repeats were NAAT-positive, in order to identify which patients might benefit from repeat NAAT for SARS-CoV-2, and the appropriate interval.

Methods: We conducted an IRB-approved retrospective review of laboratory and electronic medical record data for all patients evaluated for SARS-CoV-2 infection at the Mount Sinai Health System, whose initial NAATs were done between March 16 – March 30, 2020, and who were retested within one month. NAATs were performed on NPS in viral transport medium using the Roche Diagnostics cobas® 6800 SARS-CoV-2 Test. Baseline patient characteristics, clinical and radiographic findings were identified.

Results: Of 235 patients eligible for inclusion, 172 (70.5%) were initially NAAT-negative, and 118 (68.6%) remained NAAT-negative over 1 month follow up. 54 (31.4%) converted to NAAT-positive over the next 1-month. Of patients who became NAAT-positive, 31 (57.4%) were inpatients who converted results within a single admission; the average interval was 6d 7h between the NAAT-negative and NAAT-positive results, and the minimum interval was 10.5 h. Symptoms examined for correlation for conversion to NAAT-positive were: fever, cough, shortness of breath, and combined nausea/vomiting/diarrhea. Duration of symptoms reported at triage did not appear to affect time to conversion to NAAT-positive. No individual symptom was more likely to be associated with conversion to NAAT- positive. However, time to conversion to NAAT-positive was shorter for patients with multiple symptoms. In general, chest radiography (CXR) findings correlated with NAAT results; interval to NAAT-positive was shorter for patients with worsening CXR findings.

Conclusion: Our data supports repeat testing in patients with multiple clinical symptoms suggestive of SARS CoV-2 infection and negative initial NP test results. Further studies are needed to determine the true clinical sensitivity and specificity of SARS-CoV-2 NAAT assays.

Disclosures: **All Authors**: No reported disclosures

422. Performance Evaluation of a Rapid and Easy-to-Use COVID-19 Test

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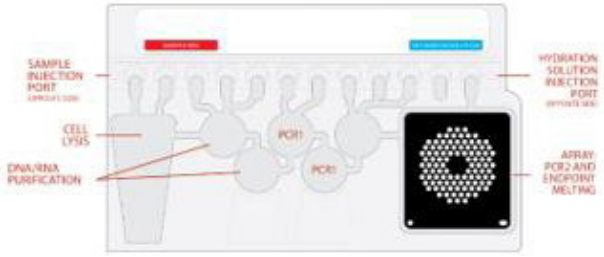
Session: P-13. COVID-19 Diagnostics

Background: The BioFire® COVID-19 Test is a qualitative test for use on the FilmArray® 2.0 and Torch systems for the detection of SARS-CoV-2 RNA in nasopharyngeal swabs (NPS) in transport media. This test received Emergency Use Authorization from the FDA.

A closed, disposable pouch contains all the necessary reagents for sample preparation, nucleic acid extraction, reverse transcription, polymerase chain reaction (PCR), and amplified nucleic acid detection to identify RNA from SARS-CoV-2 virus in an NPS specimen. Internal controls monitor all stages of the test process. Once an NPS sample (0.3 mL) is loaded into the system disposable pouch (Figure 1), the fully automated test returns results within an hour. As an additional resource, the BioFire® COVID-19 Test External Control Kit (+) includes positive external control material that may be used for quality control and laboratory verification.

Figure 1. BioFire COVID-19 Test Disposable Pouch



Methods: The following were evaluated:

- Limit of Detection (LoD)
- Positive and Negative Percent Agreement (PPA and NPA, respectively) for clinical contrived samples and a limited number of clinical specimens
- Exclusivity

Results: • LoD

The LoD was evaluated using live SARS-CoV-2 virus (cultured from the USA_WA1/2020 strain obtained from World Reference Center for Emerging Viruses and Arboviruses (WRCEVA)). The LoD was determined to be 3.3E+02 GC/mL (2.2E-02 $TCID_{50}$/mL).

- Clinical Contrived

Accurate detection of virus in clinical matrix was demonstrated at various LoD levels using thirty contrived individual unique clinical samples (PPA), and 66 individual unique negative clinical specimens (NPA).

- Clinical Samples

Positive samples were collected from patients presenting with signs or symptoms of COVID-19, and who were previously identified as positive for SARS-CoV-2 by another EUA test. Negative samples were collected in 2018, and therefore presumed negative for SARS-CoV-2.

- Exclusivity

The potential for cross-reactivity was evaluated for six viruses from the same genetic family as SARS- CoV-2, and for an additional 30 high priority organisms/viruses. No cross-reactivity was observed.

Table 1. SARS-CoV-2 Virus Test Results at 1× and 0.1× LoD for the BioFire COVID-19 Test

×LoD	Concentration Tested		Test Result (% Detection)		
	GC/mL	$TCID_{50}$/mL	Detected	Equivocal	Not Detected
1×	3.3E+02	2.2E-02	20/20 (100%)	0/20 (0%)	0/20 (0%)
0.1×	3.3E+01	2.2E-03	14/20 (70%)	5/20 (25%)	1/20 (5%)

Table 2. Clinical Contrived and Negative Testing with the BioFire COVID-19 Test

	Agreement with Known Analyte Composition			
	PPA	%	NPA	%
Overall Agreement	30/30	100%	66/66	100%
95% CI	[88.6 – 100]		[94.5-100]	

Table 3. BioFire COVID-19 Test Performance Summary

Virus	PPA			NPA		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
SARS-CoV-2	9/10 [a]	90%	59.6-98.2%	5/5	100%	56.6-100.0%

[a]One sample had a late Ct value when originally evaluated on the NECOV19 test. When the sample was retested on the NECOV19 test, the result was negative. These results indicate a near-LoD level of SARS-CoV-2 virus and/or sample degradation.

Conclusion: The BioFire COVID-19 Test reliably detects SARS-CoV-2 virus RNA in clinically relevant samples.

Disclosures: Corike Toxopeus, PhD, BioFire Defense, LLC. (Employee, stock owner) Brian Jones, PhD., BioFire Defense, LLC (Employee, own stock) Jessica Brown, BS, BioFire Defense (Employee, Stock owner) Mark Gurling, PhD, BioFire Defense, LLC (Employee) Cynthia Andjelic, PhD., BioFire Defense (Employee, Other Financial or Material Support, Own stocks) Cynthia L. Phillips, PhD, BioFire Defense (Employee, Scientific Research Study Investigator, Shareholder)BioFire Defense (Employee, Scientific Research Study Investigator, Shareholder)

423. SARS-CoV-2 NGS Assay Powered by Biotia COVID-DX Software

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Session: P-13. COVID-19 Diagnostics

Background: COVID-19 had spread quickly, causing an international public health emergency with an alarming global shortage of COVID-19 diagnostic tests. We developed and clinically validated a next-generation sequencing (NGS)-based target enrichment assay with the COVID-DX Software tailored for the detection, characterization, and surveillance of the SARS-CoV-2 viral genome.

Methods: The SARS-CoV-2 NGS assay consists of components including library preparation, target enrichment, sequencing, and a COVID-DX Software analysis tool. The NGS library preparation starts with extracted RNA from nasopharyngeal (NP) swabs followed by cDNA synthesis and conversion to Illumina TruSeq-compatible libraries using the Twist Library Preparation Kit via Enzymatic Fragmentation and Unique Dual Indices (UDI). The library is then enriched for SARS-CoV-2 sequences using a panel of dsDNA biotin-labeled probes, specifically designed to target the SARS-CoV-2 genome, then sequenced on an Illumina NextSeq 550 platform. The COVID-DX Software analyzes sequence results and provides a clinically oriented report, including the presence/absence of SARS-CoV-2 for diagnostic use. An additional research use only report describes the assay performance, estimated viral titer, coverage across the viral genome, genetic variants, and phylogenetic analysis.

Results: The SARS-CoV-2 NGS Assay was validated on 30 positive and 30 negative clinical samples. To measure the sensitivity and specificity of the assay, the positive and negative percent agreement (PPA, NPA) was defined in comparison to an orthogonal EUA RT-PCR assay (PPA [95% CI]: 96.77% [90.56%-100%] and NPA [95% CI]: 100% [100%-100%]). Data reported using our assay defined the limit of detection to be 40 copies/ml using heat-inactivated SARS-CoV-2 viral genome in clinical matrices. In-silico analysis provided >99.9% coverage across the SARS-CoV-2 viral genome and no cross-reactivity with evolutionarily similar respiratory pathogens.

Conclusion: The SARS-CoV-2 NGS Assay powered by the COVID-DX Software can be used to detect the SARS-CoV-2 virus and provide additional insight into viral titer and genetic variants to track transmission, stratify risk, predict outcome and therapeutic response, and control the spread of infectious disease.

Disclosures: Dorottya Nagy-Szakal, MD PhD, Biotia (Employee) Mara Couto-Rodriguez, MS, Biotia (Employee) Joseph Barrows, MS, Biotia, Inc. (Employee, Shareholder) Heather L. Wells, MPH, Biotia (Consultant) Marilyne Debieu, PhD, Biotia (Employee) Courteny Hager, BS, Biotia (Employee) Kristin Butcher, MS, Twist Bioscience (Employee) Siyuan Chen, PhD, Twist Bioscience (Employee) Christopher Mason, PhD, Biotia (Board Member, Employee, Shareholder) Niamh B. O'Hara, PhD, Biotia (Board Member, Employee, Shareholder)Twist (Other Financial or Material Support, I am CEO of Biotia and Biotia has business partnership with Twist)