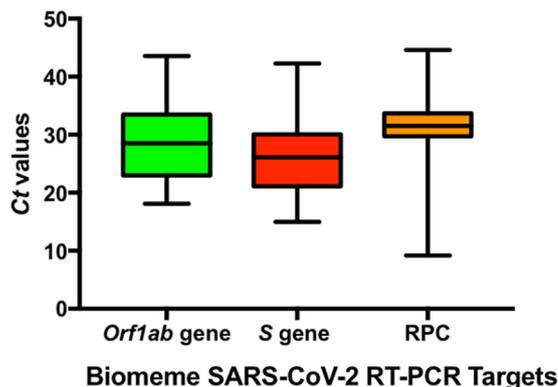


FIG 2 C_T values for the triplex targets of the deployable Biomeme SARS-CoV-2 assay.

samples that were identified as positive by the Biomeme SARS-CoV-2 system. An assessment of the C_T values for the amplified targets showed that the *S* gene had a mean C_T value of 26.08 ± 5.77 (14.97 to 42.27). The mean C_T value for the *Orf1ab* gene for the 85 samples was 24.10 ± 12.05 (18.1 to 43.57). No significant differences in the C_T values of the *S* and *Orf1ab* genes were observed ($P = 0.1374$) (Fig. 2).

The RPC was detected in 43 of 101 (42.57%) samples with positive SARS-CoV-2 results. Due to the amplification of at least one of the SARS-CoV-2-specific genes, the absent amplifications of the RPC did not impact the interpretation of the positive results, which aligned with the results of the standard-of-care tests (Table S1). Most importantly, the RPC was detected in all (100%) negative samples, including the nine samples that were initially associated with invalid results and were later confirmed as COVID-19 negative after reextraction and retesting. The negative results aligned with the results of the standard-of-care tests (Table S1). The mean C_T value of the RPC was 32.57 ± 5.1 (9.2 to 44.76) ($n = 134$) (Fig. 2).

We assessed the correlation of the deployable Biomeme SARS-CoV-2 system with the standard-of-care tests using the Pearson's correlation coefficient. Our result indicates a positive correlation between the C_T values of the *S* gene and the minimum C_T value of the matching samples generated by the standard-of-care systems with R coefficient of 0.583 and a P value of <0.0001 (Fig. 3). Thus, we excluded samples with CN values, because these values are specific values reported by the Abbott RealTime SARS-CoV-2 assay and not directly comparable to C_T values (17).

DISCUSSION

Given the current challenges of the COVID-19 pandemic, a rapid testing system is critical to mitigating the further spread of the disease (18, 19). Despite multiple tests being available widely, RT-PCR remains the current gold-standard for SARS-CoV-2 detection (3). However, limitations such as the requirement of large laboratory equipment with continuous power supply, available trained professionals, sample throughput restrictions, and specific biosafety requirements impact the accessibility of the tests in remote and low-resourced environments and restrict point-of-care testing.

Our study independently evaluated a completely portable solution to test COVID-19 that can be easily made available in remote locations. The portable Biomeme SARS-CoV-2 system combined with a portable glove box could rapidly test samples within 80 min while adhering to strict biosafety measures. We tested clinical samples and compared the results against the results from the routine standard-of-care testing for COVID-19. The Biomeme SARS-CoV-2 system demonstrated superior diagnostic performance with 99.0% positive percent agreement and 97.8% negative percent agreement. The overall percent agreement between the two systems was excellent (98.4%).

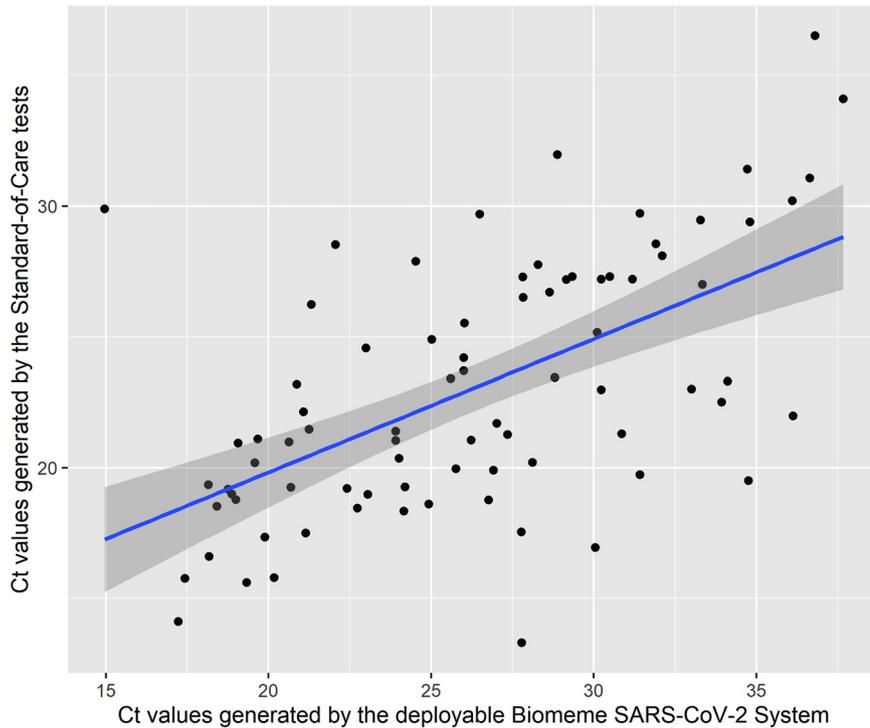


FIG 3 Pearson's correlation coefficient shows a positive correlation between the C_T values of the *S* gene from the deployable Biomeme SARS-CoV-2 assay and the C_T values that occurred with the standard-of-care tests for SARS-CoV-2.

Also, having only around 5.2% of samples that provided invalid results demonstrated the test's efficiency to mostly (94.79%) deliver valid and reliable results from the first run. The observed high performance, correlated C_T values between the Biomeme SARS-CoV-2 system and the reference methods, and their comparable characteristics demonstrate the reliable clinical testing of COVID-19.

Three discordant samples were identified in our study using the Biomeme system. First was a false negative, which might have occurred due to the use of a previous sample that was subjected to initial testing and several thawing and freezing cycles. It is well known that freeze-thaw cycles can affect the nucleic acid stability leading to RNA degradation, which may affect the sensitivity of RT-PCR (20). In an independent run, we tried to retest the same sample using the deployable Biomeme SARS-CoV-2 system, and it tested positive for both genes. The two other discrepant results were false positives, which might have resulted from accidental contamination, viral load below the detection limit of the reference test, or unspecific amplification due to primer dimers. The latter is suspected due to the exceptionally high C_T values, including one sample with only one *S* gene amplification. It is worth mentioning that the manufacturer has reported the limit of detection of the Biomeme SARS-CoV-2 assay as 1.8 genome equivalents per μl , but no further validation for the limit of detection of the assay was conducted as part of this study.

Due to the increasing global concerns related to the emergence and spread of new SARS-CoV-2 variants, the U.S. FDA issued a letter to health care providers on 8 January 2021 warning that the genetic variants of SARS-CoV-2 may lead to false-negative results with molecular tests for the detection of SARS-CoV-2 (21). Biomeme has later confirmed, through *in silico* analysis of the genetic sequences, that the SARS-CoV-2 assay detects the new variants reported by the United Kingdom, lineage B.1.1.7, South Africa, lineage B.1.351, and Brazil, lineage B.1.1.28 (22).

Also, our study validates the portable Biomeme SARS-CoV-2 system to be suitable

for molecular point-of-care testing with high accuracy for the detection of SARS-CoV-2 compared to that of the routine standard-of-care testing. The total sample-to-result time, including hands-on time and run time, was 80 min for a batch of nine samples and was comparable to the Xpert Xpress SARS-CoV-2 platform, which could test several samples at once within 45 min in a multimodule configuration instrument (such as GX-XVI) (23). However, multimodule GenXpert models are large, are not easily affordable, require a continuous power supply, and cannot be easily deployed at point-of-care settings.

In addition to diagnostic performance, sample throughput, simplicity of use, and biosafety measures are also critical factors to consider. The M1 sample prep cartridge has several benefits besides being a quick, user-friendly, and equipment-free method. It offers an immediate virus deactivation by placing the UTM directly into the lysis buffer. This alternative step to heat deactivation, as described by Harrington et al. (24), adds at least 30 min to the testing turnaround-time, which could affect the RNA stability (24–26). Also, rapid RNA extraction can be performed directly on fresh nasal swabs without collecting in viral transport medium, which could minimize sample dilution. Most importantly, this protocol is timely, as it will not be affected by the global limitation of transport media (27–29).

Collecting nasopharyngeal swabs for COVID-19 testing can be associated with stress, anxiety, and hazardous exposure for health care workers (30, 31). Hence, samples from nasal swabs (mid-turbinate) and saliva have been studied as alternative specimens for detecting SARS-CoV-2 using RT-PCR (32–37). Here, we demonstrated an agreement in SARS-CoV-2 testing between the fresh nasal swabs (mid-turbinate) versus the nasopharyngeal swabs collected in viral transport medium from patients at the emergency ward and COVID-19 ward. However, more nasal swabs will need to be tested using the Biomeme SARS-CoV-2 system to ensure their robustness as an alternative to nasopharyngeal swabs, which may later allow patients to self-collect their samples and thereby minimize exposure to health care workers.

Handling COVID-19 samples can place laboratory staff at significant risk for laboratory-acquired infections by SARS-CoV-2. Hence, biosafety prevention and control measures have been placed to limit any risks. These include performing procedures that may entail potential exposure to aerosols or droplets (such as vortexing) in a certified class II biological safety cabinet placed inside a negative pressure environment (38). However, these procedures require large equipment and expensive infrastructure that can be absent in low-resourced settings, which can centralize the testing to be limited only to well-established infrastructures such as major public health laboratories. The use of the RNA extraction protocol and performing the entire testing process inside a portable and fully enclosed glove box can provide a safe solution to conduct field-testing and at the point of care for COVID-19 anywhere in the world.

The Biomeme master mixes combined with primers and probes are lyophilized into beads that are prepackaged in quantitative PCR (qPCR) tube “Go-Strips” and are shelf stable for up to 18 months. This feature eliminates the need for a cold supply chain and further enhances this platform’s field utility to conduct testing for infectious diseases in the community and low-resourced settings. The ultracompact system is even flexible enough to use other RNA extraction kits and test in-house assays using the Franklin thermocycler (validated by our team, but data not shown in this study). Also, the use of a smartphone-enabled geolocation feature can even help in the epidemiological surveillance and tracking of pathogens, including SARS-CoV-2. Lastly, if an Internet connection is available, Biomeme Cloud offers a platform for remote access to the results and raw data (e.g., amplification curves) for each run.

The main limitation of this study is the restriction to a single site. A multicenter evaluation using several deployable Biomeme SARS-CoV-2 systems would provide a comprehensive analysis of the system’s reproductivity for COVID-19 testing. A multicenter evaluation would also allow testing for more samples, allowing for a lower margin of error for the performance matrix. This is particularly important when

testing the nasal swabs (mid-turbinate) to fully evaluate their suitability to be used routinely for COVID-19 testing. Another limitation of our study is to evaluate the possibility for sample pooling on the Biomeme SARS-CoV-2 system to test several samples at the same time. The evaluation of pooled sample data would require a separate study to test a large number of samples, at separate runs and sites, to ensure pooling's suitability to assess the testing capacity efficiently and confidently. Our study was limited to the use of the Biomeme SARS-CoV-2 system for point-of-care testing at a clinical setting. Independent studies would need to be carried out at fields with limited resources/trainings to mimic real-life scenarios that may associate with clinical challenges. Yet, it is important to note that the EUA for the Biomeme SARS-CoV-2 system is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a that meet requirements to perform high-complexity tests. Lastly, the Franklin thermocycler has only nine sample wells. This integral feature can limit the number of samples tested per run to nine samples, except if more Franklin thermocyclers are used simultaneously. Another integral feature is the Go-Strips, which requires batching at least three samples per run.

In summary, we have evaluated a highly sensitive, specific, and deployable system that offers point-of-care testing for SARS-CoV-2 anywhere in the world, allowing for the decentralization of infectious disease testing. We have demonstrated the system's ability to rapidly perform RNA extraction with limited equipment to test nine samples at once. To eliminate any biosafety concerns, we also incorporated the fully sealed portable glove box containing the entire testing process. Given the lengthy testing times for standard-of-care tests, our results showed significantly reduced testing times to 80 min, allowing for rapid triaging decisions.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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H.M.Z., T.H.A., W.S.A., A.W., R.A.H., and S. A. Alqahtani conceptualized, designed, and performed the study. W.S.A., A.A., S.A., M.M., Z.A.M., and H.M.A. provided advice and logistical support. H.M.Z., S. A. Altamimi, M.A., and R.S.A. gathered and curated the tests and clinical data. H.M.Z., R.A.H., and O.S.A. performed the statistical analysis and interpretation. All authors have contributed to the manuscript and reviewed the submitted version.

We declare no competing interests.

REFERENCES

- World Health Organization. 2020. WHO Director-General's opening remarks at the media briefing on COVID-19 - 16 March 2020. <https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19-16-march-2020>.
- Aldibasi OS, Alharbi NK, Alkelya M, Zowawi HM, Alghnam S. 28 April 2020. The association of country-level factors with outcomes of COVID-19: analysis of the pandemic after one million cases. *Res Square* <https://doi.org/10.21203/rs.3.rs-25834/v1>.
- Dhama K, Khan S, Tiwari R, Sircar S, Bhat S, Malik YS, Singh KP, Chaicumpa W, Bonilla-Aldana DK, Rodriguez-Morales AJ. 2020. Coronavirus disease 2019—COVID-19. *Clin Microbiol Rev* 33:e00028-20. <https://doi.org/10.1128/CMR.00028-20>.
- Hoxha A, Wyndham-Thomas C, Klamer S, Dubourg D, Vermeulen M, Hammami N, Cornelissen L. 23 July 2020. Asymptomatic SARS-CoV-2 infection in Belgian long-term care facilities. *Lancet Infect Dis* [https://doi.org/10.1016/S1473-3099\(20\)30560-0](https://doi.org/10.1016/S1473-3099(20)30560-0).
- Cheng MP, Papenburg J, Desjardins M, Kanjilal S, Quach C, Libman M, Dittrich S, Yansouni CP. 2020. Diagnostic testing for severe acute respiratory syndrome-related coronavirus 2. *Ann Intern Med* 172:726–734. <https://doi.org/10.7326/M20-1301>.
- Lurie N, Saviile M, Hatchett R, Halton J. 2020. Developing COVID-19 vaccines at pandemic speed. *N Engl J Med* 382:1969–1973. <https://doi.org/10.1056/NEJMp2005630>.
- Nguyen T, Duong Bang D, Wolff A. 2020. 2019 novel coronavirus disease (COVID-19): paving the road for rapid detection and point-of-care diagnostics. *Micromachines (Basel)* 11:306. <https://doi.org/10.3390/mi11030306>.
- Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, Ren R, Leung KSM, Lau EHY, Wong JY, Xing X, Xiang N, Wu Y, Li C, Chen Q, Li D, Liu T, Zhao J,

- Liu M, Tu W, Chen C, Jin L, Yang R, Wang Q, Zhou S, Wang R, Liu H, Luo Y, Liu Y, Shao G, Li H, Tao Z, Yang Y, Deng Z, Liu B, Ma Z, Zhang Y, Shi G, Lam TTY, Wu JT, Gao GF, Cowling BJ, Yang B, Leung GM, Feng Z. 2020. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N Engl J Med* 382:1199–1207. <https://doi.org/10.1056/NEJMoa2001316>.
9. Tabah A, Ramanan M, Laupland KB, Buetti N, Cortegiani A, Mellinghoff J, Conway Morris A, Camporota L, Zappella N, Elhadi M, Povoa P, Amrein K, Vidal G, Derde L, Bassetti M, Francois G, Ssi Yan Kai N, De Waele JJ, PPE-SAFE contributors. 2020. Personal protective equipment and intensive care unit healthcare worker safety in the COVID-19 era (PPE-SAFE): an international survey. *J Crit Care* 59:70–75. <https://doi.org/10.1016/j.jcrc.2020.06.005>.
 10. Hogan CA, Garamani N, Lee AS, Tung JK, Sahoo MK, Huang C, Stevens B, Zehnder J, Pinsky BA. 2020. Comparison of the Accula SARS-CoV-2 test with a laboratory-developed assay for detection of SARS-CoV-2 RNA in clinical nasopharyngeal specimens. *J Clin Microbiol* 58:e01072-20. <https://doi.org/10.1128/JCM.01072-20>.
 11. Young S, Taylor SN, Cammarata CL, Varnado KG, Roger-Dalbert C, Montano A, Griego-Fullbright C, Burgard C, Fernandez C, Eckert K, Andrews JC, Ren H, Allen J, Ackerman R, Cooper CK. 2020. Clinical evaluation of BD Veritor SARS-CoV-2 point-of-care test performance compared to PCR-based testing and versus the Sofia 2 SARS antigen point-of-care test. *J Clin Microbiol* 59:e02338-20. <https://doi.org/10.1128/JCM.02338-20>.
 12. Fenollar F, Bouam A, Ballouche M, Fuster L, Prudent E, Colson P, Tissot-Dupont H, Million M, Drancourt M, Raoult D, Fournier PE. 2020. Evaluation of the Panbio COVID-19 rapid antigen detection test device for the screening of patients with COVID-19. *J Clin Microbiol* 59:e02579-20. <https://doi.org/10.1128/JCM.02589-20>.
 13. Deeks JJ, Dinnes J, Takwoingi Y, Davenport C, Spijker R, Taylor-Phillips S, Adriano A, Beese S, Dretzke J, Ferrante di Ruffano L, Harris IM, Price MJ, Dittich S, Emperador D, Hoof L, Leeflang MM, Van den Bruel A, Cochrane COVID-19 Diagnostic Test Accuracy Group. 2020. Antibody tests for identification of current and past infection with SARS-CoV-2. *Cochrane Database Syst Rev* 6:CD013652. <https://doi.org/10.1002/14651858.CD013652>.
 14. Esbin MN, Whitney ON, Chong S, Maurer A, Darzacq X, Tjian R. 2020. Overcoming the bottleneck to widespread testing: a rapid review of nucleic acid testing approaches for COVID-19 detection. *RNA* 26:771–783. <https://doi.org/10.1261/rna.076232.120>.
 15. Hogan CA, Sahoo MK, Huang C, Garamani N, Stevens B, Zehnder J, Pinsky BA. 2020. Five-minute point-of-care testing for SARS-CoV-2: not there yet. *J Clin Virol* 128:104410. <https://doi.org/10.1016/j.jcv.2020.104410>.
 16. Kosack CS, Page AL, Klatser PR. 2017. A guide to aid the selection of diagnostic tests. *Bull World Health Organ* 95:639–645. <https://doi.org/10.2471/BLT.16.187468>.
 17. Marais G, Naidoo M, Hsiao NY, Valley-Omar Z, Smuts H, Hardie D. 2020. The implementation of a rapid sample preparation method for the detection of SARS-CoV-2 in a diagnostic laboratory in South Africa. *PLoS One* 15:e0241029. <https://doi.org/10.1371/journal.pone.0241029>.
 18. Guan W-J, Ni Z-Y, Hu Y, Liang W-H, Ou C-Q, He J-X, Liu L, Shan H, Lei C-L, Hui DSC, Du B, Li L-J, Zeng G, Yuen K-Y, Chen R-C, Tang C-L, Wang T, Chen P-Y, Xiang J, Li S-Y, Wang J-L, Liang Z-J, Peng Y-X, Wei L, Liu Y, Hu Y-H, Peng P, Wang J-M, Liu J-Y, Chen Z, Li G, Zheng Z-J, Qiu S-Q, Luo J, Ye C-J, Zhu S-Y, Zhong N-S, China Medical Treatment Expert Group for Covid-19. 2020. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med* 382:1708–1720. <https://doi.org/10.1056/NEJMoa2002032>.
 19. Studdert DM, Hall MA. 2020. Disease control, civil liberties, and mass testing - calibrating restrictions during the COVID-19 pandemic. *N Engl J Med* 383:102–104. <https://doi.org/10.1056/NEJMp2007637>.
 20. Kraiden M, Minor JM, Rifkin O, Comanor L. 1999. Effect of multiple freeze-thaw cycles on hepatitis B virus DNA and hepatitis C virus RNA quantification as measured with branched-DNA technology. *J Clin Microbiol* 37:1683–1686. <https://doi.org/10.1128/JCM.37.6.1683-1686.1999>.
 21. United States Food and Drug Administration. 2021. Genetic variants of SARS-CoV-2 may lead to false negative results with molecular tests for detection of SARS-CoV-2 - letter to clinical laboratory staff and health care providers. <https://www.fda.gov/medical-devices/letters-health-care-providers/genetic-variants-sars-cov-2-may-lead-false-negative-results-molecular-tests-detection-sars-cov-2>.
 22. Biomeme Inc. 2021. Biomeme's SARS-CoV-2 assay detects New United Kingdom, South Africa, Brazil, and California variants. <https://blog.biomeme.com/biomemes-sars-cov-2-assay-detects-new-variants>.
 23. Loeffelholz MJ, Alland D, Butler-Wu SM, Pandey U, Perno CF, Nava A, Carroll KC, Mostafa H, Davies E, McEwan A, Rakeman JL, Fowler RC, Pawlowsky J-M, Fourati S, Banik S, Banada PP, Swaminathan S, Chakravorty S, Kwiatkowski RW, Chu VC, Kop J, Gaur R, Sin MLY, Nguyen D, Singh S, Zhang N, Persing DH. 2020. Multicenter evaluation of the Cepheid Xpert Xpress SARS-CoV-2 test. *J Clin Microbiol* 58:e00926-20. <https://doi.org/10.1128/JCM.00926-20>.
 24. Harrington A, Cox B, Snowdon J, Bakst J, Ley E, Grajales P, Maggiore J, Kahn S. 2020. Comparison of Abbott ID Now and Abbott m2000 methods for the detection of SARS-CoV-2 from nasopharyngeal and nasal swabs from symptomatic patients. *J Clin Microbiol* 58:e00798-20. <https://doi.org/10.1128/JCM.00798-20>.
 25. Brisco MJ, Morley AA. 2012. Quantification of RNA integrity and its use for measurement of transcript number. *Nucleic Acids Res* 40:e144. <https://doi.org/10.1093/nar/gks588>.
 26. Yang T-H, Chang P-L. 2012. Determination of RNA degradation by capillary electrophoresis with cyan light-emitted diode-induced fluorescence. *J Chromatogr A* 1239:78–84. <https://doi.org/10.1016/j.chroma.2012.03.070>.
 27. Rodino KG, Espy MJ, Buckwalter SP, Walchak RC, Germer JJ, Fernholz E, Boerger A, Schuetz AN, Yao JD, Binnicker MJ. 2020. Evaluation of saline, phosphate-buffered saline, and minimum essential medium as potential alternatives to viral transport media for SARS-CoV-2 testing. *J Clin Microbiol* 58:e00590-20. <https://doi.org/10.1128/JCM.00590-20>.
 28. Smith KP, Cheng A, Chopelas A, DuBois-Coyne S, Mezghani I, Rodriguez S, Talay M, Kirby JE. 2020. Large-scale, in-house production of viral transport media to support SARS-CoV-2 PCR testing in a multi-hospital healthcare network during the COVID-19 pandemic. *J Clin Microbiol* 58:e00913-20. <https://doi.org/10.1128/JCM.00913-20>.
 29. Won J, Lee S, Park M, Kim TY, Park MG, Choi BY, Kim D, Chang H, Kim VN, Lee CJ. 2020. Development of a laboratory-safe and low-cost detection protocol for SARS-CoV-2 of the coronavirus disease 2019 (COVID-19). *Exp Neurobiol* 29:107–119. <https://doi.org/10.5607/en20009>.
 30. Roxby AC, Greninger AL, Hatfield KM, Lynch JB, Dellit TH, James A, Taylor J, Page LC, Kimball A, Arons M, Munanga A, Stone N, Jernigan JA, Reddy SC, Lewis J, Cohen SA, Jerome KR, Duchin JS, Neme S. 2020. Outbreak investigation of COVID-19 among residents and staff of an independent and assisted living community for older adults in Seattle, Washington. *JAMA Intern Med* 180:1101. <https://doi.org/10.1001/jamainternmed.2020.2233>.
 31. Ruggiero A, Sanguinetti M, Gatto A, Attinà G, Chiaretti A. 2020. Diagnosis of COVID-19 infection in children: less nasopharyngeal swabs, more saliva. *Acta Paediatr* 109:1913–1914. <https://doi.org/10.1111/apa.15397>.
 32. Harikrishnan P. 2020. Saliva as a potential diagnostic specimen for COVID-19 testing. *J Craniofac Surg* 31:e653–e655. <https://doi.org/10.1097/scs.0000000000006724>.
 33. Palmas G, Moriondo M, Trapani S, Ricci S, Calistri E, Pisano L, Perferi G, Galli L, Venturini E, Indolfi G, Azzari C. 2020. Nasal swab as preferred clinical specimen for COVID-19 testing in children. *Pediatr Infect Dis J* 39:e267–e270. <https://doi.org/10.1097/inf.0000000000002812>.
 34. Pinninti S, Trieu C, Pati SK, Latting M, Cooper J, Seleme MC, Boppana S, Arora N, Britt WJ, Boppana SB. 29 June 2020. Comparing nasopharyngeal and mid-turbinate nasal swab testing for the identification of SARS-CoV-2. *Clin Infect Dis* <https://doi.org/10.1093/cid/ciaa882>.
 35. Sri Santosh T, Parmar R, Anand H, Srikanth K, Saritha M. 2020. A review of salivary diagnostics and its potential implication in detection of COVID-19. *Cureus* 12:e7708. <https://doi.org/10.7759/cureus.7708>.
 36. Srivatsan S, Han PD, van Raay K, Wolf CR, McCulloch DJ, Kim AE, Brandstetter E, Martin B, Gehring J, Chen W, Kosuri S, Konnick EQ, Lockwood CM, Rieder MJ, Nickerson DA, Chu HY, Shendure J, Starita LM. 23 April 2020. Preliminary support for a dry swab, extraction free protocol for SARS-CoV-2 testing via RT-qPCR. *bioRxiv* <https://doi.org/10.1101/2020.04.22.056283>.
 37. To KK, Tsang OT, Chik-Yan Yip C, Chan KH, Wu TC, Chan JMC, Leung WS, Chik TS, Choi CY, Kandamby DH, Lung DC, Tam AR, Poon RW, Fung AY, Hung IF, Cheng VC, Chan JF, Yuen KY. 2020. Consistent detection of 2019 novel coronavirus in saliva. *Clin Infect Dis* 71:841–843. <https://doi.org/10.1093/cid/ciaa149>.
 38. Wang K, Zhu X, Xu J. 2020. Laboratory biosafety considerations of SARS-CoV-2 at biosafety level 2. *Health Secur* 18:232–236. <https://doi.org/10.1089/hs.2020.0021>.