Comparison of Next Generation Diagnostic Systems (NGDS) for the Detection of SARS-CoV-2

< Antonio O. Sanchez, Anna R. Ochoa, Sallie L. Hall, Chet R. Voelker, Rachel E. Mahoney, Jennifer S. McDaniel, August Blackburn, Susana N. Asin, Tony Yuan >

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~~If other collaborating entities, add insignia~~

59th Medical Wing
Office of the Chief Scientist
1632 Nellis, BLDG. 5406
JBSA Lackland AFB, TX 78236-7517

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for the Detection of SARS-CoV-2

Ruben O'Neal, DAF
Program Analyst
Diagnostics and Therapeutics
~~Program~~
59MDW Office of the Chief Scientist

Antonio O. Sanchez II.
Clinical Research Molecular Scientist, CAMD
59MDW Office of the Chief Scientist

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Antonio O. Sanchez, Anna R. Ochoa, Sallie L. Hall, Chet R. Voelker, Rachel E. Mahoney, Jennifer S. McDaniel, August Blackburn, Susana N. Asin, Tony Yuan

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Over the last year, the COVID-19 pandemic has severely impacted lives of millions of US citizens, including active-duty service members and their beneficiaries. In response, the Department of Defense (DoD) began enterprise-wide testing at its medical centers, hospitals, and health clinics, which contributed and underscored the scarcity of collection and detection reagents throughout the country. Throughout the expansion of testing, it became apparent that there is need for specific and accurate detection of SARS-CoV-2 due to the overlap of its clinical symptoms with other respiratory pathogens, such as influenza and SARS-CoV-1. To circumvent the reliance on NS-based collection as well as to assess the efficiency of Next Generation Detection Systems (NGDS) with integrated sample processing to detect SARS-CoV-2 and other common respiratory pathogens, the Center for Advanced Molecular Detection (CAMD) evaluated two NGDS platforms: the Cepheid GeneXpert® IV and the BioFire® FilmArray® 2.0 systems with the objectives of (1) comparing BioFire® FilmArray® and the Cepheid GeneXpert® platforms for their limit of detection and specificity to detect SARS-CoV-2, and (2) validate the use of additional collected specimen types to include NP in saline, oropharyngeal (OP) and nasal swabs (NS), as well as saliva for SARS-Cov-2 detection. Patient specimens were provided by iSpecimen® to The Center for Advanced Molecular Detection (CAMD) at Joint Base San Antonio (JBSA) – Lackland for testing on BioFire® FilmArray® and the Cepheid GeneXpert® platforms using BioFire® FilmArray® RP2.1 and Cepheid Xpert® Xpress SARS-CoV-2 kits, respectively. After evaluating 216 unique patient pins, results demonstrated that both platforms performed similarly, demonstrating > 89% agreement on every sample type. Furthermore, it was determine that saline is an adequate substitute for VTM.

15. SUBJECT TERMS- COVID19, SARS-CoV-2, Diagnostic Testing, Polymerase Chain Reaction, Next Generation Detection Systems

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1.0 EXECUTIVE SUMMARY

Diagnostic testing for infectious diseases is essential for force health protection and mission readiness and is consequently a high priority for the United States Department of Defense. The World Health Organization declared Coronavirus Disease 2019 (COVID-19) a pandemic in March 2020. Early in the pandemic, testing procedures for COVID-19 relied heavily on the use of nasopharyngeal (NP) swabs and real-time polymerase chain reaction (RT-PCR) assays targeting SARS-CoV-2, the virus that causes COVID-19. In addition, there were supply chain disruptions coupled with increased demand that led to shortages of essential laboratory supplies like viral transport media (VTM). The overlap of clinical symptoms between COVID-19 and illness caused by other respiratory pathogens underscores the need for specific and accurate multi-target detection assays. Furthermore, the discomfort of procuring nasopharyngeal swabs highlights the necessity to expand the types of biological specimens that can be used for diagnostic testing. In this study we 1) compared the sensitivity and specificity of Cepheid GeneXpert® IV and the BioFire® FilmArray® 2.0 systems, Next Generation Detection Systems (NGDS) with integrated sample processing, to detect SARS-CoV-2, 2) evaluated the performance of these NGDS using different sample types, and 3) assessed saline as an alternative to VTM for sample storage and shipping. Limit of detection testing indicated that the Cepheid GeneXpert® IV is more sensitive than the BioFire® FilmArray® 2.0. Comparative testing using 1) nasopharyngeal swabs in VTM, 2) nasopharyngeal swabs in saline, 3) nasal swabs, and 4) oropharyngeal swabs from 216 study participants are consistent with the Cepheid GeneXpert® being more sensitive than the BioFire® FilmArray® RP2.1. Conversely, testing saliva on the Cepheid GeneXpert® IV demonstrated statistically significant lower sensitivity compared to the BioFire® FilmArray® RP2.1, counter to other results in this study. Nasopharyngeal swabs stored and shipped in saline were non-inferior (McNemar test) to VTM and Cohen's kappa statistic showed "substantial agreement" or "almost perfect agreement"based on comparative testing on both platforms. This finding supports the use of saline in place of VTM when VTM is not readily available. Overall, our results indicate that SARS-CoV-2 was detected in all five biospecimen types and there was agreement between the two RT-PCR platforms in the numbers of positive and negative samples. Both qualitative RT-PCR tests allowed for the rapid and specific identification of SARS-CoV-2 in a wide range of biological samples, providing the Military Health System with reliable and accurate diagnostic platforms to detect a variety of respiratory tract infections.

2.0 INTRODUCTION

In late 2019, a novel respiratory pathogen appeared in Wuhan city, Hubei province, China with symptoms resembling both influenza and pneumonia [1, 2]. Initial sequencing determined the respiratory pathogen was a new virus of the genus Betacoronavirus, within the family Coronaviridae [3], which was subsequently named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). SARS-CoV-2 spread across China, Europe, and the rest of the world, and by March 2020 the World Health Organization declared COVID-19 a pandemic. The COVID-19 pandemic has been consequential, most notably for overwhelming health care systems and causing increased mortality year over year compared to years prior to the pandemic. As a result, governments around the world have been forced to implement and depend on non-pharmaceutical, public health interventions such as lockdowns, social distancing, and mask mandates to help slow the spread of COVID-19. Early in the pandemic, diagnostic testing for SARS-CoV-2 was delayed due to global supply chain disruptions not only with the availability

of testing kits but also transport media, swabs and collection tubes. Due to these limitations, COVID-19 testing in the U.S. was initially provided only to those with acute respiratory symptoms, severe respiratory distress, and health care workers. Real Time Polymerase Chain Reaction (RT-PCR), the gold standard for diagnosis of SARS-CoV-2, requires 1-2 days from sample processing to results leading to delayed public health interventions and increased community spread of the virus [4]. Consequently, the U.S. Food and Drug Administration (FDA) worked with diagnostic developers to help fast-track development of assays with quicker turnaround times [5, 6]. The requests for Emergency Use Authorization (EUA) included in many instances the modification of previously FDA-approved assays, which had the capability to detect and differentiate between several types of upper respiratory pathogens. However, these diagnostic tests come with limitations including limited sample and transport matrices validation studies. To prevent sustained spread of the SARS-CoV-2 within the Military Health System and overcome some of the supply chain obstacles, military public health laboratories implemented widespread testing using next generation diagnostic systems (NGDS) already in practice for infectious disease surveillance. The use of currently approved devices allowed for the rapid integration of newly designed, EUA-approved SARS-CoV-2 assays. The Biofire® FilmArray® 2.0 system is one such NGDS that has been previously utilized for the detection of upper respiratory pathogens in the military population. Using the BioFire® FimArray® Respiratory Panel 2.1 (RP2.1), biological respiratory samples can be tested for 22 respiratory pathogens including SARS-CoV-2 in as little as 45 minutes. Another NGDS platform used by military labs is the Cepheid GeneXpert®, a multiplex PCR system capable of detecting SARS-CoV-2 in 25 minutes. The ability to rapidly detect SARS-CoV-2 is critical for effective triage and infection control, thereby minimizing the burden on health care resources both in the military and civilian populations. The purpose of this study was to define if the Biofire® FilmArray® RP 2.1 and Cepheid Xpert® SARS-CoV-2\Flu\RSV assays were equally effective at detecting SARS-CoV-2 in upper respiratory tract samples. We evaluated the limit of detection and clinical sample concordance between both diagnostic tests. In addition, we compared five upper respiratory secretion sample collection sites, including the already validated nasopharyngeal (NP) swab in VTM, to determine if these specimen types could be used with the BioFire® RP2.1 and Cepheid SARS-CoV-2/Flu/RSV panels for COVID-19 testing.

3.0 METHODS, ASSUMPTIONS AND PROCEDURES

Study design and sample processing

A total of 1080 specimens were collected from 216 enrollees (Table 2.) were recruited by iSpecimen, Inc. (Lexington, MA) and consented to submit five separate upper respiratory tract specimens under an iSpecimen, Inc. approved Institutional Review Board (IRB) approved protocol (Figure 1). This 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). Sampling was conducted at three separate collection sites. The specimen types included a nasal swab, two nasopharyngeal swabs, stored and transported in either VTM or saline, an oropharyngeal swab in VTM, and a saliva sample. After collection, samples were shipped overnight on ice and then stored at -80°C until time of testing. The day of testing, all samples were thawed at 4°C and tested with BioFire® RP2.1 pouches. The following day, Cepheid Xpert® Xpress

SARS-CoV-2/Flu/RSV cartridges were run on the GeneXpert system. Sample testing with both assays was completed in accordance with the respective company instructions for use (IFU) [13] [14].

Limit of detection (LoD) testing

Previously tested, SARS-CoV-2 negative clinical samples were pooled for LoD quantification. After confirmation of negative results, samples were spiked with known concentrations of SARS-CoV-2 viral RNA [15] [16}. Concentrations ranged from 50-150 copies/mL for the Cepheid Xpert® SARS-CoV-2/Flu/RSV assay and 150-500 copies/mL for the RP2.1 testing.BioFire® FilmArray® RP2.1 and Cepheid Xpert® Xpress SARS-CoV-2/Flu/RSV (4-in-1) assays

The BioFire® FilmArray® System (BioFire Diagnostics, LLC, Salt Lake City, UT) is a multiplex Polymerase Chain Reaction (PCR) instrument that utilizes a patented pouching system. The Respiratory Panel 2.1 (RP2.1) contains integrated freeze-dried reagents which include primer sets for 22 upper respiratory tract pathogens. The closed system pouch has all the necessary reagents on-board for automated sample preparation, and pathogen detection by RT-PCR. The RP2.1 test detects the SARS-CoV-2 Spike (S) and Membrane (M) proteins (Table 1). A result is considered positive for SARS-CoV-2 when either one or both proteins is detected. The IFU states that the RP2.1 Positive Percent Agreement (PPA) was determined to be 98% and the Negative Percent Agreement (NPA) was 100% in archived specimens.

The Cepheid GeneXpert system (Cepheid, Sunnyvale, CA) is a fully automated, multiplex PCR system which uses a new cartridge technology that has all reagents on-board for a completely hands-off workflow. The Xpert® SARS-CoV-2/Flu/RSV assay integrates sample preparation, nucleic acid extraction, and amplification and detection of respiratory viruses in nasopharyngeal, mid-turbinate, and nasal swabs. The benefit of this new 4-in-1 combination assay is that it allows for the simultaneous detection of three common upper respiratory pathogens in addition to SARS-CoV-2. The integrated primers are designed to detect RNA from two SARS-CoV-2 proteins: the nucleocapsid (N) and the envelope protein (E) (Table 1). The Xpert® SARS-CoV-2/Flu/RSV test stated a PPA of 97.9% and a NPA of 100.0% in archived samples.

Data Analyses

Statistical analyses were performed using R version 4.0.3 and the R packages ‘epiR’ and ‘fmsb’. For comparative analyses between platforms and between sample types we used Cohen’s kappa statistics to estimate agreement and test the null hypothesis that agreement was random (i.e. kappa statistic equals zero) [7]. We used McNemar’s Chi-square test to test the null hypothesis that the platforms are equivalent in terms of sensitivity and specificity.

As a result of the recruitment plan for this study, there was a time lag between initial RT-PCR Clinical Laboratory Improvement Amendment (CLIA) testing and sample collection. For comparative analyses a Welch two sample t-test was used to test the null hypothesis of no difference in the number of days between CLIA testing and sample collection (lag time) between concordant positive results and discordant results. A Welch two sample t-test was used to test the null hypothesis of no difference between Cepheid Ct values between concordant positive samples and discordant samples which were positive on the Cepheid but negative on the BioFire. We encoded detected and not detected as 1 and 0 respectively and used locally weighted scatterplot smoothing implemented within the loess.smooth R

function with an alpha of 0.1 to visually investigate the relationship between the percentage of samples that tested positive and the length of time that passed between positive CLIA testing and sample collection.

Linear regression and visual interpretation of scatter plots were used to understand the relationship between Ct values before and after centrifugation of saliva samples. We used a paired t-test to test the null hypothesis of no difference in the mean Ct values before and after centrifugation.

4.0 MAJOR EVENTS/MILESTONES/SUCCESS

In preparation for the execution of this project,

- Kick Off Meeting – 04 DEC 2020
- IRB Approval – 23 NOV 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 – Diagnostic Microbiology and Infectious Diseases - Submission Pending
- Dissemination of Results – IPR briefing of CARES Act FY20 COVID-19 projects to DHA and DASD HRP & O, and internal 59th Programmatic meetings at the CAMD

5.0 RISK ASSESSMENT

5.1 Risk Analysis:

Mitigating Agent hazards and laboratory procedure hazards?	• Eye wash and shower stations are flushed and cleaned
	• Basic PPE provided for all personnel working in the laboratory including
	  ○ gloves
	  ○ laboratory coats
	  ○ gowns
	  ○ face masks or shields
	  ○ show covers
	• laboratory coats available for all staff who may enter area
	• Laboratory personnel receive training in the Biosafety Laboratory Competencies

Risks associated with specimen source and likely organisms	• BSCs are used effectively and scientists prevent unnecessary risk of harm by doing the following
	  ○ BSCs free of clutter and the front grate kept clear
	  ○ closed centrifuge carriers opened only in the BSC

Elimination of Transmission, route of exposure, infectivity and infectious dose	• There is controlled access to biosafety level 2 • Centrifuge rotors sealed with O-rings to prevent aerosolization • Written policy for when to change gloves • Written procedure for appropriate donning and doffing PPE including laboratory coats, gloves, protective eyewear, face shields, N95 and/or PAPRs • There is a policy restricting eating, drinking, storing food, applying cosmetics and handling contact lenses to areas outside of the laboratory
Epidemiological information available to personnel such as signs of symptoms and occupational hazards	• Policy in place for hand washing • Policy in place for decontaminating surfaces • Personnel offered appropriate vaccinations for working in their occupation • Biohazard signs posted by the entrance of laboratories where infectious agents are processed and tested
Risk factors and experience of individual performing the assay	• The following certified at least annually o Biosafety Cabinets (BSCs) o Autoclaves o HVAC o HEPA Filters • N95 respirators or PAPRs available to appropriately trained staff to use when working with organisms requiring their use? • Policy in place for safe handling of sharps • Annual biosafety training program for all personnel
Possible risk when assays require inactivating BSL-2 agents	• Autoclaves tested for efficacy using biological material. • Scheduled autoclave efficacy testing during high autoclave usage. • Policy in place for proper disposal of biomedical waste and sharps

5.2 Technical Challenges

Technical challenges related to utilization of new platform systems

- Technical issues caused by specimen types with high amounts of cellular debris caused while running assays.
- Unavailability of necessary consumables and reagents
- Insufficient sample volume upon delivery of specimens
- A previously known tendency for several assay cartridges to fail during testing per supply case.
- Biosafety cabinet motor burned out during testing, which left laboratory with only one BSC.
- Lag in time for delivery of instrument rack to shore up lab bench space.

6.0 TRANSITION PLAN

6.1 Military Relevance

Next generation diagnostics are limited in their rapid deployment by critical sample processing step that is time-consuming and costly as necessary reagents becomes unavailable during the current SARS-CoV-2 pandemic. The need for sample preparation has also limited the deployment of these diagnostics in a more forward role.

6.2 Transition Strategy

- Expansion of Usable Specimen Type of for GeneXpert® and BioFire® FilmArray on nasopharyngeal swab (with saline/PBS), nasal swab, throat swab, and saliva samples.
- Validation of limit of detection and specificity of Cepheid GeneXpert® and BioFire® FilmArray on nasopharyngeal specimen.
- Report to Cephid and BioFire® in support of EUA amendments.

7.0 RESULTS

Cohort recruitment, demographics, and other characteristics

iSpecimen, Inc. recruited and consented 216 study participants between Nov. 6th 2020 and Jan. 7th 2021 at three sites: California, New Jersey, and New York. Study participants donated a nasal swab, an oropharyngeal swab in VTM, a saliva sample and two nasopharyngeal swabs, one of each which were stored and transported in VTM and saline. Study participants also donated a blood sample. Demographic information for the study participants is shown in Table 2.

Based on the results of an initial CLIA SARS-CoV-2 RT-PCR test, study participants were separated into one of three cohort groups. The first cohort group consisted of SARS-CoV-2 positive enrollees who were instructed to submit their samples sometime between the day of testing and up to 14 days after RT-PCR

testing. The second cohort was also SARS-CoV-2 Positive, but participants were asked to submit samples from 15 to 30 days after initial RT-PCR result. The third and final cohort group consisted of SARS-CoV-2 negative individuals who donated samples up to 30 days after CLIA testing.

Figure 2 depicts the percentage samples from cohorts 1 & 2 that tested positive for SARS-CoV-2 on the BioFire RP2.1 with respect to days post-CLIA testing. As expected, 100 % of all biological samples tested positive for SARS-CoV-2 on or nearest the day of CLIA testing. The percentage of SARS-CoV-2 positive samples was significantly reduced when more time passed between the initial positive CLIA testing and the day of sample collection.

LoD testing

Table 3 depicts the range of viral concentrations used to estimate the LoD for each diagnostic testing platform. The lowest viral concentration with a positivity rate of ≥ 99% was observed at 387.5 copies/mL for the BioFire® FilmArray® RP2.1 and 250 copies/mL for the Cepheid Xpert® Xpress SARS-CoV-2/Flu/RSV test. In comparison each company reported a LOD of 500 copies/mL and 131 copies/mL, respectively (BioFire Diagnostics, 2021) (Cepheid, 2020).

Assessment of SARS-CoV-2 agreement findings in clinical samples evaluated by BioFire® FilmArray® RP2.1 and Cepheid Xpert® Xpress SARS-CoV-2/Flu/RSV assays

Given that BioFire® FilmArray® RP2.1 and Cepheid Xpert® Xpress SARS-CoV-2/Flu/RSV diagnostic platforms are not quantitative, testing results for both platforms are reported qualitatively indicating SARS-CoV-2 as either detected or not. Table 3 depicts the results of comparative testing of both platforms in different sample types. Kappa statistics indicate “nearly perfect correlation” for nasal swab, NP swabs in either VTM or saline, and oropharyngeal swabs, and “substantial agreement” for saliva samples.

The Cepheid and BioFire platforms were non-equivalent in sensitivity at detecting SARS-CoV-2 in nasal swabs (p=0.004; McNemar test), and NP swabs in VTM (p=0.002; McNemar test). Nine of 10 nasal swabs and 12 of 15 nasopharyngeal swabs in VTM with discordant results were from the CLIA positive group. Most discordant results were detected by the Cepheid compared to the BioFire; all 10 discordant results from nasal swabs and 14 of 15 discordant results from NP swabs. Furthermore, the mean Ct values for the samples that were positive on the Cepheid and negative on the BioFire were higher on average (mean nasal swab Ct = 42.34; mean NP swab VTM Ct = 40.32) than the samples that were concordant positive (mean nasal wash Ct = 30.98; mean NP swab VTM Ct = 30.68). These differences were statistically significant (nasal swab $p < 2.2x10\text{-}16$; NP swab VTM $p = 8.18 \times 10\text{-}11$).

Initial testing using saliva samples led to the qualitative observation that the Cepheid platform was detecting fewer SARS-CoV-2 positive samples compared to the BioFire. We hypothesized that cellular debris in saliva samples was interfering with the performance of this platform. To test this hypothesis, saliva samples used for testing on the Cepheid were briefly centrifuged to sediment cellular debris and reanalyzed. As shown in Figure 2, Ct values of SARS-CoV-2 detection from Cepheid were strongly correlated before and after centrifugation. A paired t-test indicated that the effect of centrifugation on Ct values (mean [95% CI] = -1.047 [-0.35,2.44]) was not statistically significant (p=0.14). Furthermore, five additional samples that tested negative before centrifugation, tested positive after centrifugation.

Table 3 presents the results of comparative testing of the devices in saliva in which the saliva samples used for the Cepheid platform were centrifuged. Kappa statistics indicate “substantial agreement” for saliva. However, the Cepheid and BioFire platforms were non-equivalent for saliva (p=3.0 x 10-5; McNemar test). 19/23 saliva samples with discordant results were from the CLIA positive group, with 22 discordant results detected by the BioFire but not the Cepheid.

Comparative analysis of transport matrices for SARS-CoV-2 detection

The initial shortages in VTM availability at the beginning of the pandemic indicate a need to validate additional transport matrices. Here we compared SARS-CoV-2 detection in NP swabs stored and transported in either VTM or saline. On the Xpert® SARS-CoV-2/Flu/RSV assay, Kappa statistic (k=0.82) indicates “nearly perfect agreement” between nasopharyngeal samples stored and transported in saline and VTM. The results using saline were non-inferior to VTM (p=0.10; McNemar test). There was a relatively high PPA of 84% (95% CI, 74%-91%) and NPA of 96% (95% CI, 91%-99%) between NP swabs diluted in VTM and Saline. On the BioFire® FilmArray® RP2.1, Kappa statistic (k=0.75) indicates “substantial agreement” between nasopharyngeal samples stored and transported in saline and VTM. The results using saline were non-inferior to VTM (p=0.67; McNemar test). Lastly, there was a relatively high PPA of 81% (95% CI, 70%-89%) and NPA of 93% (95% CI, 88%-97%) between NP swabs diluted in VTM and Saline.

8.0 CONCLUSION/DISCUSSION

Here, we report on the feasibility of using upper respiratory tract specimen types other than NP swabs in VTM, the gold standard, as well as Next Generation Detection Systems to accurately detect SARS-CoV-2. By using cross-tabulation tables and chi-square analyses we determine if statistically significant differences exist among results generated through molecular testing on the Cepheid SARS-CoV-2\Flu\RSV assay and the BioFire® FilmArray® RP2.1 platforms. Our findings indicate that additional specimen types, including saliva, NP swabs in saline, and oropharyngeal swabs in VTM are suitable alternatives for molecular diagnostic testing on both platforms.

Early in the pandemic testing procedures relied only on the use of NP swabs transported in VTM [8]. However, NP collection is inherently uncomfortable and likely to deter some individuals from being tested (Pondaven-Letourmy, 2020). Therefore, the validation of additional upper respiratory specimens could circumvent the need for healthcare workers to rely solely on NP swabs in VTM, not only overcoming patient testing hesitancy but also collection supply shortages. Results from both the BioFire RP2.1 and Cepheid Xpert® SARS-CoV-2/Flu/RSV assays, using specimen types not currently validated for testing such as nasal swabs, OP swabs, and saliva yield estimates for PPA and PNA that ranged between 70-100%. These estimates provide evidence that these alternative respiratory sample matrices can serve as acceptable candidate specimens for SARS-CoV-2 testing.

Additionally, given that some VTM formulations have been reported to yield false negative results (add reference), we addressed the feasibility of using saline as an alternative medium to transport NP swabs.

We found no difference in SARS-CoV-2 detection in NP swabs transported in either saline or VTM. We observed a high positive and negative concordance between the two transport matrices suggesting that both transport media types are equally viable options for collection of nasopharyngeal samples for SARS-CoV-2 testing.

We also investigated the long-term viability of saliva as a suitable sample type for diagnostic testing of individuals experiencing symptoms of COVID-19. One impediment to the use of saliva for clinical testing, is the necessity of including a sample processing step prior to testing as centrifugation may prove difficult or unsuitable in rural testing sites, at home, or in austere environments. Nagura-Ikeda et al suggests that results using saliva can be highly variable and that better saliva processing techniques may improve testing sensitivity [9]. As such, we wanted to determine if the centrifugation of cellular debris increased the detectability of SARS-CoV-2 in clinical samples as has been reported by others [10]. Interestingly, our data showed no significant difference in assay results using saliva samples with and without a centrifugation step prior to testing. Our results support previous studies proposing saliva as a candidate clinical specimen for the detection of SARS-CoV-2 [11, 12].

One limitation of this study is that biological samples were not collected at the time of CLIA laboratory testing. Indeed, we observed a sharp decline in SARS-CoV-2 positive samples when there was more time between CLIA testing and that of sample collection. This finding suggests that study participants who were infected with SARS-CoV-2 mounted innate and/or adaptive immune responses to clear the virus from their system as time passes. As a result, the positive samples in this study are enriched for samples that have SARS-CoV-2 viral loads that are near or below the limit of detection for the devices in this study in comparison to samples from study designs in which samples are collected on the same day that patients present to the clinic. Thus, estimates of Positive Percent Agreement (PPA) in this study are conservative.

The high sensitivity of both the BioFire® FilmArray® RP2.1 and the Cepheid Xpert® Xpress SARS-CoV-2/Flu/RSV assays reported in this study correspond to results generated by internal testing from BioFire and Cepheid. These two Multiplex PCR devices offer a rapid and easy to operate molecular diagnostic option for both point-of-care settings and in hospitals where frequent COVID-19 testing of certain populations, like healthcare workers, is desired. Our study results indicate that efforts to reprocess existing FDA-approved assays for the purpose of mounting an immediate response to emerging pathogens, can be an effective tool amidst an ongoing pandemic.

CONCLUSION

The COVID-19 pandemic altered how the world reacts to a highly transmissible RNA virus, including producing highly effective deployable, diagnostic tests to help curb spread of the novel virus. This study has shown that both the Biofire® FilmArray® RP2.1 and Cepheid Xpert® Xpress SARS-CoV-2/Flu/RSV assays serve as ideal candidates for rapid testing and reliable detection of SARS-CoV-2 in a variety of clinical matrices.

9.0 DELIVERABLES

9.1 Publications: Diagnostic Microbiology and Infectious Diseases, Submission Pending

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

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11.0 REFERENCES

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FIGURES AND TABLES:

Figure 1. Flowchart of study design and cohort group classification

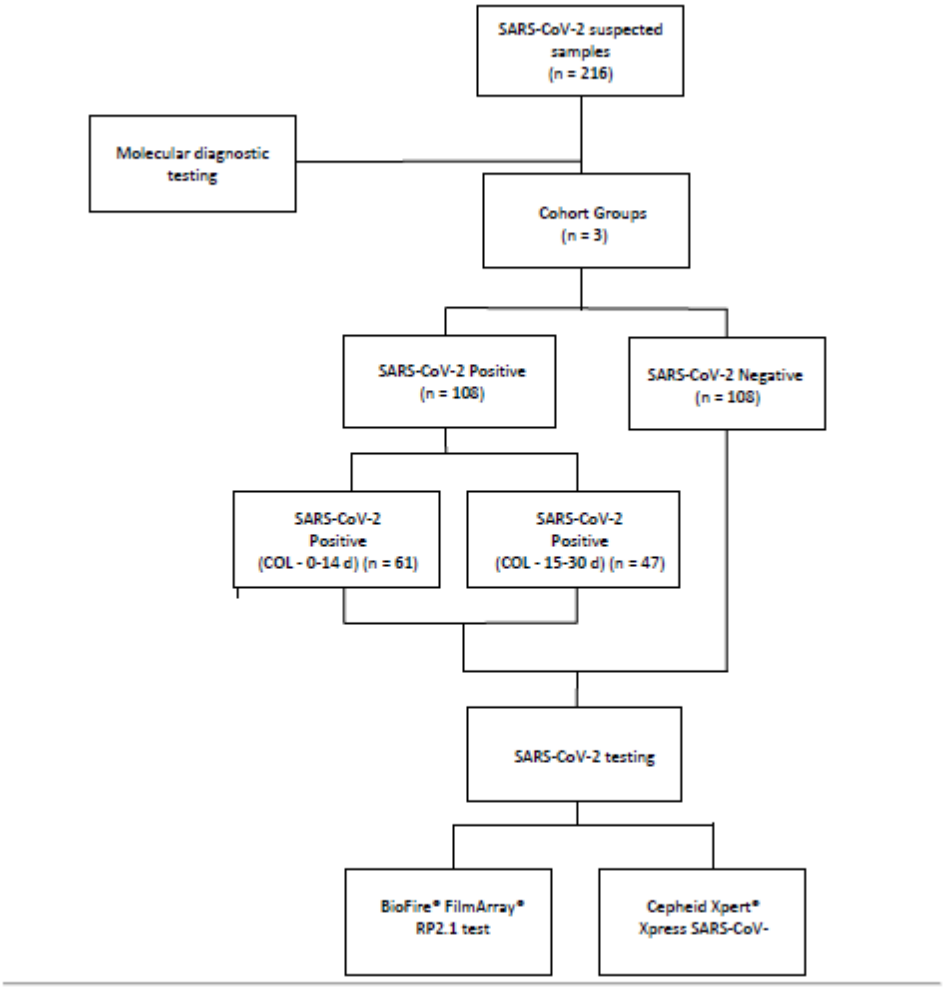

Figure 1. Flowchart showing an overview of study design. Enrollees were assigned to cohort group according to an initial PCR test result. Testing of specimen samples was completed using two molecular diagnostic assays with EUA designation. Abbreviations: COL, collection; d, days.

Table 1. Comparison of two diagnostic tests for the detection of SARS-CoV-2.

Brand Name	Method	Sample type	Assay run time (min)	Sample vol (µL)	Analytical sensitivity per IFU	Target
Xpert® Xpress SARS-CoV-2/Flu/RSV test (Cepheid)	Real Time RT-PCR	NP-VTM, NW/A*, NS*	45	300	131 copies/mL	E and N2 genes
FilmArray® RP2.1 test (BioFire Defense, LLC)	Multiplex RT-PCR test	NP-VTM	45	300	500 copies/mL	S and M genes

Table 1 Details of the BioFire and Cepheid assays including classification, length of test, validated sample types, published LoD, and gene targets. Abbreviations: E, Envelope; N2, Nucleocaspid; S, Spike protein gene; M, Membrane protein gene. *Nasal wash/ aspirate and nasal swab sample performance has not been assessed or established by company as per IFU

Table 2. Patient Characteristics

Sex, (*n*)	Male (98)	Female (118)	All (216)
Age, year, mean (SD)	49 (15.2)	48 (15.5)	48 (15.6)
Range	21 - 80	20 - 75	20 - 80

Table 2 Demographic summary of the characteristics of all enrolled participants of this current study. 5 sample types were collected per patient (n = 1080). Abbreviation: SD, Standard deviation

Figure 2. Post CLIA positive reduction in positivity by sample using locally weighted smoothing

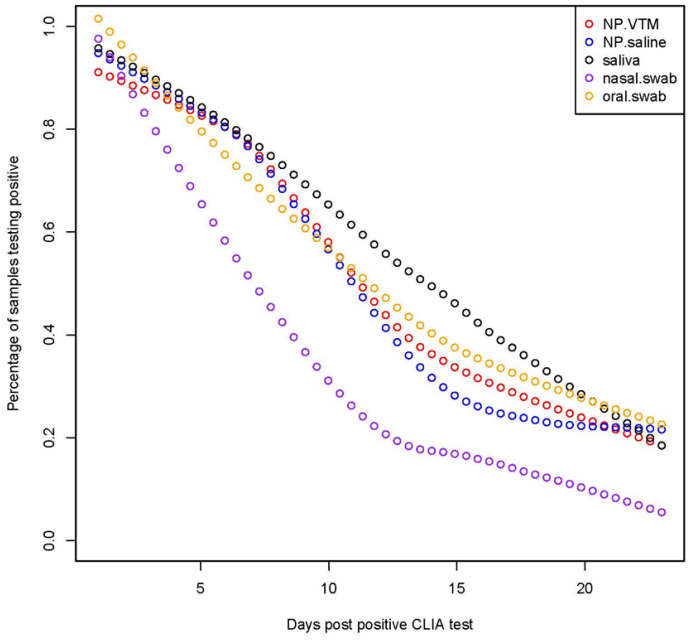

Table 3. Dilution series to estimate level of detection for SARS-CoV-2

Dilution	Copies/mL	Cepheid Xpert Xpress SARS-CoV-2/Flu/RSV[1]	Dilution	Copies/mL	BioFire RP 2.1[2]
3.0×10^{-2}	150	6/6	1.2×10^{-1}	500	6/6
2.0×10^{-2}	100	6/6	1.0×10^{-1}	425	6/6
1.8×10^{-2}	88	6/6	9.2×10^{-2}	387.5	6/6
1.6×10^{-2}	81	6/6	8.3×10^{-2}	350	5/6
1.5×10^{-2}	75	5/6	6.0×10^{-2}	250	4/6
1×10^{-2}	50	3/3	3.6×10^{-2}	150	4/6
NC	0	0/6	NC	0	0/6

[1]SeraCare AccuPlex SARS-CoV-2 Reference Material Kit # 0505-0126

[2]ATCC Heat-inactivated SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020 # VR-1986HK .

Figure 2. Ct value distribution in Cepheid Xpert GeneXpert® results in relationship to centrifugation

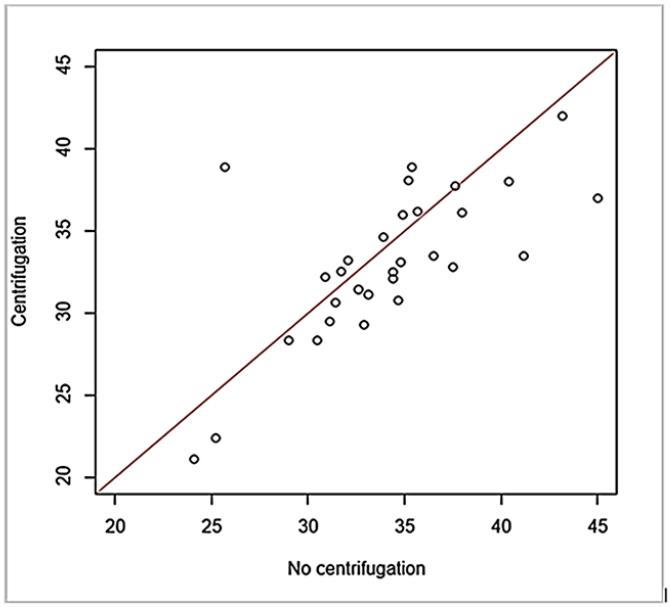

***Figure 2.** A scatterplot of the Ct values before and after centrifugation, for those that were detected on both BioFire and Cepheid platforms. The red line is a slope of 1 and an intercept of 0.*

Table 4. SARS-CoV-2 target performance between BioFire RP2.1/Cepheid Xpert SARS-CoV-2/Flu/RSV

Specimen Type	Detected RP2.1/4plex	Detected RP2.1 only	Detected 4plex only	Not Detected RP2.1/4plex	PPA (95% CI)	PNA (95% CI)	Cohen's κ (95% CI) (p-value)	McNemar test p-value
NS	52	10	0	154	1.00 [0.93,1.00]	0.94 [0.89,0.97]	0.881 [0.81,0.95] (< 2.2e-16)	0.004427
NP-S	62	3	11	136	0.95 [0.87,0.99]	0.93 [0.87,0.96]	0.852 [0.77,0.926] (< 2.2e-16)	0.06137
NP-V	67	1	14	134	0.99 [0.92,1.00]	0.91 [0.85,095]	0.847 [0.77,0.922] (< 2.2e-16)	0.001946
OP	65	3	8	140	0.96 [0.88,0.99]	0.95 [0.90,0.98]	0.884 [0.818,0.951] (< 2.2e-16)	0.2278
Saliva	53	22	1	119	0.71 [0.59,0.81]	0.99 [0.95,1.00]	0.737 [0.636,0.838] (< 2.2e-16)	3.04E-05

Table 4. Contingency table of frequencies showing the comparison of qualitative assessment between RP2.1 and Xpert SARS-CoV-2/Flu/RSV assays for the evaluation of SARS-CoV-2 in five specimen types. Abbreviations: NS, Nasal swab; NP, Nasopharyngeal; OP, Oropharyngeal; PPA, percent positive agreement; PNA, Percent negative agreement; 1; 4plex, Xpert SARS-CoV-2/Flu/RSV.

Table 5. Comparison of Nasopharyngeal swab transport matrices for detection of SARS-CoV-2

Test Method	Detected VTM / Saline	Detected Saline only	Detected VTM only	Not Detected VTM / Saline	PPA (95% CI)	PNA (95% CI)	Cohen's κ (95% CI) (p-value)	McNemar test p-value
BioFire RP2.1	55	10	13	138	0.81 [0.70,0.89]	0.93 [0.88,0.97]	0.750 [0.654,0.847] (< 2.2e-16)	0.6767
Cepheid Xpert SARS-CoV-2/Flu/RSV	68	5	13	126	0.84 [0.74,0.91]	0.96 [0.91,0.99]	0.817 [0.736,0.898] (< 2.2e-16)	0.09896

Table 5. 2 x 2 contingency table showing results of comparison analysis between VTM and saline after testing of all nasopharyngeal swabs samples on both the BioFire RP2.1 and Cepheid Xpert SARS-CoV-2/Flu/RSV assays. Abbreviations: PPA, percent positive agreement; PNA, percent negative agreement; VTM, viral transport media; RP2.1, respiratory panel v.2

12.0 LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

SARS-CoV-2

COVID-19

RT-PCR

DNA

RNA

PCR

FDA

E

S

SAR-CoV-1

RP2.1

CLIA

IRB

NS

NP-S

NP-VTM

OP

Saliva

BioFire

Cepheid

Xpert

Flu

RSV