ABSTRACT

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.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) was first identified in late December 2019 in Wuhan, the capital of Central China's Hubei Province. Within the United States, from 3 January 2020 to 30 June 2021, there have been 33,317,803 confirmed cases of COVID-19 with 599,089 fatalities, which are both highest in the world. In response to rapid spread of SARS-CoV-2, there was 249 diagnostic kits have been approved by the FDA under Emergency Use Authorization (EUA). Overall, reverse transcription-polymerase chain reaction (RT-PCR) was one of the primary laboratory method employed by these diagnostic kits. However, with traditional RT-PCR **(Fig. 1),** sample processing can add significant time as well as the risk of contamination leading unreliable results.

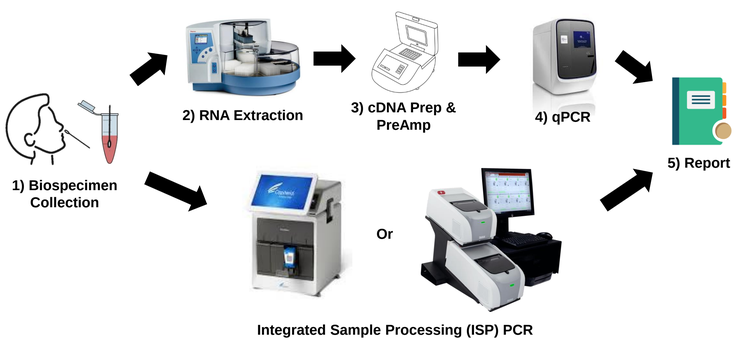


Figure 1: Traditional RT-PCR workflow and RT-PCR with ISP

Subsequently, one-step RT-PCR with integrated sample processing (ISP) have been developed meet this critical gap. Briefly, these are close-loop systems that extracts and processes raw samples prior to PCR. Overall, PCR with ISP significantly reduced operating time and lowers the risk of contamination leading to unreliable results, which are critical needs for meeting the current high-throughput needs.

OBJECTIVES

1. Assess the performance of BioFire® FilmArray and the Cepheid GeneXpert® integrated platforms for their limit of detection and specificity for the SARS-CoV-2 virus.
2. To validate the use of various patient specimens: nasopharyngeal swabs, oropharyngeal swabs, nasal wash, and saliva.

METHODS

Limit of Detection (LoD)

Uninfected sample was spiked with various concentrations of the SARS-CoV-2 reference material kit, up to LoD as reported by EUA.

Patient Sample Collection and Analysis

5 biospecimen types from 216 unique patients were collected and provided iSpecimen® (**Table 1**). Positive and negative COVID patient were determined by iSpecimen® by CLIA-approved RT-PCR tests available at one of the 3 enrollment sites.

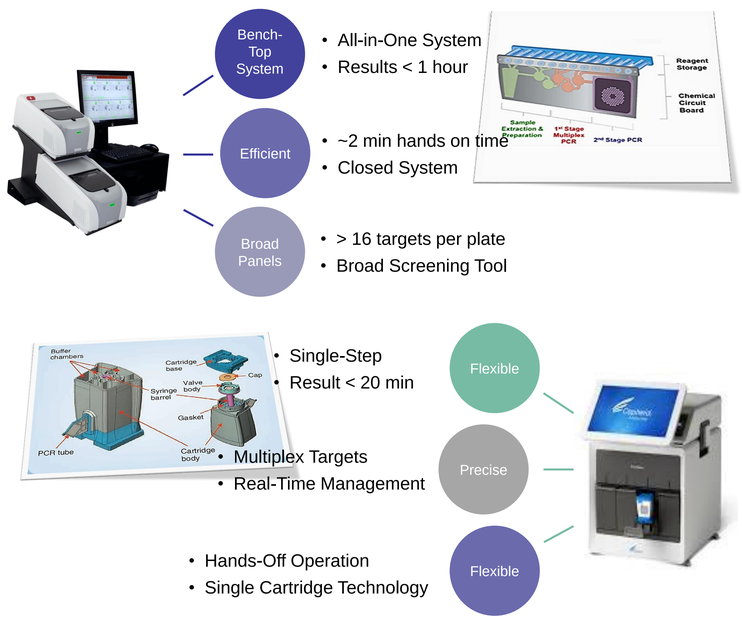
Table 1: Overview of patient cohort assignment (n = 216)

Cohort	No. of Patient	Description
1	61	COVID-19 Positive (0 – 14 days)
2	47	COVID-19 Positive (15 – 30 days)
3	108	COVID-19 Negative

*Days determined based from the first day symptoms

Collected Biospecimen Types
Nasopharyngeal Swab in Saline (NP-S)
Nasopharyngeal Swab in VTM (NP-VTM)
Oropharyngeal Swab (OP)
Nasal Swab (NS)
Saliva

- All biospecimens were shipped to and stored at CAMD under -80°C.
- All five biospecimen types were evaluated by **(Fig. 2)**:
  1. Cepheid GeneXpert®
     - Xpert Xpress SARS-CoV-2/Flu/RSV test
  2. BioFire® FilmArray®
     - FilmArray® RP2.1 panel.



RESULTS

Limit of detection testing was carried out using the Seracare Accuplex Reference material kit (#0505-0126) that includes:

- Human RdRp (Negative Control)
- Positive control
- Viral Primer for Envelope, Nucleocapsid, RdRp, and ORF1a.

LoD for Xpert® Xpress and BioFire ® RP2.1 were **81.25** and **720** copies/mL, respectively **(Table 2).** LoD was determined when all six replicates reported positive.

Table 2. Results of LOD dilution testing

Dilution	Copies/mL	Cepheid Xpert® Xpress	Dilution	Copies/mL	BioFire® RP 2.1
3.00E-02	150	6/6	2.00E-01	1000	6/6
2.00E-02	100	6/6	1.50E-01	750	6/6
1.75E-02	88	6/6	1.44E-01	720	6/6
1.63E-02	81	6/6	1.37E-01	687.5	5/6
1.50E-02	75	5/6	1.25E-01	325	5/6
1.00E-02	50	3/3	1.00E-01	500	3/6
NC	0	0/6	7.00E-02	350	3/6
			5.00E-02	250	2/6
			2.72E-02	136	2/6
			NC	0	0/6

[1]SeraCare AccuPlex SARS-CoV-2 Reference Material Kit #0505-0126 (5000 copies/mL)
[2]Only Membrane target detected.

Percentage Positive and Negative for each biospecimen type are shown in **Fig 2** & **3**. There was no statistical differences between the two NGS-ISP platforms **(Table 4).** Furthermore, all specimen types were equally adequate as biomaterial for the detection of SARS-CoV-2. However, due to volume challenges, not all Saliva samples were tested.

Figure 2: Percentage Positive for Each Biospecimen Type

Figure 3: Negative Positive for Each Biospecimen Type

Table 3. Comparative Pe
2/Flu/RSV to BioFire® R

Specimen Type	+/+	+/-	-/
NS	53	9	
NP-Saline	62	11	
NP-VTM	66	14	
OP	65	8	
Saliva	54	1	

[1]Twelve patients had insufficie

**Table 4 . Ct value distri
Discordant BioFire® Re**

N and M gene Ct value	Nasal Swab (NS)	
	+/+	+/-
Ct ≤ 20	2	
20 < Ct ≤ 25	5	
25 < Ct ≤ 30	12	
30 < Ct ≤ 35	20	
35 < Ct ≤ 40	12	
40 < Ct ≤ 45	2	9

- Nasopharyngeal swab significant differences saline would be a suita
- Both platforms perform every sample type.
- Cepheid GeneXpert® Biofire® platform acr demonstrated difficult components. Technical remove contaminants.
- Higher C_t values in dis system is more sensitiv
- Oropharyngeal swab taking patient samples swab sample, and is le

R

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ACKN

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The views expressed in this article reflect the results of research conducted by the authors and do not necessarily reflect the official views, policy or position of the Department of the Navy, Department of Air Force, Department of Defense, nor the United States Government.