Evaluation of the Biomeme Next Generation Molecular Detection System Comparing Go-Strips to Go-Plates

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ABSTRACT

- The COVID-19 pandemic has impacted military readiness, creating the need for accessible RT-PCR assays that can rapidly detect SARS-CoV-2.
- Biomeme has developed a mobile, real-time molecular detection platform, the Franklin three9, that accommodates Biomeme's Go-Strip assay which is pre-loaded with lyophilized PCR reagents for the detection of SARS-CoV-2. Biomeme also produces the SARS-CoV- 2 assay in a 96-well plate format to be run on other PCR devices.
- The focus of this research is to compare the sensitivity and specificity of the Biomeme SARS-CoV-2 Go-Strips to the SARS-CoV-2 Go-Plates in five upper respiratory sample types using the BioFire Respiratory Panel (RP2.1) assay as a reference standard.
- Our results demonstrate consistency in SARS-CoV2 detection between the two formats. Using Biofire RP2.1 as a reference standard, our results demonstrate high specificity in the assays and reduced sensitivity in both systems.

INTRODUCTION

- The COVID-19 pandemic has negatively impacted military readiness, causing the delay of military operations, quarantine of military personnel, and closure of military facilities[1]. Rapid, early detection of SARS-CoV-2 can prevent the outbreaks[2] by informing infected personnel on the need to quarantine. RT-PCR provides a reliable, sensitive, and rapid method for detecting SARS-CoV2 in clinical upper respiratory samples[3].
- The Biomeme Franklin three9 (**Figure 1**) is a small lightweight, portable, battery-powered qPCR device that can test biological samples in the field without centrifugation, the use of frozen reagents, or a power source. The device can detect three targets in up to nine samples in a single run.
- The Biomeme SARS-CoV-2 Go-Strips are designed specifically for use with the Franklin three9 instrument. The assay reagents are lyophilized for the detection of SARS-CoV-2 gene targets open reading frame 1ab (ORF1ab) and spike (S). The ORF1ab gene is FAM-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.
- The Biomeme SARS-CoV-2 Go-Plates are designed for use on the Thermo QuantStudio qPCR platforms. The lyophilized master mix has the same chemistry as the Go-Strip but in a 96-well plate format. The only difference between the Go-Strips and the Go-Plates are the fluorescent dyes used. The Texas RedX dye is replaced with ROX dye and the ATTO647N dye is replaced with Cy5 dye on the Go-Plate. The FAM dye is the same on both platforms.
- Before RT-PCR amplification can be performed on Biomeme assays, RNA must be extracted and purified. Biomeme developed a filtration-based manual extraction method, the M1 Sample Prep Cartridge, 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.
- The BioFire Respiratory Panel 2.1 (RP2.1), run on the BioFire FilmArray 2.0, is a separate molecular platform for detecting SARS-CoV-2. The RP2.1 is a real-time, nested multiplexed PCR test that identifies targets from 22 different respiratory pathogens. The SARS- CoV-2 targets detected in the RP2.1 are spike protein (S) gene and membrane protein (M) gene.
- In the work reported here, we compare detection of SARS-CoV-2 between Biomeme Go-Strips run on the Franklin three9 and Biomeme Go-Plates run on the QuantStudio7 using the BioFire RP2.1 as the reference standard in five different upper respiratory sample types.

Figure 1: Biomeme Franklin three9 System

METHODS

Sample Preparation:

- iSpecimen, Inc. (Lexington, MA), enrolled and collected five different sample types from more than 200 individuals. 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 61 individuals who initially tested SARS-CoV-2 positive and were recollected within 0 to 14 days. Cohort #2 included 47 individuals who initially tested SARS-CoV2 positive and were recollected within 15 to 30 days, and cohort #3 included 108 individuals who were SARS-CoV-2 negative.

RNA extraction:

- RNA purification and extraction was performed with the M1 sample prep cartridge (**Figure 2**) that consists of pre-aliquoted buffers (Biomeme Protein Wash, Biomeme Wash Buffer, Biomeme Drying Wash, Biomeme Elution Buffer) for a six simple step manual extraction method.
- Using a 1mL luer lock syringe, samples were lysed with Biomeme Lysis Buffer and passed through the M1 Sample Prep column, binding RNA to the silica membrane inside the column.
- Each subsequent wash removes unwanted salts and material until the final step where the RNA bound to the column is eluted in Biomeme Elution Buffer.
- RNA from all of the iSpecimen samples was extracted and purified using the M1 extraction cartridge.
- Purified RNA from a single extraction was used for testing on both the Biomeme Go-Strips and Go-Plates.

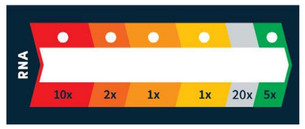

Figure 2: M1 Extraction Cartridge

Biomeme Go- Strips:

- Biomeme Go-Strips contain three reaction wells. 20uL of extracted RNA are pipetted into each reaction well of the Go-Strip.
- Once all wells of a single Go-Strip are used, a void filling cap is placed into the Go-Strip to minimized contamination.
- Go-Strips are then placed into the 3-well slots on the Franklin three9 thermocycler where target genes are identified through RT-PCR and fluorescent detection utilizing three fluorophore channels.
- The run is setup on a smartphone installed with the Biomeme Go app. The phone connects by to the Franklin three 9 by Bluetooth or USB and allows you to run, monitor, and analyze your test results.

Biomeme Go-Plates:

- Biomeme Go-Plates consist of a 96-well reaction plate where each well contains the same lyophilized master mix as the Biomeme Go-Strips.
- As in the Go-Strips, 20uL of purified RNA sample is added to each well and mixed with the lyophilized master mix.
- Plates are then loaded and run on Thermo QuantStudio 7, using QuantStudio Flex software for analysis.
- Quantstudio RT-PCR uses FAM dye as its target gene for Orf1ab, Cy5 dye as a target gene for RPC, and ROX dye for a target gene for S gene. Analysis of qPCR results is performed on Quantstudio for detection of SARS-CoV-2.

Specificity/Sensitivity Testing

- Specificity/Sensitivity of the Biomeme Go-Strips and Go-Plates were calculated using the BioFire RP2.1 as the reference standard.

RESULTS

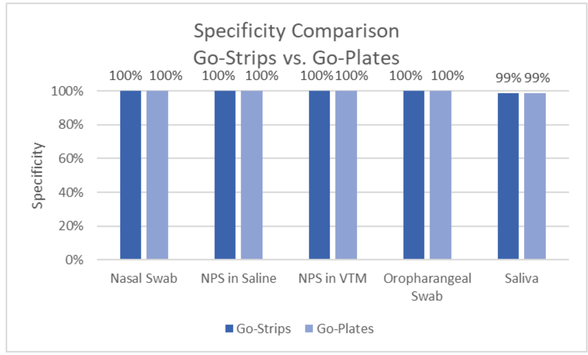

Figure 3: Comparison of specificity between the Biomeme Go-Strips and Go-Plates stratified by sample type. BioFire RP2.1 used as reference standard.

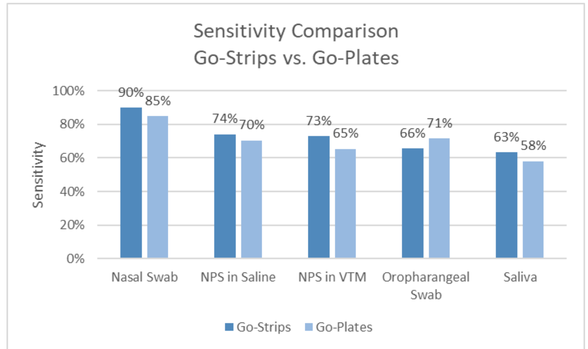

Figure 4: Comparison of sensitivity between the Biomeme Go-Strips and Go-Plates stratified by sample type. BioFire RP2.1 used as reference standard.

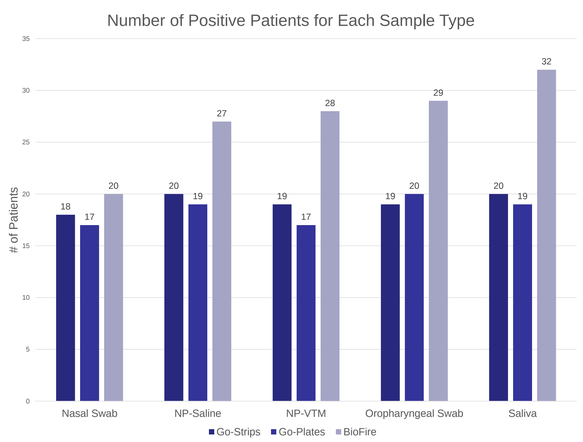

Figure 5: Comparison of overall positives between the Biomeme Go-Strips, Biomeme Go-Plates, and BioFire RP2.1 stratified by sample type.

DISCUSSION

- The BioFire RP2.1 was chosen as the reference standard for sensitivity/specificity testing on the Biomeme SARS-CoV-2 Go-Strips and Go-Plates because the assay has received De Novo clearance from the FDA.
- Both Biomeme SARS-CoV-2 Go-Plates and Go-Strips are highly specific (**Figure 3**) in detection of the SARS-CoV-2. In only one sample, SARS-CoV-2 was detected in the Biomeme Go-Strips and Go-Plates that was not detected in the BioFire RP2.1 assay.
- Some variation can be seen between the sensitivity (**Figure 4**) of the Biomeme SARS-CoV-2 Go-Strips and Go-Plates. Discrepancies in detection may be due to RNA concentrations near the limit of detection threshold.
- Reduced sensitivity is seen in both SARS-CoV-2 Go-Plates and Go-Strips. This result is expected since Biomeme reports a lower limit of detection for the Go-Strips and Go-Plate assays than BioFire reports for the RP2.1.
- Although the Nasal Swab sample type has the highest sensitivity, this does not indicate Nasal Swab specimens are an optimal collection method for detection of SARS-CoV-2. Overall SARS-CoV-2 was not detected as frequently in this matrix (**Figure 5**), even in the BioFire 2.1, causing the sensitivity to be elevated.
- Reduced sensitivity could be due to the large elution volume used in the M1 extraction cartridge.
- Once all samples have been tested on all platforms, statistical analyses will be performed to determine significance of results.

Future Aims

- Biomeme has developed the next iteration of their mobile PCR device, the Franklin three9 ISP (**Figure 6**). The new system has integrated sample prep built into the assay, eliminating the need for manual extraction using the M1 Sample Prep Cartridge. DNA/RNA is automatically extracted and purified from crude liquid samples and 27 PCR targets are detected in one sample.
- We aim to acquire the Franklin three9 ISP system and compare performance to other commercially developed PCR platforms. We predict that the new system will be more sensitive than previous generations of Biomeme platforms because of the elimination of the manual extraction method.

Figure 6: Biomeme Franklin three9 ISP

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