Rapid Respiratory Panel Testing for SARS-CoV-2: Experience in a Private Tertiary Hospital

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ABSTRACT

SARS-CoV-2 has infected more than 643 million individuals worldwide and accounts for close to 64,950 deaths in the Philippines. Due to COVID-19's clinical overlap with other diseases and non-specific radiologic findings, its diagnosis rests primarily on laboratory methods, including reverse transcription polymerase chain reaction (RT-PCR) and multiplexed molecular platforms for rapid syndromic testing. Compared to RT-PCR which has a turnaround time of 24 to 72 hours, multiplexed molecular platforms can provide alternative diagnoses to COVID-19 in an average of one hour, providing meaningful data that can impact clinical and resource management when handling acute surge of patients with respiratory symptoms.

Key words: COVID-19, SARS-CoV-2, diagnostics, film array, RT-PCR

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INTRODUCTION

COVID-19 is a highly infectious disease that broke out in Wuhan, China in December 2019. Caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), it eventually spread to become a public health emergency of international concern on January 30, 2020 and a pandemic on March 11, 2020. As of December 12, 2022, there have been 643,875,406 confirmed cases around the world, including 6,630,082 deaths.[1] In the Philippines, there has been a total of 4,050,045 confirmed cases with 64,902 deaths.[2] Aside from the clinical effects of the disease, COVID-19 has also led to economic damage and changes in the socio-political climate.

The clinical spectrum of COVID-19 ranges from asymptomatic to mild disease to respiratory failure necessitating mechanical ventilation to multiorgan dysfunction. When symptomatic, the primary clinical presentation of COVID-19 is fever and dry cough.[3] Other common symptoms include sore throat, nasal congestion, malaise, loss of taste and/ or smell, and diarrhea.[3]

Since the disease often manifests as pneumonia, radiologic imaging has a pivotal role in the diagnosis and management of infected patients. Chest x-ray, chest computed tomography (CT) and lung ultrasound may show multifocal alveolar opacities; patchy, multifocal, bilateral ground glass areas with consolidation; and pleural effusion.[3] However, the patterns seen on imaging are often non-specific. Coupled with the significant overlap in clinical presentation with a host of other diseases, diagnosis rests primarily on laboratory methods.

The most sensitive, specific, and widely used test is the reverse transcription polymerase chain reaction (RT-PCR), which involves the amplification of genetic material isolated from upper and/ or lower respiratory tract samples. Current laboratory methods for RT-PCR for COVID-19 have a turnaround time of 24 hours to 3 days, due to

tests being run in batches and to give time allowance for repeat testing in cases of initially indeterminate results. This long turnaround time (TAT) has led to complaints from patients in our institution, especially those from the emergency room awaiting admission, as results are required for triaging and determination of which ward to admit the patient. Clinicians in our institution also raised the question of false negative results in patients presenting with respiratory symptoms and a negative RT-PCR result.

In contrast, multiplexed molecular platforms for rapid syndromic testing, such as the BioFire® Respiratory Panel 2.1 plus (RP2.1*plus*) (Cepheid, USA), has an average TAT of one hour. These platforms are real time, nested, multiplexed nucleic acid tests that, in addition to detecting SARS-CoV-2, can also simultaneously identify other respiratory viral and bacterial nucleic acids in nasopharyngeal swab samples (Table 1).[4] All necessary reagents for isolation, amplification, and detection of nucleic acids from the aforementioned respiratory pathogens are contained within a closed system disposable pouch. In RP2.1*plus*, the sample is prepared by bead beating and chemical lysis.[4] Extraction and purification of nucleic acids occurs via magnetic bead technology.[4] Endpoint melting curve data are then used to detect target-specific amplicons which are analyzed to generate a result.[4]

RP2.1*plus* received emergency-use authorization (EUA) from the US Food and Drug Administration last May 4, 2020 for use on clinical samples and is considered a confirmatory test for SARS-CoV-2 infection by the Philippine Department of Health as part of the national laboratory response. The test has a reported clinical sensitivity of 98% and specificity of 100% for SARS-CoV-2, with a limit of detection at $1.6x10^2$ copies/mL.[4] It has a 98% percent positive agreement and a 100% percent negative agreement when compared to other SARS-CoV-2 EUA assays.[5]

In our institution, both RT-PCR and RP2.1*plus* results are made available to the attending physician through the electronic records and to the patient via an electronic portal.

METHODOLOGY

We retrieved the results and demographic data of patients tested for SARS-CoV-2 using RP2.1*plus* in our institution's electronic records. The study covers data gathered over three months of testing, which covers an initial period of RP2.1*plus* being offered in our institution (November 2020) and a subsequent surge in COVID-19 cases in the country (September and October 2021). Our report was deemed exempt from ethical clearance by our institutional review board as it does not include identifiable personal information or patient photographs.

RESULTS AND DISCUSSION

Our institution received 2,325 clinical samples for SARS-CoV-2 testing using the RP2.*1plus* panel during the study period. The majority were samples from adult patients [mean age: 45 years (Table 2)] being admitted through the emergency department.

Table 1. Respiratory viral and bacterial nucleic acids that can be detected by the BioFire® Respiratory Panel 2.1*plus*

Viruses	Bacteria
Adenovirus	*Bordetella parapertussis*
Coronavirus 229E	*Bordetella pertussis*
Coronavirus HKU1	*Chlamydia pneumoniae*
Coronavirus NL63	*Mycoplasma pneumoniae*
Coronavirus OC43	
Middle East Respiratory Syndrome Coronavirus (MERS-CoV)	
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	
Human Metapneumovirus	
Human Rhinovirus / Enterovirus	
Influenza A	
Influenza B	
Parainfluenza Virus 1	
Parainfluenza Virus 2	
Parainfluenza Virus 3	
Parainfluenza Virus 4	
Respiratory Syncytial Virus	

Table 2. Demographic data of patients whose samples were tested using RP2.1*plus*

	Number of samples (n)	Percentage of total samples (%)
Sex		
Male	1,015	43.7
Female	1,310	56.3
Age		
Pediatric (0-18 years)	197	8.5
Adult (> 18 years)	2,128	91.5
Total samples	***2,325***	***100***

SARS-CoV-2 was detected in 19.23% of the samples tested while 1.85% had co-infection with other viruses, the most common being Human Rhinovirus / Enterovirus (Table 3). Almost eight percent were positive for an infectious agent other than SARS-CoV-2 while 71.1% of the samples were negative for all viral and bacterial nucleic acids included in the panel. The most common infectious agent in the SARS-CoV-2-negative samples was the Human Rhinovirus / Enterovirus. Note that a negative SARS-CoV-2 result was seen in majority of cases, which then facilitated patient admission to non-COVID wards in our institution.

The human rhinovirus / enterovirus is the most common infectious agent worldwide, afflicting both children and adults.[6] This could then account for the high incidence of this strain in our clinical samples. With an average incubation period of two days, symptom duration of seven to ten days, and a clinical presentation that includes nasal congestion, cough, malaise, and pneumonia, it has considerable clinical overlap with SARS-CoV-2. The prevalence of this causative agent could account for cases who present with a clinical picture suspicious for COVID-19 but who subsequently test negative for SARS-CoV-2 on RT-PCR. For cases that ultimately tested negative for all viruses and bacteria included in the panel, possible explanations include infection with pathogens not detected by RP2.1*plus* and lower respiratory tract infection which may not be detected with a nasopharyngeal swab.[4]

Viral co-infection in patients with COVID-19 has been previously documented, seen in 4.3% to as many as 47% of SARS-CoV-2 infected patients.[7,8] The most common

Table 3. Summary of test results obtained using RP2.1*plus*

	Number of samples (n)	Percentage of total samples (%)
Positive for SARS-CoV-2 only	447	19.23
Positive for SARS-CoV-2 with co-infection	43	1.85
SARS-CoV-2 + Adenovirus	8	—
SARS-CoV-2 + Coronavirus HKU1	1	—
SARS-CoV-2 + Human Rhinovirus / Enterovirus	16	—
SARS-CoV-2 + Parainfluenza Virus 2	2	—
SARS-CoV-2 + Parainfluenza Virus 3	1	—
SARS-CoV-2 + Parainfluenza Virus 4	1	—
SARS-CoV-2 + Respiratory Syncytial Virus	8	—
SARS-CoV-2 + Influenza B	1	—
SARS-CoV-2 + Bordetella pertussis	1	—
SARS-CoV-2 + Multiple other viral strains	4	—
Positive for other viral strain	179	7.70
Adenovirus	16	—
Coronavirus 229E	1	
Coronavirus HKU1	4	—
Coronavirus NL63	6	—
Human Metapneumovirus	1	—
Human Rhinovirus / Enterovirus	83	—
Parainfluenza Virus 1	2	—
Parainfluenza Virus 2	2	—
Parainfluenza Virus 3	3	—
Parainfluenza Virus 4	3	—
Respiratory Syncytial Virus	37	—
Influenza A	3	—
Influenza B	4	—
Positive for multiple other viral strains	14	—
Positive for bacteria	1	0.04
Bordetella parapertussis	1	—
Negative for all viruses and bacteria included in the panel	1655	71.1
Total samples	***2325***	***100***

causative agent in SARS-CoV-2 co-infection varies according to study population. For example, the most common co-infective agent in a cross-sectional study in Indonesia was influenza A virus, followed by influenza B virus.[7] In contrast, the most common co-infective agent in our sample population as well as in a study conducted by Le Glass et al., is human rhinovirus / enterovirus.[8] Of note, in silico analyses of RP2.1*plus* from the manufacturer did not show any loss of sensitivity in detecting SARS-CoV-2, despite the presence of co-infection.

The human rhinovirus / enterovirus causes a predominantly mild and self-limited infection, just like COVID-19.[3,6] However, in patients co-infected with SARS-CoV-2 and rhinovirus, reported cough and dyspnea is significantly more common compared to rhinovirus monoinfection.[8] On the other hand, the likelihood of being transferred to an intensive care unit is not statistically significant between SARS-CoV-2 and rhinovirus co-infection and rhinovirus monoinfection.[8] The impact of co-infection with other causative agents of upper respiratory infections on SARS-CoV-2 replication and transmission is unknown, but a study has shown that human rhinovirus can block SARS-CoV-2 replication by triggering an interferon response.[9]

One limitation of our study is that at the time the study was conducted, circulating SARS-CoV-2 variants of concern only included alpha (B.1.1.7), beta (B.1.351), gamma (P.1), and delta (B.1.617.2). Whether or not the aforementioned frequencies will be similar as SARS-CoV-2 continues to evolve can be a subject of further study. Additional studies can also expand the dataset to include additional months (or years) of testing; it should be noted that this was only intended to furnish initial data on the utility of rapid syndromic molecular testing in the Philippine setting.

There is no considerable difference between the management of patients with mild SARS-CoV-2 infection and infection with other respiratory pathogens, which consists mainly of symptomatic therapy. However, the ability to definitively rule out SARS-CoV-2 and to simultaneously identify an alternative diagnosis for the patient has potential impact on clinical and resource management when handling such cases.

CONCLUSION

With a shorter turnaround time and the ability to detect alternative diagnoses and SARS-CoV-2 co-infection, rapid syndromic molecular testing provides meaningful data that can impact clinical and resource management when handling patients with respiratory symptoms. In the emergency room setting, this can facilitate the triaging of patients being admitted to designated hospital wards.

STATEMENT OF AUTHORSHIP

The authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

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