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REVIEW ARTICLE

# Flu Viral Multiplex Testing and its Implication for COVID-19 Testing

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**Abstract:**

***Background:***

The diagnosis of viral causes for flu-like syndromes have positively been impacted by the availability of molecular assays. In recent years, syndromic multiplex panels have been able to give rapid turn-around-times and highly accurate results. We examine the use of this test during the first four months of 2020 during the COVID-19 pandemic.

***Methods:***

A retrospective review of 2145 patient results from a multiplex syndromic flu panel using Biofire RP2 was performed. Cases in which parallel testing for COVID-19 by real-time polymerase chain reaction (RT-PCR) was compared.

***Results:***

53% of the patients tested identified as a viral agent. 13% of the positive cases were coinfection with more than a single virus. The most frequently detected virus(es) were rhinovirus/enterovirus, followed by coronaviruses (non-MERS, non-COVID-19). One hundred patients had simultaneous testing for COVID-19. Seventeen (17%) had positive COVID-19 by RT-PCR. Three of these patients had coinfection with rhinovirus/enterovirus and COVID-19. The negative predictive value for COVID-19 based on a positive non-COVID agent was 95% in our sample.

***Conclusion:***

Viral syndromic panels are useful for the rapid detection and appropriate treatment of patients. Our results suggest coinfection is infrequent, and we discuss the impact of COVID-19 on patient testing strategy. The use of multiplex panels is useful to provide accurate diagnosis and rule out important pathogens that have different treatment approaches.

**Keywords:** PCR, Flu, Lab, Molecular, COVID-19, Virus.

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|---|---|---|---|

## 1. INTRODUCTION

The diagnosis of viral illnesses has significantly been impacted by the advent of molecular diagnosis [1 - 3]. Multiplex syndromic panels have facilitated the identification of viral aetiologic agents from a single sample. Validated, FDA-approved, CE-marked kits are readily available, and the rapid turn-around-time (TAT), gives clinicians quick actionable information, and provides relief to patients and parents of children to know that they will be receiving the appropriate treatment and medical care [1 - 3].

In this past calendar year, the novel Coronavirus (COVID-19) has presented challenges to laboratory diagnostics, public health efforts, and treatment of symptomatic patients [4 - 7]. The rapid identification of infected patients has been provided by the gold standard polymerase chain reaction (PCR) testing using specific primer sets. This has been made possible by rapid identification of the COVID-19 genome and validation of PCR tests for detection with high sensitivity and specificity [4, 8, 9]. In addition, clinical parameters and epidemiologic factors continue to inform our triage of patients and identify appropriate individuals and cohorts for testing.

We examine our experience with patients tested for flu-like symptoms panel during the 2020 year and looked at the relationship between COVID-19 and the syndromic panel test outcomes with specific attention to cases with parallel testing. Based on our data, we make recommendations for future testing strategies with multiplex platforms to apply to similar

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novel flu-like outbreaks in the future.

## 2. MATERIALS AND METHODS

We retrospectively reviewed the molecular viral lab test results of patients evaluated for flu-like symptoms at a private hospital in Dubai during January to April 2020.

Multiplex molecular analysis was performed using the Biofire RP2 machine. Nasopharyngeal swabs were used for specimen source.

The multiplex test detects twenty-one separate organisms, including 17 viruses and 4 non-viral and bacterial organisms, including adenovirus, coronaviruses (HKU1, NL63, 299E, OC43, MERS), human metapneumovirus, human rhinovirus/enterovirus, influenza (A, A/H3, A/H1-2009, B), parainfluenza virus [1 - 4], respiratory syncytial virus (RSV); Bordetella parapertussis, Bordetella pertussis, Chlamydia pneumoniae, Mycoplasma pneumoniae. Specimens were tested by using the sample testing pouch as per manufacturer instructions, with the quality control is built into the test system [2, 3].

COVID-19 results were based on tests for SARS-CoV-2 by a real-time quantitative polymerase chain reaction in the central Dubai Health Authority Virology Laboratory where viral RNA was extracted from nasopharyngeal swabs and amplified using standard protocols. using the QIAamp Viral RNA Mini or the EZ1 DSP Virus Kits (Qiagen, Hilden, Germany).

Calculation of sensitivity, specificity, negative (NPV) and positive predictive values (PPV) used RVP+ as a predictor of COVID- as an inverse relationship. COVID-19 PCR was considered the gold standard for viral status. Thus, sensitivity was 14/17 = 82% (RVP-COVID+/COVID+); specificity 60/83 = 72% (RVP+COVID-/COVID-); NPV 60/63 = 95% (RVP+COVID-/RVP+); PPV 14/37 = 38%(RVP-COVID+/RVP-).

Institutional Ethics Committee permission was granted and based on quality assurance and audit activity under Clinical Governance with the maintenance of patient privacy of information.

## 3. RESULTS

### 3.1. Testing Volumes for Flu Syndromic Panel

Viral syndromic multiplex testing was performed on patients presenting with flu-like symptoms. Most testing and positive cases occurred in the first three months (Table **1**). The maximum number of tests was in March, with 787 sampled patients. The highest number and percentage of positive specimens occurred in January with a decrease over the next three months. An average of one in seven patients (13% average) of the positive cases were co-infected with more than one virus (monthly range 7-17%).

### 3.2. Relative Frequency of Type of Virus Infection

Rhinovirus/Enterovirus species were most frequently seen in over 40% of the positive cases. This was followed in frequency by influenza species, then common alpha-and beta-coronaviruses (229E, HKU1, NL63, OC43). No cases of MERS-coronavirus were detected in our patient population. The current viral multiplex system does not detect the agent for COVID-19 (Table **2**).

The most common coinfecting virus was also rhinovirus/enterovirus species (44%) followed by influenza and common coronaviruses (non-MERS, non-COVID) seen in 16% of the coinfected cases.

Most cases of multiple infections were detected in the paediatric population. Fifteen patients were coinfected with three agents. All of these were in the paediatric age group (range 3-months to 10-years). In the 15 patients with three agents, all had rhinovirus, followed by adenovirus seen in five patients.

**Table 1. Summary of respiratory syndromic panel testing volumes.**

| Month | Volume | Positive | Coinfection |
|---|---|---|---|
| Jan | 578 | 426 (74%) | 71 (17%) |
| Feb | 597 | 392 (66%) | 44 (11%) |
| March | 787 | 304 (39%) | 33 (11%) |
| April | 203 | 15 (7%) | 1 (7%) |
| Total | 2145 | 1137 (53%) | 149 (13%) |

**Table 2. Frequency of viruses detected.**

| Virus | Single Virus | Co-infection |
|---|---|---|
| Rhino/enterovirus | 42% | 44% |
| Influenza A/B | 27% | 16% |
| Coronavirus | 12% | 16% |
| Metapneumovirus | 5% | 1% |
| Parainfluenza | 5% | 5% |
| Adenovirus | 4% | 11% |
| Respiratory syncytial virus | 2% | 5% |

**Table 3. Paired COVID19 and multiplex viral testing results.**

| - | COVID- | COVID+ |
|---|---|---|
| Respiratory Panel negative | 23 | 14 |
| Respiratory Panel Positive | 60 | 3 |

### 3.3. Paired COVID Testing Results

One hundred patients who had multiplex testing also were tested for COVID-19 by real time PCR (Table **3**). Sixty-three of these patients were positive on the multiplex panel and seventeen were positive for COVID-19. Coinfection with viruses by paired testing were seen in three patients; all had COVID-19 coinfection with rhino/enterovirus. Single infection by COVID-19 was the predominant finding. If we used the multiplex as a predictor of COVID-19 infection and assume an exclusive inverse relationship, then the negative predictive value (NPV) for COVID-19 when another virus is present by our multiplex panel is 95% (sensitivity 82%, specificity 72%, positive predictive value PPV 38%).

## 4. DISCUSSION

We have characterized the experience of syndromic panel virus testing during the first months of 2020 when we also dealt with the COVID-19 pandemic. Our study is also consistent with reports in the literature report of the high prevalence of Rhinovirus/enterovirus species as the main agent for flu-like symptoms. This is generally the reported epidemiology and agrees with a prior report from the Kingdom of Saudi Arabia [10]. One would also expect the rate of coinfection to reflect this based on pure stochastic mechanisms and models have examined this using mathematical schemes [11]. Coinfection and superinfection by other agents have been studied [12]. Our focus was not on clinical findings or severity of illness. However, a prior study from Egypt confirmed that most co-infections occur in children, and that superinfection with bacterial organisms leads to a more severe clinical course [13]

Prior to the availability of widespread COVID-19 molecular testing, the triage strategy was predicated on clinical findings, travel history, and exposure [5, 6]. Prior to index cases and widespread available COVID-19 PCR testing, we had some experience on the prevalence of coinfection among patients presenting with flu-like symptoms. This proportion was consistent during the flu season. This likely reflects ongoing competition of viruses for receptors and other means of entry into the cell to account for productive infection and symptoms [11]. During the time prior to documented community transmission of COVID-19, one strategy was to focus on additional testing and attention on patients with high risk for exposure who were negative for other viruses by our multiplex assay. This has proven to be a valid approach as the negative predictive value is quite high when another viral cause for flu-like symptoms is identified. Based on our results, the cases of coinfection were only seen with rhinovirus/enterovirus in three of seventeen patients (18%). This is similar to the data from the Stanford study and other PCR based detection studies [14]. The rate of coinfection based on serologic evidence of infection is much higher, almost 90% in some cases [14, 15]. Prior studies have focused on coinfection with influenza, which can present clinical challenges [16, 17]. We did not identify any patients in our cohort with influenza coinfection, as all our COVID-19 coinfections were with rhinovirus/enterovirus.

Our recommendation for settings where molecular COVID-19 data is not readily available or there is a time urgency, a multiplex assay can be used, and a positive identification of a viral agent would rule out COVID-19 infection with a high degree of certainty. A negative result on symptomatic patients should be treated as suspicious for COVID-19 until PCR results are returned. In our setting, we used additional clinical information and diagnostic information to triage and prioritize high-risk patients for additional testing as needed. High risk clinical criteria include as fever, anosmia, travel history, exposure to known or high-risk patients [6, 7]. In recent months, dermatologic and gastric symptoms and orchitis have been reported as markers of COVID-19 [18-21]. Diagnostic findings such as neutrophil to lymphocyte ratio, C-reactive protein, procalcitonin, and troponin levels have been used, in addition to imaging ground-glass opacities in lung.

## CONCLUSION

The use of multiplex panels provides timely diagnosis and identification of organisms with different treatment approaches. The possibility of co-infection, which requires multiple assays, especially in the paediatric population, should be of concern. Future studies as we enter the next flu season will be important to determine the optimum testing strategy in various settings.

## CONSENT FOR PUBLICATION

Not applicable.

## FUNDING

None.

## CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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