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

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NOTICE AND SIGNATURE PAGE

COMPARISON OF NEXT GENERATION DIAGNOSTIC SYSTEMS (NGDS) FOR THE DETECTION OF SARS-COV-2

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14. ABSTRACT

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.

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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 that the etiological respiratory agent was a new virus of the genus *Betacoronavirus*, within the family *Coronaviridae* (3), subsequently named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). By March of 2020, the World Health Organization (WHO) declared COVID-19 a pandemic, and as of October 28, 2021, SARS-CoV-2 has infected 245 million people worldwide and claimed 4.97 million lives. As a result, world governments have implemented public health mitigation strategies (i.e. lockdowns, social distancing, and mask mandates) along with rapid diagnostic testing systems to accurately detect, isolate, and trace infected individuals to help slow the spread of COVID-19.

Initial diagnostic testing for SARS-CoV-2 relied on the use of Real Time-Polymerase Chain Reaction (RT-PCR) as the gold standard; however, early on delayed testing times and availability of laboratory supplies were inadequate to meet testing demands. Specifically, typical RT-PCR requires 1-2 days from sample collection to results leading to long delays in public health interventions, and increasing person-to-person viral spread (4). Furthermore, global supply chain disruptions adversely impacted the availability of testing kits, viral transport media (VTM), nasopharyngeal (NP) collection swabs, and general laboratory supplies. Consequently, the U.S. Food and Drug Administration (FDA), through the Emergency Use

Authorization (EUA) process, worked quickly to approve new diagnostic platforms and systems with faster turnaround times (5, 6). Various EUA requests included modifications of previously FDA-approved assays for respiratory infections, such as the addition of specific SARS-CoV-2 molecular targets. However, only a few of the EUA-approved systems addressed the critical supply shortages brought upon by the pandemic, including the significant reliance on NP sampling and VTM.

To overcome supply chain obstacles and improve turnaround times, many healthcare systems including the military began relying on the use of next generation diagnostic systems (NGDS) for infectious disease testing and surveillance. A significant benefit from the use of these systems is the reduced use of reagents and samples due to the integrated sample processing. Within the military health system (MHS), two NGDS have been previously used for the detection of upper respiratory pathogens and are currently used for SARS-CoV-2: the BioFire® FilmArray® 2.0 and Cepheid GeneXpert®. The BioFire® FilmArray® 2.0 system uses the FimArray® Respiratory Panel 2.1 (RP2.1), which can detect 22 respiratory pathogens including SARS-CoV-2 in as little as 45 minutes while the Cepheid GeneXpert® uses the Xpress SARS-CoV-2/Flu/RSV assay and detects SARS-CoV-2 in 25 minutes.

The purpose of the current study was to 1) determine if the BioFire® FilmArray® RP 2.1 and Cepheid Xpert® SARS-CoV-2\Flu\RSV assays were comparable at detecting SARS-CoV-2 in clinical upper respiratory tract samples, 2) perform an independent validation of their limit of detection of these assays, and 3) assess clinical sample concordance as specified by their respective EUAs. Additionally, BioFire® RP2.1 and Cepheid SARS-CoV-2/Flu/RSV panels were used to compare four upper respiratory biospecimen collection sites as well as the use of saline as an alternative transport medium to the previously validated nasopharyngeal (NP) swab in VTM.

3.0 METHODS, ASSUMPTIONS AND PROCEDURES

3.1 Study Design and Sample Processing

A total of 1,080 specimens were collected from 216 enrollees, who were recruited by iSpecimen, Inc. (Lexington, MA) under an Institutional Review Board (IRB) approved protocol; and

consented to submit five separate specimens from four upper respiratory tract locations. 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 MDW Institutional Review Board (IRB) Chairperson or designee via the exempt review/determination process. The overview of the study design is shown in **Figure 1**. The specimen types collected included a nasal swab, an oropharyngeal (OP) swab in VTM, a saliva sample, and two NP swabs, stored in either VTM or saline. After collection, samples were shipped overnight on dry 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, samples were tested using the GeneXpert system and Cepheid Xpert® Xpress SARS-CoV-2/Flu/RSV cartridges. Sample testing with both assays was completed in accordance with each company's instructions for use (IFU).

3.2 Limit of Detection Testing

Previously identified SARS-CoV-2 negative samples were pooled and tested by RT-PCR. After confirmation of negativity, samples were spiked with known concentrations of SARS-CoV-2 viral RNA. 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

3.3 BioFire® FilmArray® RP2.1 and Cepheid Xpert® Xpress SARS-CoV-2/Flu/ RSV (4-in-1) Assays

The BioFire® FilmArray® System Respiratory Panel 2.1 (RP2.1; BioFire® Diagnostics, LLC, Salt Lake City, UT) contains integrated lyophilized reagents which include primer sets for 22 upper respiratory tract pathogens. The closed pouch system has all the necessary reagents on-board for automated sample preparation and pathogen detection by RT-PCR. Specifically, RP2.1 targets the SARS-CoV-2 Spike (S) and Membrane (M) proteins (**Table 2**). A sample is considered positive

for SARS-CoV-2 when either one, or both proteins are detected. According to the IFU provided by the manufacturer as part of the EUA approval, the RP2.1 Positive Percent Agreement (PPA) was determined to be 98% and the Negative Percent Agreement (NPA) was 100% in archived specimens.

Similarly, the Cepheid GeneXpert® Xpert® SARS-CoV-2/Flu/RSV assay (Cepheid, Sunnyvale, CA) is fully automated and based on cartridge technology with all the reagents on-board for a completely hands-off workflow that integrates sample preparation, nucleic acid extraction, amplification, and detection of respiratory viruses in nasopharyngeal, mid-turbinate, and nasal swabs. Unlike the RP2.1, the Xpert® SARS-CoV-2/Flu/RSV assay can only simultaneously detect three common upper respiratory pathogens (Influenza A, B, and Respiratory Syncytial Virus) in addition to SARS-CoV-2. The specific integrated primers for SARS-CoV-2 proteins target the nucleocapsid (N) and the envelope protein (E) (**Table 2**). The EUA IFU for Xpert® SARS-CoV-2/Flu/RSV assay states a PPA of 97.9% and a NPA of 100.0% in archived samples.

3.4 Concordance between Nasopharyngeal (NP) Swab Transport Media Types

Differences in SARS-CoV-2 detection between the two transport media types (VTM and saline) used for NP swabs was determined. A regression analysis was conducted to compare the cycle threshold (C_t) values from NP swabs diluted in VTM to NP swabs diluted in saline. The comparative analysis was limited to SARS-CoV-2 positive samples detected by the Cepheid Xpert® SARS-CoV-2/Flu/RSV assay because SARS-CoV-2 negative samples and BioFire® FilmArray® system do not produce a C_t value.

3.5 SARS-CoV-2 Detection in saliva samples before and after centrifugation

Given that saliva had not been validated as a sample type for SARS-CoV-2 detection by either the Cepheid or BioFire® assays at the time of our testing and is known to contain cellular debris, we

evaluated whether sample centrifugation to remove cellular debris reduced SARS-CoV-2 detection. C_t values from saliva samples evaluated before centrifugation were compared to those obtained from identical samples which were not centrifuged.

3.6 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). McNemar's Chi-square test was used to test the null hypothesis that the platforms are equivalent in terms of sensitivity and specificity. (8)

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 also used to test the null hypothesis of no difference between Cepheid C_t 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 loss. smooth R function with an alpha of 0.1 to visually evaluate 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 C_t values before and after centrifugation of saliva samples. A paired t-test

was used to test the null hypothesis of no difference in the mean C_t values before and after centrifugation.

4.0 MAJOR EVENTS/MILESTONES/SUCCESS

In preparation for the execution of this project,

- Kick-Off Meeting – 4th June 2020
- IRB/IACUC Approval – 23rd November 2020
- All experimental procedures completed – 14th June 2021
- Data Analysis – 1st August 2021
- Poster presentation:
  - 59MDW Commander's Immersion Brief (16th August 2021)
  - MHSRS Cancelled, Accepted (23rd – 25th August 2021)
- Manuscript accepted Journal of Clinical Laboratory Analysis – March 2022
- Dissemination of Results – Submission Pending

5.0 RISK ASSESSMENT

5.1 Risk Analysis:

Risk	Probability	Consequence	Mitigation Strategy
Potential skin puncture with needle cannula during pouch loading	Seldom (2) A risk in this category is very rare but more common than those in the unlikely category. These risks still need to be considered and cannot be ruled out yet	Marginal (2) Consequences are marginal and may cause only minor risk. This risk is unlikely to have a huge impact	Provide protocol and safety training on handling reagent properly.
Possible exposure to blood borne pathogens during sample loading	Seldom (2) A risk in this category is very rare but more common than those in the unlikely category. These risks still need to be considered and cannot be ruled out yet	Marginal (2) The consequences are marginal and may cause only minor risk. This risk is unlikely to have a huge impact	• Wear Personal Protective Equipment (PPE) • All samples should be processed in BSC. • Decontaminate hood with wipe down hood with 70% cidehol.

5.2 Technical Challenges

We experienced a technical challenge when processing saliva samples for clinical testing on the Cepheid. During testing, saliva samples generated invalid results due to cellular debris within the collected sample. To resolve this issue we centrifuged saliva for one minute and aliquoted 300 µL of the supernatant for testing. This modification in the protocol facilitated pipetting saliva samples into the Cepheid assay. Samples were retroactively tested using this method to ensure consistency in testing.

6.0 TRANSITION PLAN

6.1 Military Relevance

COVID-19 negatively impacted mission readiness and the health of military personnel. Military operations were delayed due to current testing protocols and limited reagents for SARS-CoV-2 testing. Biofire® and Cepheid® were able to provide a quick and accurate multi-target detection assay to detect multiple respiratory pathogens including COVID-19. Biofire and Cepheid contain a qualitative rapid real-time qPCR system that can diagnose each patient where frequent testing is necessary. Both qualitative RT-PCR tests provide the Military Health System with reliable and accurate diagnostic platforms to detect a variety of respiratory tract infections. Another advantage is a person without laboratory experience can easily be trained to complete the protocols. Both companies have received an Emergency Use Authorization to the Food and Drug Administration (FDA) for laboratory sample prep, nucleic extraction and polymerase chain reaction (PCR) amplification using multiplex PCR and detection of the SARS-CoV-2 targeted sequences in a single use cartridge on assays using nasopharyngeal swabs.

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

7.1 Cohort Recruitment and Assignment

iSpecimen, Inc. recruited and consented 216 study participants between November 6, 2020 and January 7, 2021 at three sites located in California, New Jersey, and New York. Each study participant provided a nasal and OP swabs, two NP swabs and a saliva sample. For the evaluation of the effects of transport media on SARS-CoV-2 detection, the two NP swabs were stored and transported in either VTM or saline, individually. The age and sex information for the study participants is shown in **Table 1.**

Based on the results of an initial CLIA SARS-CoV-2 RT-PCR test, study participants were placed into one of three cohorts. Cohort 1 consisted of SARS-CoV-2 positive enrollees, who were instructed to submit their samples between the day of RT-PCR testing and up to 14 days thereafter. Cohort 2 was also SARS-CoV-2 positive, but these enrollees were asked to submit samples from 15 to 30 days after initial RT-PCR testing. The final cohort group consisted of SARS-CoV-2 negative individuals, who donated samples up to 30 days after RT-PCR testing.

Figure 2 depicts the percent of samples from cohorts 1 & 2 that tested positive for SARS-CoV-2 on the BioFire® RP2.1 post-CLIA testing. As expected, 100% of all biological samples tested positive for SARS-CoV-2 when collection was done soon after CLIA testing. The

percentage of SARS-CoV-2 positive samples began to decline as more time passed between the initial positive CLIA testing and the day of sample collection.

7.2 Limit of Detection Testing

Table 3 depicts the range of known 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 81 copies/mL for the Cepheid Xpert® Xpress SARS-CoV-2/Flu/RSV assay. In comparison each company reported a LOD of 500 copies/mL and 131 copies/mL, respectively.

7.3 NGDS Comparative Testing

BioFire® FilmArray® RP2.1 and Cepheid Xpert® Xpress SARS-CoV-2/Flu/RSV diagnostic platforms are not intended to be quantitative tests, results are reported qualitatively as either "detected" or "not detected" indicating SARS-CoV-2 presence. **Table 4** summarizes 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 OP 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 C_t values for the samples that were positive on the Cepheid and negative on the BioFire® were higher on average (mean nasal swab $C_t = 42.34$; mean NP swab VTM $C_t = 40.32$) than the samples that were concordant positive (mean nasal wash $C_t =$

30.98; mean NP swab VTM C_t = 30.68). These differences were statistically significant (nasal swab $p < 2.2x10^{-16}$; NP swab VTM $p = 8.18 \times 10^{-11}$).

7.4 Saliva Comparative Testing

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®. It was hypothesized that cellular debris in saliva samples was interfering with the performance of this platform. To test this hypothesis, saliva samples used for the Cepheid were briefly centrifuged to sediment cellular debris and re-analyzed. As shown in **Figure 3** C_t 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 C_t 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. Kappa statistics indicated "substantial agreement" for saliva. However, the Cepheid and BioFire® platforms were non-equivalent for saliva (**Table 4**, $p = 3.0 \times 10^{-5}$; 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.

7.5 Comparative Analysis of Transport Matrices for SARS-CoV-2 Detection

The initial shortages in VTM availability at the beginning of the pandemic highlighted the need to validate additional transport matrices. Thus, 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 ($k = 0.82$) indicated "nearly perfect agreement" between nasopharyngeal samples stored and transported in saline and VTM (**Table 5**). 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) indicated "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 (**Table 5**).

8.0 CONCLUSION/DISCUSSION

The current study demonstrates the utility of using upper respiratory tract specimen types other than NP swabs in VTM, the gold standard, as well as NGDS to accurately and quickly detect SARS-CoV-2. Early on in the pandemic, testing procedures relied only on the use of NP swabs transported in VTM (9). As NP collection is inherently uncomfortable it may be likely to deter some individuals from being tested (9). 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 hesitancy but also future 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 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 we addressed the practicality of using saline as an alternative medium to transport NP swabs (10, 11). 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 media types are equally viable options for collection of nasopharyngeal samples for SARS-CoV-2 testing.

We also evaluated the utility of using 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 additional centrifugation step may prove difficult or unsuitable in rural testing sites, at home, or in austere environments. Nagura-Ikeda *et al* suggested that results using saliva can be highly variable and that better processing techniques may improve testing sensitivity (12). 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 (13). Interestingly, our data showed no significant difference when 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 (14, 15).

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 positivity rates when there was more time between CLIA testing and that of sample collection suggesting that SARS-CoV-2 positive study participants mounted an innate and/or adaptive immune response to clear the virus from their system. As a result, the positive samples collected for this study may be enriched for SARS-CoV-2 viral loads near or below the limit of detection for the devices compared to clinical samples from studies in which samples are collected on the same day that participants present to the clinic. Thus, estimates of 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 with results provided within the EUA from both manufacturers. These two multiplex PCR devices offer a rapid and easy-to-operate molecular diagnostic option for both point-of-care settings where frequent COVID-19 testing is desired. Our study suggests that efforts to reprocess existing FDA-approved assays for the purpose

of mounting an immediate public health response to emerging pathogens can be an effective tool amidst an on-going pandemic.

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:

Journal of Clinical Laboratory Analysis, March 2022

9.2 Presentations:

59MDW Commanders Immersion Brief, 16 AUG 2021

10.0 COST

This work was funded by the Defense Health Agency (DHA) CARES Act

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12.0 FIGURES AND TABLES:

12.1 Tables

Table 1: Participant Demographics

	Sex		
	Male (n = 98)	**Female (n = 118)**	**All (N = 216)**
Age (Mean±SD)	49 (19.2)	48 (15.5)	48 (15.6)
Range	21 – 80	20 – 75	20 – 80

Abbreviation: SD, standard deviation

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

Brand Name	EUA Validated Sample Types	Assay Run Time (min)	Sample Volume (µL)	Analytical sensitivity per IFU	SARS-CoV-2 Targets
Cepheid Xpert® Xpress	NP-VTM, NW/A*, NS*	25	300	131 copies/mL	E & N2
BioFire® FilmArray®	NP-VTM	45	300	500 copies/mL	S & M

Abbreviations: E, Envelope; N2, Nucleocaspid; S, Spike protein gene; M, Membrane protein gene; IFU, instructions for use; Flu, influenza; RSV, Respiratory Syncytial Virus; RP2.1, respiratory panel 2.1; RT-PCR, Reverse Transcriptase Polymerase Chain Reaction. *Nasal wash/ aspirate and nasal swab sample performance has not been assessed or established by company as per IFU.

Table 3: Analytical Limits of Detections for NGDS SARS-CoV-2 Assays

Cepheid Xpert® Xpress SARS-CoV-2/Flu/RSV*			**BioFire® FilmArray® RP2.1†**		
Dilution	**Copies/mL**	**No. of replicates detected / total replicates**	**Dilution**	**Copies/mL**	**No. of replicates detected / total replicates**
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	3/6	3.6×10^{-2}	150	4/6
NC	0	0/6	NC	0	0/6

Abbreviations: NC, negative control; No., number; Flu, influenza; RSV, Respiratory Syncytial Virus; RP2.1, respiratory panel 2.1.

*SeraCare AccuPlex SARS-CoV-2 Reference Material Kit # 0505-0126

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

Table 4: Comparison of two NGDS for detection of SARS-CoV-2 in different biospecimen types.

Specimen Type	"Detected" RP2.1/4plex	"Detected" RP2.1 only	"Detected" 4plex only	"Not Detected" RP2.1/4plex	PPA [95% CI]	NPA [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 [0.77,0.93] (< 2.2e-16)	0.06137
NP-V	67	1	14	134	0.99 [0.92,1.00]	0.91 [0.85,095]	0.847 [0.77,0.92] (< 2.2e-16)	0.001946
OP	65	3	8	140	0.96 [0.88,0.99]	0.95 [0.90,0.98]	0.884 [0.82,0.95] (< 2.2e-16)	0.2278
Saliva	53	22	1	119	0.71 [0.59,0.81]	0.99 [0.95,1.00]	0.737 [0.64,0.84] (< 2.2e-16)	3.04e-05

Abbreviations: NS, Nasal swab; NP, Nasopharyngeal; OP, Oropharyngeal; PPA, positive percent agreement; NPA, negative percent agreement; RP2.1, BioFire® Respiratory panel 2.1; 4plex, Cepheid Xpert SARS-CoV-2/Flu/RSV.

Table 5: Comparison of two transport media for the detection of SARS-CoV-2

Test Method	"Detected" VTM/Saline	"Detected" Saline only	"Detected" VTM only	"Not Detected" VTM/Saline	PPA [95% CI]	NPA [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 [0.65,0.85] (< 2.2e-16)	0.6767
Cepheid Xpert®	68	5	13	126	0.84 [0.74,0.91]	0.96 [0.91,0.99]	0.817 [0.74,0.90] (< 2.2e-16)	0.09896

Transport media does not affetc SARTS-CoV-2 detection in nasopharyngeal swabs. Nasopharyngeal swabs were diluted in either VTM or saline and were tested on the BioFire® RP2.1 and Cepheid Xpert® SARS-CoV-2/Flu/RSV assays. Abbreviations: PPA, positive percent agreement; NPA, negative percent agreement; VTM, viral transport media; RP2.1, respiratory panel 2.1

12.2 Figures

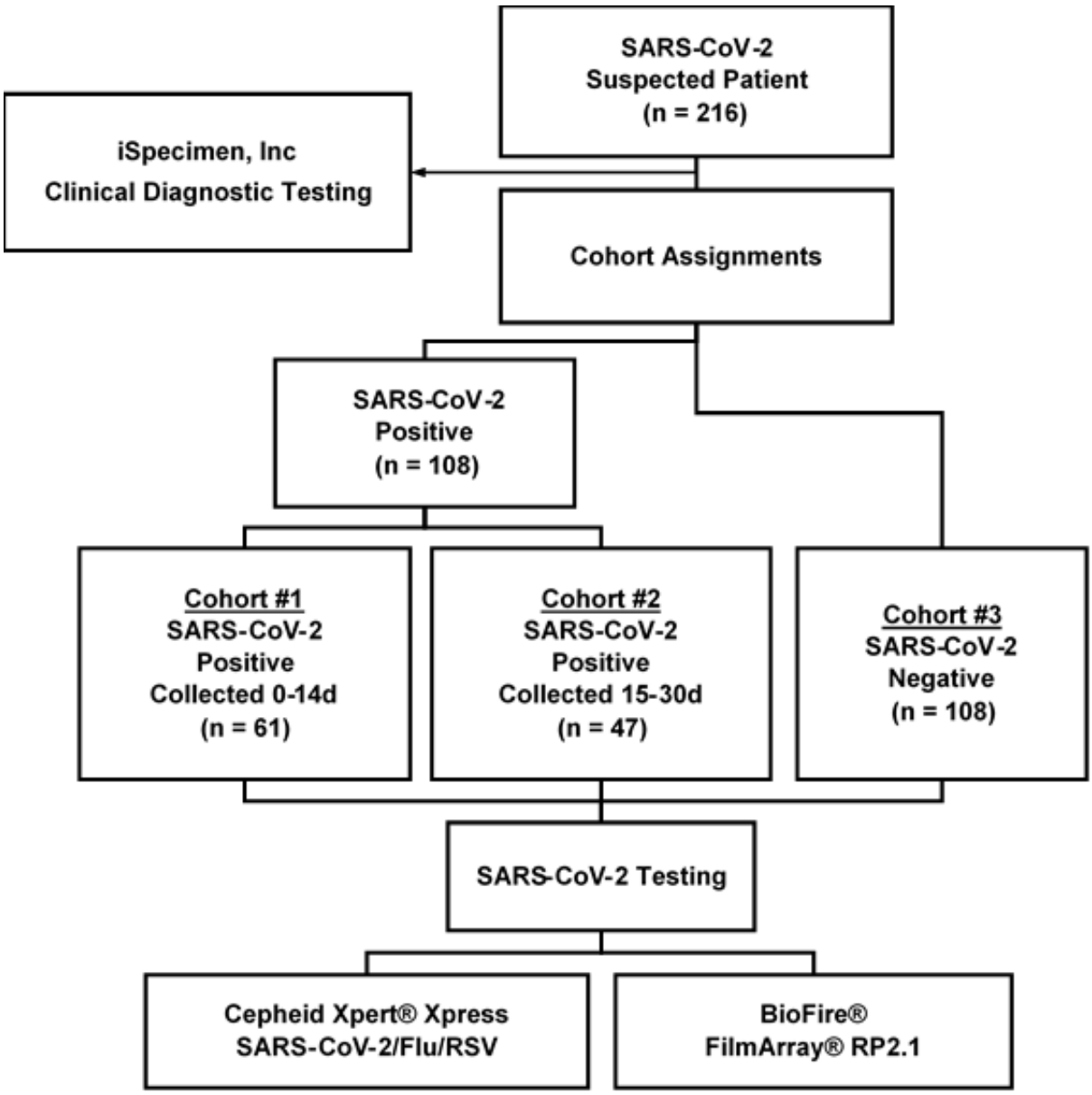

Figure 1: Overview of Study Design. Participants were assigned to each cohort according to initial clinical RT-PCR test.

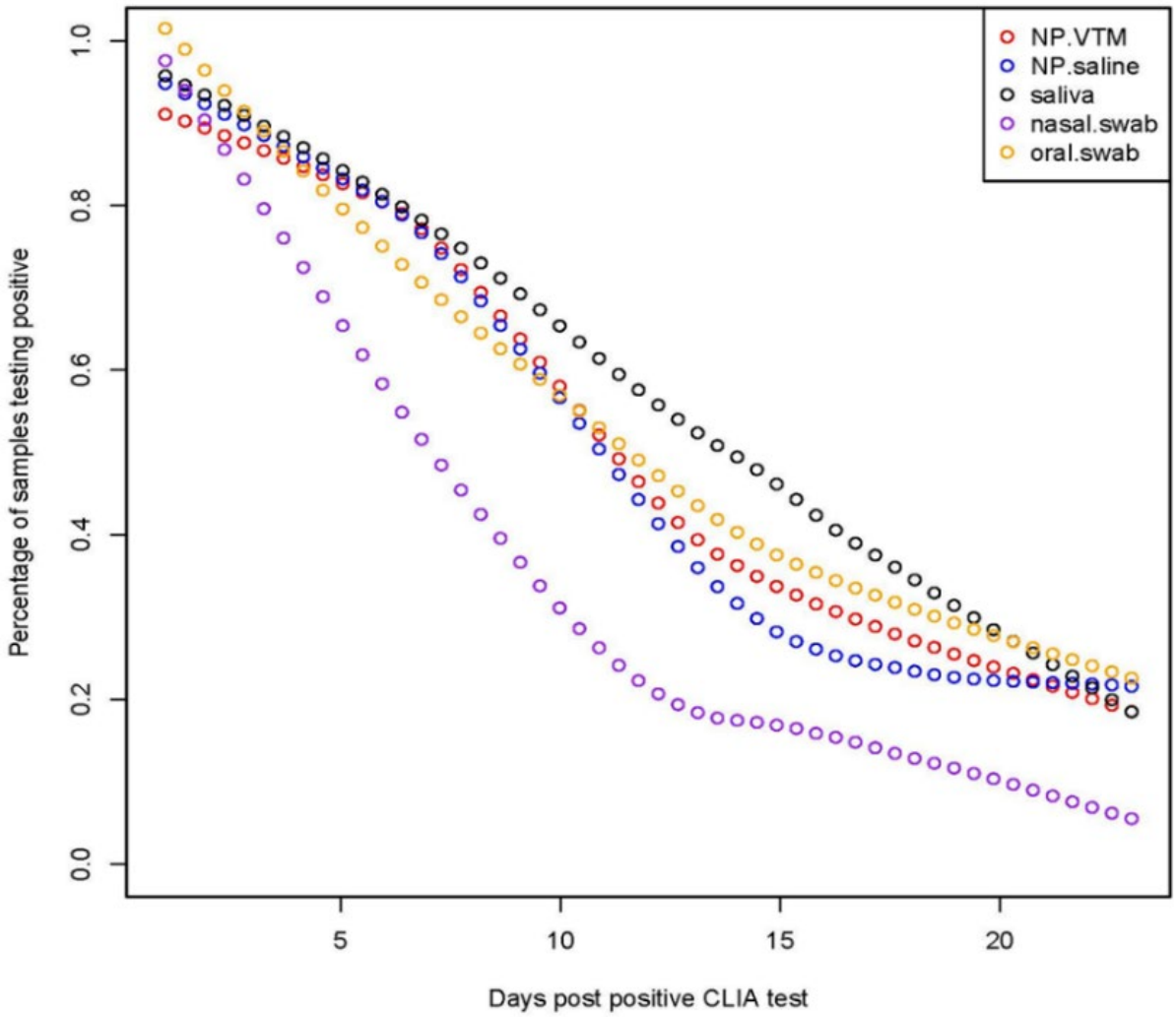

Figure 2: Analysis of the time-lag effect on positivity in respiratory samples. The graph shows the frequency of samples testing positive in relation to an interval of time following a previous positive COVID-19 PCR test. The study estimate is that the time gap caused a decline in the percentage of tests reported as positive by the RP2.1 test. Abbreviations: NP VTM, nasopharyngeal swab in VTM; NP saline, nasopharyngeal swab in saline; oral swab, oropharyngeal swab in VTM; CLIA, Clinical Laboratory Improvement Amendments.

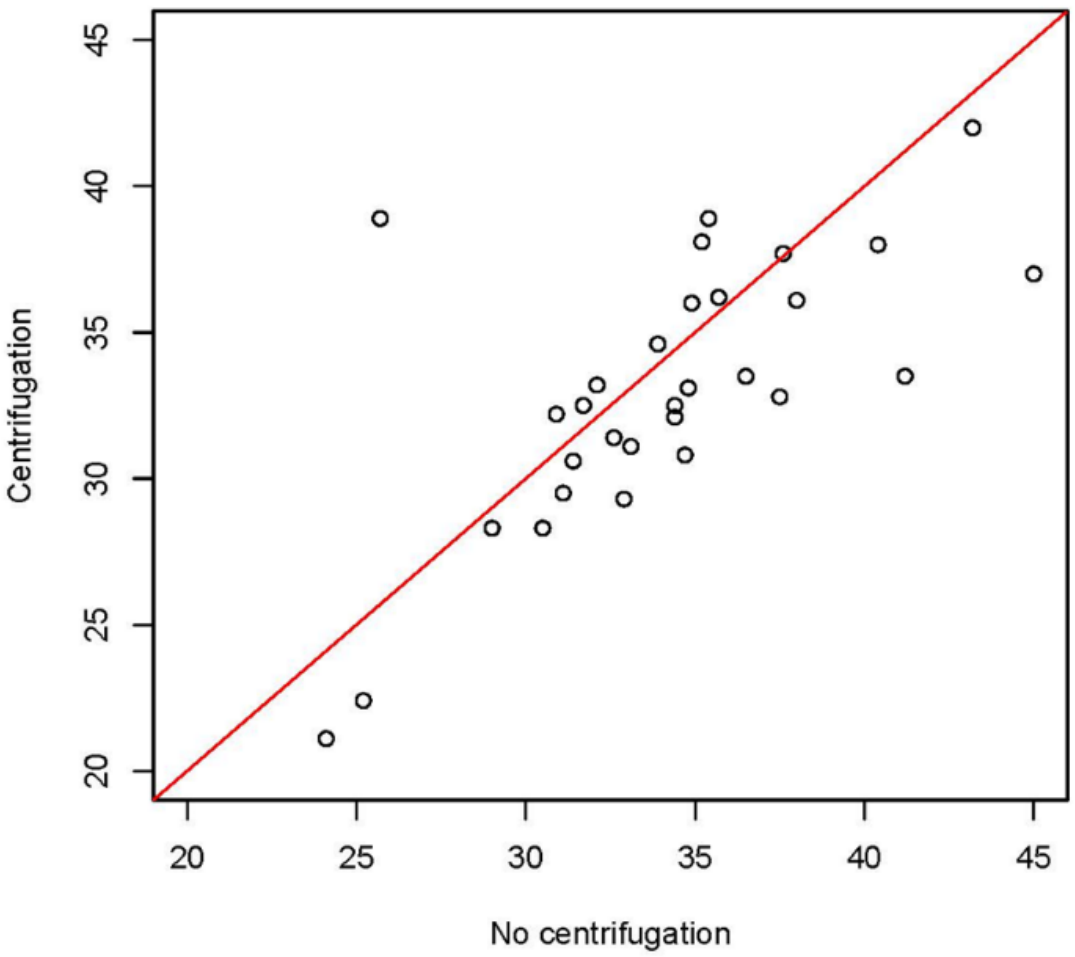

Figure 3: The effect of centrifugation on the detection of SARS-CoV-2 in saliva. The C_t values shown were produced on the Cepheid Xpert SARS-CoV-2/Flu/RSV test and characterize specimens before (x-axis) and after (y-axis) centrifugation. The effect of centrifugation on Ct value, was not statistically significant ($p = 0.14$).

13.0 LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
COVID-19	Coronavirus Disease 2019
RT-PCR	Reverse Transcription Polymerase Chain Reaction
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
PCR	Polymerase Chain Reaction
FDA	Food and Drug Administration
SAR-CoV-1	Severe Acute Respiratory Syndrome Coronavirus 1
RP2.1	Respiratory Panel 2.1
CLIA	Clinical Improvement Amendments
IRB	Institutional Review Board
NS	Nasal Swab
NP-S	Nasopharyngeal Swab in Saline
NP-VTM	Nasopharyngeal Swab in Viral Transport Media
OP	Oropharyngeal Swab
NGDS	Next Generation Diagnostic Systems
VTM	Viral Transport Media
NP	Nasopharyngeal Swab
MHSRS	Military Health System Research Symposium
RSV	Respiratory Syncytial Virus
IFU	Instruction for Use
N	Nucleocapsid Protein
E	Envelop Protein
LoD	Limit of Detection
PPA	Positive Percentage Agreement
C_t	Cycle Threshold