SARS-CoV-2 Identification and Characterization on the Biomeme Franklin Polymerase Chain Reaction System

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We evaluated the sensitivity and specificity of the Biomeme Franklin™ three9 Real-Time PCR Thermocycler and Biomeme SARS-CoV-2 Go-Strips in the detection of SARS-CoV-2. The Biomeme Franklin™ three9 platform is a portable, battery-operated system that could be used in remote settings. We assessed performance of the Biomeme SARS-CoV-2 detection system at a wide range of viral concentrations, examined cross-reactivity of the SARS-CoV-2 Go-Strips against several near-neighbor respiratory pathogens, and evaluated agreement against the BioFire® Respiratory Panel 2.1 in four clinical sample types. Our data indicate the Biomeme Go-Strips can reliably detect SARS-CoV-2 at a concentration of 4.2 x 10^3 copies/mL. No cross reactivity of the Go-Strips targets was detected against any of the tested near-neighbor respiratory pathogens. Cohen's kappa statistics ranged from 0.68 to 0.92 between results from the Biomeme SARS-CoV-2 Go-Strips and the BioFire® Respiratory Panel 2.1 in all the different sample types. Compared to the BioFire® Respiratory Panel 2.1, the Biomeme SARS-CoV-2 Go-Strips demonstrated statistically significantly lower sensitivity in 3 out of 5 sample types. Overall, our study demonstrates the Biomeme Franklin™ three9 used with the SARS-CoV-2 Go-Strips is an effective system for the detection of SARS-CoV-2 that could potentially be used in a remote or austere environment.

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1.0 EXECUTIVE SUMMARY

We evaluated the sensitivity and specificity of the Biomeme Franklin™ three9 Real-Time PCR Thermocycler and Biomeme SARS-CoV-2 Go-Strips in the detection of SARS-CoV-2. The Biomeme Franklin™ three9 platform is a portable, battery-operated system that could be used in remote settings. We assessed performance of the Biomeme SARS-CoV-2 detection system at a wide range of viral concentrations, examined cross-reactivity of the SARS-CoV-2 Go-Strips against several near-neighbor respiratory pathogens, and evaluated agreement against the BioFire® Respiratory Panel 2.1 in four clinical sample types. Our data indicate the Biomeme Go-Strips can reliably detect SARS-CoV-2 at a concentration of 4.2 x 10^3 copies/mL. No cross reactivity of the Go-Strips targets was detected against any of the tested near-neighbor respiratory pathogens. Cohen's kappa statistics ranged from 0.68 to 0.92 between results from the Biomeme SARS-CoV-2 Go-Strips and the BioFire® Respiratory Panel 2.1 in all the different sample types. Compared to the BioFire® Respiratory Panel 2.1, the Biomeme SARS-CoV-2 Go-Strips demonstrated statistically significantly lower sensitivity in 3 out of 5 sample types. Overall, our study demonstrates the Biomeme Franklin™ three9 used with the SARS-CoV-2 Go-Strips is an effective system for the detection of SARS-CoV-2 that could potentially be used in a remote or austere environment.

2.0 INTRODUCTION

The World Health Organization declared Coronavirus Disease (COVID-19) a global pandemic on March 11th, 2020 [1]. Since then, the pandemic has disrupted the lives of people worldwide and placed a significant burden on health care systems. As of August 4, 2021, more than 200 million COVID-19 cases have been confirmed worldwide, including approximately 4.7 million deaths [2]. The unprecedented global impact of the COVID-19 pandemic highlights the need for rapid, reliable diagnostic tests for the detection of SARS-CoV-2 that can lead to early intervention, improved health outcomes, and reduced spread of the disease.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) assays for the detection of viral RNA targets are the gold standard for SARS-CoV-2 diagnosis. Large healthcare facilities and laboratories in suburban and metropolitan areas have access to high-throughput, sample-to-answer RT-PCR platforms; however, more remote settings typically cannot access these technologies [3]. Rural areas, which on average, consist of older populations with higher rates of underlying health conditions lack access to testing due to limited resources and hospital closures as a result of the pandemic [4]. Military populations that operate in austere environments far removed from health care facilities also need access to adaptable molecular diagnostic approaches that enable early detection of SARS-CoV-2 or other highly contagious pathogens [5]. A cost-effective, easy-to-use application could address the capability gap faced by these communities.

The Biomeme Franklin™ three9 Real-Time PCR Thermocycler (Philadelphia, PA) system is a lightweight, portable, battery-powered qPCR device that can test biological samples without centrifugation, the use of frozen reagents, or a power source. Furthermore, the device is capable of multiplex detection of up to three targets in each sample, where nine samples can be tested in a single run. Real-time PCR results are displayed on a smartphone that is connected to the device via Bluetooth or a USB cable. The Biomeme SARS-CoV-2 Go-Strip assay is a qualitative test for the detection of SARS-CoV-2 viral RNA in nasopharyngeal, nasal, and oropharyngeal swab specimens, and nasopharyngeal washes or aspirates. Biomeme received Emergency Use Authorization for their SARS-CoV-2 Go-Strip assay from the FDA on August 11, 2020 [6].

The objective of this study was to evaluate the sensitivity and specificity of the Biomeme SARS-CoV-2 Go-Strips assay tested on the Biomeme Franklin™ three9 Real-Time PCR Thermocycler. We assessed the performance of the Biomeme SARS-CoV-2 Go-Strips assay utilizing Biomeme's M1 extraction method at a range of viral concentrations and tested several upper respiratory near-neighbor pathogens in the SARS-CoV-2 Go-Strips to evaluate cross-reactivity. Finally, we evaluated sensitivity and specificity of the Biomeme SARS-CoV-2 Go-Strips by testing clinical upper respiratory samples using the BioFire®

Respiratory Panel 2.1 (RP2.1; Salt Lake City, UT) as a reference standard and validated results from the Biomeme SARS-CoV-2 Go-Strips using Biomeme SARS-CoV-2 Go-Plates tested on the ThermoFisher Quantstudio^{TM} 7 Flex Real-Time PCR System (Waltham, MA).

3.0 METHODS, ASSUMPTIONS AND PROCEDURES

2.1. Sensitivity testing of the Biomeme system

RNA was extracted and purified using Biomeme's M1 Sample Prep Cartridge, a filtration-based manual extraction method where nucleic acid binds to a silica membrane inside of a piercing tool attached to a syringe. Sample is pumped through the membrane along the sealed cartridge chambers which contain lysis buffer, wash buffers, and an elution buffer. Following RNA extraction, Biomeme SARS-CoV-2 Go-Strips were used to detect SARS-CoV-2 in clinical samples. The Go-Strips are designed specifically for use with the Franklin^{TM} three9 instrument. The assay reagents are lyophilized for the detection of SARS-CoV-2 gene targets including open reading frame 1ab (ORF1ab) and spike (S). The ORF1ab gene is FAM (Carboxyfluorescein)-labeled and the S protein is ATTO647N-labeled. An internal control, the RNA Process Control (RPC), which determines if the M1 Sample Prep Cartridge RNA extraction has worked or failed, is Texas RedX-labeled. All three targets are multiplexed primer/probes that are triplex reactions in one Go-Strip.

To evaluate the sensitivity of the Biomeme SARS-CoV-2 detection system, a dilution series was prepared. Six 1:10 serial dilutions of Heat Inactivated 2019 Novel Coronavirus (ATCC® VR-1986HK^{TM}; Manassas, VA) were prepared in RNase-free water from a stock concentration of 4.2×10^8 genome copies/mL. Additional concentrations of 2.10×10^3 and 1.05×10^3 copies/mL were prepared to assess performance near the threshold of detection. ATCC® VR-1986HK^{TM} is a preparation of strain 2019-nCoV/USA-WA1/2020 inactivated by heating to 65°C of 30 minutes [7]. Each concentration was extracted and purified using the Biomeme M1 Sample Prep Cartridge Kit for RNA 2.0 according to manufacturer's instructions for use [8]. RNA from the M1 extraction process was tested in triplicate by adding 20 µL of RNA to the lyophilized master mix contained in each Go-Strip well. Amplification and detection of amplicon was performed in the Biomeme Franklin™ three9 Real-Time PCR device. Upon completion of RT-PCR, the Biomeme provides an interpreted result regarding which gene targets have been detected. No analysis of amplification plots is required to interpret results. Sample results with neither ORF1ab nor S detection but with RPC amplification are considered negative for SARS-CoV-2. Samples results with only ORF1ab detection are considered presumptive positive for SARS-CoV-2. Sample results with S or both S and ORF1ab amplification are considered positive for SARS-COV-2. Sample results with no targets detected are considered invalid and need to be retested.

2.2. Near-neighbor testing to assess cross-reactivity

Nineteen near-neighbor upper respiratory viral and bacterial pathogens were tested on the Biomeme SARS-CoV-2 Go-Strips assay. Genomic material for each near-neighbor pathogen was purchased from ATCC (Table 2). A single 1:10 dilution was prepared for each pathogen. Prepared dilutions were tested in the Biomeme SARS-CoV-2 Go-Strips assays in triplicate by adding 20 µL of dilution to the lyophilized master mix contained in each Go-Strip well. Amplification and detection of amplicon was performed on the Biomeme Franklin™ three9 Real-Time PCR device. Each near-neighbor pathogen was tested on the Bio Fire RP2.1 to confirm presence of the genomic material, with the exception of MERS-CoV and SARS-CoV-1 which are not included in the BioFire® RP2.1.

2.3. Clinical sample collection

Between November 6, 2020 and January 7, 2021, iSpecimen, Inc. (Lexington, MA) enrolled and consented individuals in California, New Jersey, and New York who tested positive or negative for SARS-CoV-2 by a CLIA approved PCR test at baseline, and collected four different sample types from under an approved Institutional Review Board (IRB) protocol. Additionally, the nasopharyngeal specimen type was collected in viral transport media and saline for each subject. The samples were shipped to the Center for Advanced Molecular Detection at Lackland Air Force Base in San Antonio, TX on dry ice for processing and testing. The study was determined to be EXEMPT from research regulation 32 CFR 219 regarding the protection of human subjects Category 4 [32 CFR 219.104(d)(4)], by the 59th Medical Wing (59 MDW), via the exempt review/determination process by the 59th MDW Institutional Review Board (IRB) Chairperson or designee, based on 32 CFR 219.104(d). Samples collected were nasal swab (NS), nasopharyngeal swab in saline (NP-S), nasopharyngeal swab in viral transport media (NP-VTM), oropharyngeal swab (OP), and saliva. There were three cohort groups: Cohort #1 included 22 individuals (NS 21, NP-S 22, NP-VTM 17, OP 22, Saliva 17) who initially tested SARS-CoV-2 positive and were recollected within 0 to 14 days, cohort #2 included 37 individuals (NS 37, NP-S 36, NP-VTM 31, OP 37, Saliva 29) who initially tested SARS-CoV-2 positive and were recollected within 15 to 30 days, and cohort #3 included 94 individuals (NS 93, NP-S 87, NP-VTM 66, OP 93, Saliva 68) who tested negative for SARS-CoV-2 and were recollected within 30 days.

2.4. Performance comparison between the Biomeme SARS-CoV-2 Go-Strips and BioFire® RP2.1 SARS-CoV-2 component using clinical samples

Clinical iSpecimen samples were tested simultaneously on the Biomeme SARS-CoV-2 Go-Strips and BioFire® RP2.1. Each sample was extracted using the M1 Sample Prep Cartridge. Purified RNA from the M1 extraction procedure was amplified on the SARS-CoV-2 Go-Strips using the Biomeme Franklin^{TM} three9 platform.

In parallel, amplification and pathogen detection of the iSpecimen clinical samples were performed on the RP2.1 via the BioFire® FilmArray® 2.0 according to manufacturer’s instructions for use [9]. The RP2.1 is a sample-to-answer, nested multiplexed PCR test that identifies targets from 22 different respiratory pathogens. RNA extraction and purification is performed within the RP2.1 assay pouch. The SARS-CoV-2 targets detected in the RP2.1 are spike protein (S) gene and membrane protein (M) gene. Results from the BioFire® RP2.1 (submitted manuscript) were used as the reference standard for calculating sensitivity and specificity on the Biomeme SARS-CoV-2 Go-Strips. The BioFire® RP2.1 was chosen as the reference standard because the assay was the first to receive De Novo marketing authorization from the FDA [10].

2.5. Validation testing of the Biomeme Go-Strips

To validate the results on the Go-Strips, the same purified RNA was amplified and detected on Biomeme SARS-CoV-2 Go-Plates using the ThermoFisher Quantstudio^{TM} 7 Flex. Biomeme SARS-CoV-2 Go-Plates contain the same reagents as the SARS-CoV-2 Go-Strips but in a 96-well format.

2.6. Statistical analyses

Statistical analyses were performed using R version 4.0.3 and the R packages ‘epiR’ and ‘fmsb’. We used Cohen’s kappa statistics to estimate agreement and test the null hypothesis that agreement was random (i.e. kappa statistic equals zero) [11]. We used McNemar’s Chi-square test to test the null hypothesis that the platforms are equivalent in terms of sensitivity and specificity. We used probit regression to estimate the concentration for which 95% of tests would be positive for each of the two targets in the Biomeme Go-Strips. Confidence intervals for the 95% probit estimate were calculated using the inverse method in the ‘investr’ R package [12].

4.0 MAJOR EVENTS/MILESTONES/SUCCESS

In preparation for the execution of this project,

- Kick Off Meeting – 4 JUNE 2020
- IRB Approval – 15 OCT 2020
- All experimental procedures completed – 19 MAY 2021
- Data Analysis – 12 SEP 2021
- Poster presentation –59MDW Commander’s Immersion Brief – 16 AUG 2021
- Manuscript submitted to – Journal of Clinical Virology – 08 OCT 2021
- Dissemination of Results – 25 NOV 2021

5.0 RISK ASSESSMENT

5.1 Risk Analysis:

Risk	Probability	Consequence	Mitigation Strategy
Testing in field conditions could reduce the sensitivity and	3. Occasional - When a risk has a 50% chance or occurring	4. Critical - The consequences are critical and may cause	Perform studies that analyze performance of the Biomeme system in

specificity of the system due to environmental challenges (i.e. contamination)	or a 50% chance of not occurring, it is an occasional risk	a great deal of risk. This risk must be addressed quickly	an austere environment to identify challenges and develop operating procedures to address negative impacts of testing in the field
The Biomeme SARS-CoV-2 Go-Strip assay requires an external reagent, provided in the kit that has a room temperature shelf-life of 7 days after being reconstituted. If reagent expires before using all the Go-Strips in the kit QC will not be available	2. Seldom - A risk in this category is still very rare but more common than those in the unlikely category. These risks still need to be considered and cannot be ruled out yet	2. Marginal - The consequences are marginal and may cause only minor risk. This risk is unlikely to have a huge impact	Request additional RPC kits from the vendor be included with Biomeme SARS-CoV-2 Go-Strips

5.2 Technical Challenges

We experienced technical challenges with the exogenous internal quality control (RPC) of the Biomeme SARS-CoV-2 Go-Strips. The RPC control failed to amplify in several Go-Strips where neither the ORF1ab target nor S target amplified leading to an invalid result. This pattern was also seen in the Go-Plate results indicating that the problem is not a performance issue with the Franklin™ three9, but the Biomeme SARS-CoV-2 assays chemistry or the M1 Sample Prep RNA extraction. We suspect that the failure of the RPC to amplify is caused by reduced sensitivity due to the high volume of elution buffer in the M1 cartridge. We also experienced a higher rate of failure of the RPC control in the NP-VTM samples indicating there are interfering substances in this sample that prevent the RPC from amplifying.

We also experienced amplification of the RPC in our no template control (NTC) after switching to a new lot number of Biomeme SARS-CoV-2 Go-Strips. Biomeme indicates in the user manual for Go-Strips that the RPC control can become airborne and contaminate laboratory spaces. We performed several tests comparing the old lot of Go-Strips to the new lot of Go-Strips to rule out contamination in our laboratory. The same quality control failure was experience in a replacement lot that was sent by Biomeme. Biomeme indicated that this problem is caused by a malfunction of the hardware where the sample is being read as a false positive. We experienced this quality control failure in the three platforms that we originally purchased from the vendor and three replacement units. We suspect that the two lots of SARS-CoV-2 Go-Strips were contaminated with RPC. Biomeme is working to replace the exogenous internal control (RPC) with an endogenous internal control that will amplify human RNA in the sample.

6.0 TRANSITION PLAN

6.1 Military Relevance

The COVID-19 pandemic has negatively impacted military readiness, causing the delay of military operations, quarantine of military personnel, and closure of military facilities. The inability to reliably and rapidly identify microbial pathogens has led to illness with lost duty days and decreased productivity in the military sector. Biomeme, Inc. offers the smallest mobile real-time qPCR system that detects pathogenic diseases using a one-minute field sample prep, lyophilized shelf-stable reagents and test kits, a mobile thermocycler, and software that enables assay analysis in the field. The devices are controlled via a smart device and an iOS application interface for wireless or wired communication. Personnel without any laboratory experience can be trained to operate the machinery in about 30 minutes. For the SARS-CoV-2 assay, Biomeme received an Emergency Use Authorization to the Food and Drug Administration (FDA) for laboratory sample prep, thermocyclers, and assays using oropharyngeal or nasopharyngeal swabs [6].

6.2 Transition Strategy

Accelerate user deployment: External validation of analytical performance with multiple specimen types and collection devices; Share data with vendor to enable FDA EUA and/or 510(k) submission; Prepare guidance for operational use; Final report; Presentation of findings at MHSRS.

7.0 RESULTS

3.1. Performance of Biomeme Go-Strips at low viral loads

Eight dilutions, ranging from 4.20 x 10^7 copies/mL to 420 copies/mL were tested to evaluate the performance of the Biomeme SARS-CoV-2 Go-Strip assay including M1 extraction at low viral concentrations (Table 1). At 4.20 x 10^3 copies/mL all replicates yield a positive result with the S gene detected in all replicates (average Ct of 36.7, SD: 0.9) and ORF1ab gene detected in five out of six replicates (Ct values of 37.0, 38.4, 38.5, 38.3, 37.5). SARS-CoV-2 was inconsistently detected in replicates at concentrations of 2.10 x 10^3 copies/mL and lower, with a significant drop off at 1.05 x 10^3 copies/mL. Probit regression indicates that the ORF1ab gene target would be positive in 95% of tests at a concentration of 6135.6 (95% CI = [3872.7, 75916.8]) copies/mL and the S gene would be positive in 95% of tests at a concentration of 3349.8 (95% CI = [2415.8, 9942.5]) copies/mL.

3.2. *Assessment of cross-reactivity between SARS-CoV-2 Go-Strip primers and near-neighbor pathogens*

We tested genomic material from nineteen near-neighbor upper respiratory bacterial and viral pathogens, including six strains of other coronaviruses, for cross-reactivity with targets in the Biomeme SARS-CoV-2 Go-Strips assay (Table 2). For each near-neighbor pathogen, no targets were detected in the SARS-CoV-2 Go-Strips. As a positive control, all near-neighbors upper respiratory pathogens that are target on the BioFire® RP2.1 were detected.

3.3. Agreement between Biomeme SARS-CoV-2 Go-Strips compared to BioFire® Respiratory Panel 2.1

We performed comparative diagnostic testing using four different clinical upper respiratory sample types (NS, NP-VTM, NP-S, OP, and saliva) to estimate positive percent agreement and negative percent agreement of the Biomeme SARS-CoV-2 Go-Strips when compared to the BioFire® RP 2.1. The clinical samples were collected from 1 to 28 days after an initial CLIA test, resulting in a range of viral loads from participants including samples with viral loads below the detection threshold for the BioFire RP2.1. Agreement between the Biomeme SARS-CoV-2 Go-Strips and the BioFire® RP 2.1 as measured by

Cohen’s kappa coefficients were 0.92, 0.82, 0.79, 0.75, and 0.68 for NS, NP-VTM, NP-S, OP, and saliva respectively. There were statistically significant differences in the marginal frequencies as measured by McNemar test in three of the five sample types. Discordant results between the two platforms were overwhelming in Cohort #1 and Cohort #2 samples. Taken together this indicates that the Biomeme SARS-CoV-2 Go-Strips were not as sensitive in detecting SARS-CoV-2 as the BioFire® RP2.1. Counts for the comparative testing, positive percent agreement, and negative percent agreement are shown in Table 3.

3.4. Validation of Biomeme SARS-CoV-2 Go-Strips against Biomeme SARS-CoV-2 Go-Plates

We validated our results on the Biomeme SARS-CoV-2 Go-Strips by analyzing the same clinical samples on the Biomeme SARS-CoV-2 Go-Plates. There was a high concordance between the Go-Strip and Go-Plate results. Cohen’s kappa estimates ranged from 0.86 to 0.97 across specimen types. There were no statistically significant differences between the marginal frequencies, indicating that the Biomeme SARS-CoV-2 Go-Strips are equivalent in sensitivity to the Biomeme SARS-CoV-2 Go-Plates. Counts for the comparative testing, positive percent agreement, and negative percent agreement are shown in Table 4.

8.0 CONCLUSION/DISCUSSION

As the Biomeme Franklin^{TM} three9 Real-Time PCR device has been successfully deployed for military environmental surveillance applications [13], we sought to evaluate if the system could potentially be used in remote settings for the detection of SARS-COV-2. This study demonstrates that the Biomeme Franklin^{TM} three9 Real-Time PCR Thermocycler and SARS-CoV-2 Go-Strip Assay may be an effective platform for the detection of SARS-CoV-2 in remote settings. We showed that the assay can reliably detect SARS-CoV-2 at viral concentrations of 4.20 x 10^3 copies/mL and found no cross-reactivity with select near-neighbor upper respiratory tract pathogens. We also demonstrated agreement that is statistically significantly greater than chance between the Biomeme SARS-CoV-2 Go-Strips and BioFire® RP2.1 in all four clinical upper respiratory sample types tested including nasopharyngeal swab in VTM, the current gold standard sample used for RT-PCR detection of SARS-CoV-2. The Go-Strips showed reduced sensitivity as compared to the BioFire® RP2.1; however, these results were expected based on the differences in limit of detections reported by the manufacturers. BioFire® reports a limit of detection of 500 copies/mL for the RP2.1 and Biomeme reports a limit of detection of 1,800 copies/mL [8, 9]. A high number of false negatives occurred in oropharyngeal swabs and saliva which we suspect is due to differences in viral load at the various collection sites [14, 15].

We speculate that the sensitivity of the Biomeme SARS-CoV-2 Go-Strip assay could be improved by lowering the volume of elution buffer in the M1 Sample Prep Cartridge. The cartridge contains approximately 800 µL of Biomeme Elution Buffer, where other extraction kits elute in approximately 1/16th of the volume [16]. The high elution volume in the M1 extraction process is necessary to ensure complete saturation of the binding column. The advantage of the M1 extraction process is that no centrifugation of external reagents, aside from external endogenous controls, are required for RNA isolation and purification. Biomeme recently commercialized the next iteration of their mobile PCR device, the Franklin™ three9 ISP [17]. The new system has integrated sample processing built into the assay, where DNA/RNA is automatically extracted and purified from crude liquid samples and up to 27 PCR targets are detected in one sample. Eliminating the need for manual extraction using the M1 Sample Prep Cartridge could increase the sensitivity and performance of Biomeme assays.

This study should be interpreted in light of the follow limitations. It is possible that the near-neighbor pathogens could be positive in the Biomeme SARS-CoV-2 Go-Strips if the genomic concentrations were higher. Testing in this study was done in a controlled laboratory environment so it is possible that performance of the Biomeme system would be negatively impacted in an austere environment. Further studies should be conducted to evaluate the Biomeme system in field conditions. Lastly, the sample collection in this study lagged identification of CLIA testing. In positive samples many participants may have cleared the virus or have substantially lower viral loads than they would upon an initial presentation to a clinical setting for testing earlier in the course of illness. As a result, we speculate that there is an enrichment for samples around or below the limit of detection for the devices in this study. Thus, the positive percent agreement between the Biomeme SARS-CoV-2 Go-Strips and the BioFire® RP2.1 might be expected to be lower than if the study design compared clinic samples collected at the peak of viral shedding (earlier in the course of illness, closer to the typical presentation to the clinic for illness).

The Biomeme Franklin™ three9 and SARS-CoV-2 Go-Strip assay provide healthcare facilities, especially those in rural areas, and personnel in remote settings an affordable, easy-to-use molecular diagnostic platform that uses almost no space on the benchtop. The Biomeme system does not require specialized laboratory equipment or cold-storage reagents. Results for up to nine samples can be obtained in less than two hours, including the time for processing and extraction and minimal training is required to learn the procedure. Our evaluation of the sensitivity and specificity of the Biomeme SARS-CoV-2 Go-Strips supports results reported in the Food and Drug Administration to support Emergency Use Authorization. Future studies should be performed comparing the sensitivity and specificity of the Biomeme SAR-CoV-2 Go-Strips assay to other SARS-CoV-2 detection platforms to provide a better understanding of the performance of the system.

9.0 DELIVERABLES

9.1 Publications: Journal of Clinical Virology, 25 NOV 2021

9.2 Presentations: 59MDW Commanders Immersion Brief, 16 AUG 2021

10.0 COST

$500,000 of funding was received from the Defense Health Agency CARES Act.

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FIGURES AND TABLES:

Table 1

Dilution Series of Heat-Inactivated SARS-CoV-2 Tested on Biomeme SARS-CoV-2 Go-Strips

Dilution	Copies/mL	Biomeme Go-Strips[a] (Orf1ab)[b]	Biomeme Go-Strips[a] (S)[b]
1×10^{-1}	42000000	6/6 (24.2 ± 5.2)	6/6 (23.7 ± 0.3)
1×10^{-2}	4200000	6/6 (28.0 ± 0.8)	6/6 (26.8 ± 0.4)
1×10^{-3}	420000	6/6 (29.8 ± 0.3)	6/6 (29.3 ± 0.4)
1×10^{-4}	42000	6/6 (33.2 ± 1.6)	6/6 (32.8 ± 0.3)
1×10^{-5}	4200	5/6 (37.0, 38.4, 38.5, 38.3, 37.5)	6/6 (36.7 ± 0.9)
2×10^{-5}	2100	2/6 (39.8, 37.3)	4/6 (37.7, 37.6, 36.4, 36.7)
4×10^{-5}	1050	3/6 (38.0, 38.5, 37.9)	0/6 (N/A)
1×10^{-6}	420	1/6 (38.1)	1/6 (36.3)

[a] Number of replicates that tested positive divided by total replicates tested

[b] Mean Ct value ± standard deviation where all replicates test positive or individual Ct values

Table 2

Near-Neighbor Pathogen Panel Tested on Biomeme SARS-CoV-2 Go-Strips and BioFire® RP2.1

Near-Neighbor Organism	Reference#	Biomeme SARS-CoV-2 Go-Strip	BioFire RP2.1
Coronavirus 229E	VR-740D	Negative	Detected
Coronavirus OC43	VR-1558D	Negative	Detected
Coronavirus NL63	VR-3263SD	Negative	Detected
Coronavirus HKU1	VR-3262SD	Negative	Detected
Parainfluenza 2	VR-92D	Negative	Detected
Parainfluenza 3	VR-93D	Negative	Detected
Parainfluenza 4	VR-1377D	Negative	Detected
Bordetella pertussis	9797DQ	Negative	Detected
Bordetella parapertussis	15311DQ	Negative	Detected
Mycoplasma pneumoniae	15531D	Negative	Detected
Human Rhinovirus 17	VR-1663D	Negative	Detected
MERS-CoV	VR-3248SD	Negative	N/A[a]
SARS-CoV-1	VR-3280SD	Negative	N/A[a]
Enterovirus	VR-1775DQ	Negative	Detected
Human metapneumovirus	VR-3250SD	Negative	Detected
Influenza A	VR-1736D	Negative	Detected
Influenza B	VR-1813	Negative	Detected
Respiratory Syncytial virus	VR-1580DQ	Negative	Detected
Chlamydia pneumoniae	VR-1360D	Negative	Detected

[a] Organisms not included on the BioFire® Respiratory Panel 2.1

Table 3

Clinical Evaluation of Biomeme SARS-CoV-2 Go-Strips with BioFire® RP2.1 as Reference Standard

Sample Type	Detected BioFire/ Biomeme	Detected BioFire Only	Detected Biomeme Only	Not Detected BioFire/ Biomeme	Kappa	McNemar test p-value	PPA[a]	NPA[b]
Nasal Swab	20	3	0	128	0.919 [0.828,1.01], $p <$ 6.77e-11	0.2482	0.87 [0.66, 0.97]	1.00 [0.97, 1.00]
NP saline	23	8	0	114	0.819 [0.697,0.941], $p <$ 3.382e-11	0.0133	0.74 [0.55, 0.88]	1.00 [0.97, 1.00]
NP VTM	20	7	1	86	0.790 [0.649,0.930], $p <$ 1.195e-9	0.0771	0.74 [0.54, 0.89]	0.99 [0.94, 1.00]
Oral Swab	21	11	0	120	0.751 [0.609,0.893], $p <$ 2.2e-16	0.002569	0.66 [0.47, 0.81]	1.00 [0.97, 1.00]
Saliva	19	12	1	82	0.676 [0.510,0.842], $p <$ 5.215e-8	0.005546	0.61 [0.42, 0.78]	0.99 [0.93, 1.00]

[a] Positive percent agreement

[b] Negative percent agreement

Table 4

Comparison of Biomeme SARS-CoV-2 Go-Strips with Biomeme SARS-CoV-2 Go-Plate

Sample Type	Detected Go-Strip/ Go-Plate	Detected Go-Plate Only	Detected Go-Strip Only	Not Detected Go-Strip/ Go-Plate	Kappa	McNemar test p-value	PPA[a]	NPA[b]
Nasal Swab	19	0	1	129	0.971 [0.913,1.03], $p < 6.42e-11$	1	1.00 [0.82, 1.00]	1.00 [0.96, 1.00]
NP saline	22	0	1	122	0.974 [0.922,1.025], $p < 1.376e-12$	1	1.00 [0.85, 1.00]	0.99 [0.96, 1.00]
NP VTM	20	0	1	67	0.968 [0.906,1.031], $p < 6.258e-12$	1	1.00 [0.83, 1.00]	0.99 [0.92, 1.00]
Oral Swab	19	3	2	125	0.864 [0.747,0.981], $p < 7.673e-10$	1	0.86 [0.65, 0.97]	0.98 [0.94, 1.00]
Saliva	17	0	3	91	0.903 [0.794,1.011], $p < 1.77e-9$	0.2482	1.00 [0.80, 1.00]	0.97 [0.91, 0.99]

[a] Positive percent agreement

[b] Negative percent agreement

12.0 LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

SARS-CoV-2

COVID-19

RT-PCR

DNA

RNA

PCR

FDA

ORF1ab

S

RPC

MERS-CoV-1

SAR-CoV-1

RP2.1

CLIA

IRB

NS

NP-S

NP-VTM

OP