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Performance Characteristic of COVID-19 and Flu A/B Multiplex Panel for Home use (Nasal Swab) Evaluated by Comparative RT-PCR Experiments

Zhang Lei[1*], **Yang Feng**[1] **and Zhu Junzhe**[2]

[1]*Department of Nursing Sciences, Zhejiang Gongshang University, Hangzhou, China*

[2]*Department of Nursing Sciences, Wenzhou Medical University, Wenzhou, China*

[*]**Corresponding author:** Dr. Zhang Lei, Department of Nursing Sciences, Zhejiang Gongshang University, Hangzhou, China, E-mail: zhanglei@zjgsu.edu.cn

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Abstract

Background: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) emerged in late 2019 and rapidly evolved into the current coronavirus pandemic. SARS-CoV-2 is a respiratory virus that causes symptoms similar to those caused by influenza A and B viruses. Although patients infected with SARS-CoV-2, influenza A and influenza B show comparable or similar manifestations, the therapeutic approaches of these respiratory viral infections are different, which requires an accurate diagnosis. Identification of viral RNA by real-time Reverse Transcription-Polymerase Chain Reaction (RT-PCR) remains the gold standard for diagnosing SARS-CoV-2 infection. However, laboratory equipment cost, availability and the need for trained personnel limit testing capacity. Through an unprecedented research effort, new diagnostic techniques such as rapid diagnostic testing, isothermal amplification techniques, and next-generation sequencing were developed, enabling accurate and accessible diagnosis. Influenza viruses are responsible for seasonal outbreaks infecting up to a quarter of the global human population. Influenza and SARS-CoV-2 present with flu-like symptoms making the differential diagnosis challenging solely on clinical presentation. Healthcare systems are likely to be faced with overlapping SARS-CoV-2 and Influenza outbreaks. On July 2, 2020, the U.S. Food and Drug Administration (FDA) granted emergency use authorization for an *in vitro* diagnostic multiplex assay for SARS-CoV-2 influenza.

Objective: The main purpose of this evaluation report is to assess the accuracy of the COVID-19 and flu A/B multiplex panel for home use (Nasal Swab) for the rapid qualitative detection of SARS-CoV-2 nucleocapsid protein, influenza A and influenza B nucleoprotein antigens in nasal swab specimens.

Methods: Run a rapid *in vitro* diagnostic test device for the detection of antigens to SARS-CoV-2 nucleocapsid protein, Influenza A and Influenza B nucleoproteins in nasal swab and compare with a leading commercial RT-PCR test for validation of performance.

Results: The results show that in the comparative experiment with RT-PCR, the relative accuracy of COVID-19 and flu A/B multiplex panel for home use (Nasal swab) in the qualitative detection of SARS-CoV-2 nucleocapsid protein, Influenza A and Influenza B nucleoproteins antigens are respectively 98.69%, 99.10% and 98.92%.

Conclusion: CITEST COVID-19 and flu A/B multiplex panel for home use (Nasal swab) is a rapid test that is a qualitative membrane-based immunoassay for the detection of SARS-CoV-2 nucleocapsid protein, Influenza A and Influenza B nucleoproteins antigens in human nasal swab specimen. The product is simple to operate and has been validated against an industry leading commercial RT-PCR test to give results within 10 minutes of the sample being tested. The COVID-19 and flu A/B multiplex panel for home use (Nasal swab) has been evaluated with specimens obtained from patients. In the comparison test with RT-PCR, the accuracy rate of the SARS-CoV-2 test reached 98.69%, the accuracy rate of influenza A antigen test reached 99.10%, and the accuracy rate of influenza B antigen test reached 98.92%. In the case of simultaneous outbreaks of the SARS-CoV-2 and influenza, these test kits can be used to obtain accurate results.

Keywords: COVID-19; SARS-CoV-2; Influenza A; Influenza B; Rapid test; RT-PCR; Accuracy

Introduction

About COVID-19 and influenza

SARS-CoV-2 infection exhibits a wide range of clinical presentations, often characterized by fever, dyspnea, lymphopenia, and lower respiratory tract infection [1]. Although COVID-19 is considered mainly a viral pneumonia, patients may also present gastrointestinal involvement such as diarrhea (30%-40% of patients) caused by the active replication of SARS-CoV-2 in enterocytes, splenic atrophy, and gastrointestinal lymphadenopathy [2,3].

The clinical presentation of influenza varies from asymptomatic infection to fulminant illness, depending on the characteristics of the virus and the host. Typical influenza usually strikes suddenly after an incubation period of 1 days-2 days. Systemic symptoms dominate the initial presentation and typically include fever, chills, headache, myalgia, malaise and anorexia. Ocular symptoms include ocular muscle pain, tearing, and a burning sensation in the eyes. Respiratory symptoms also occur during flare-ups, including dry cough, sore throat, runny and stuffy nose and more. Children and the elderly are more susceptible to influenza infection and are often accompanied by persistent high fever and other symptoms after infection. Children may also experience gastrointestinal symptoms after infection with influenza.

Materials and Methods

Complications from COVID-19 and influenza

Acute Respiratory Distress Syndrome (ARDS) is one of the most frequent and potentially severe complications of SARS-CoV-2. As the disease progresses, ARDS causes diffuse alveolar damage leading to hypoxemia, pulmonary infiltrates and fibrosis. Other respiratory complications are secondary bacterial and fungal co-infections and sepsis which may occur in 8% of cases [4]. COVID-19 is also associated with several cardiovascular complications, myocardial injury and myocarditis, acute myocardial infarction, heart failure and cardiomyopathy, arrhythmia, and thrombosis. In addition, other severe neurologic complications associated with COVID-19 include meningitis, encephalitis, Guillain-Barré syndrome, Miller-Fischer syndrome, acute myelitis, and posterior reversible encephalopathy syndrome levy. Mild neurologic manifestations, including taste and smell disturbances, headache, myalgia, dizziness, and confusion, occurred in 82% of patients throughout the course of the disease [5].

Influenza is often accompanied by two types of pulmonary complications—primary influenza viral pneumonia and secondary bacterial pneumonia. In addition to pulmonary complications, other organs and systems are also associated with influenza. Children are more likely than adults to develop myositis, which is often associated with IVb infection. For severely infected patients, influenza can also cause renal and liver damage, and myocarditis.

Diagnosis for COVID-19 and influenza

For COVID-19: Highly accurate detection methods are one of the main tools to improve the efficiency of COVID-19 control. Currently, nucleic acid-based RT-PCR molecular diagnosis is still considered the gold standard for early diagnosis of SARS-CoV-2 infection. However, due to the high specificity of this method, which requires sufficient viral RNA concentration in patient samples, the focus of qRT-PCR research is to improve sensitivity, processing of specimens, time and cost efficiency.

In addition to RT-PCR, current testing capabilities are augmented with rapid antigen and antibody tests that are readily available, inexpensive can be used in a point-of-care setting, and provide rapid results. The rapid antigen diagnostic test for SARS-CoV-2 detects SARS-CoV-2 antigens in clinical samples collected from the respiratory tract of infected individuals and this test is based on the immune response of antibodies to specific SARS-CoV-2 antigens found in the samples.

For influenza A and B: Clinical diagnosis provides acceptable accuracy in healthy young and middle-aged adults with acute influenza-like symptoms during seasonal epidemics. In addition, the symptoms of influenza are similar to those of other respiratory pathogens such as adenovirus, coronavirus, rhinovirus, Para influenza virus and respiratory syncytial virus, so specific laboratory diagnostic tests are required to confirm influenza infection. For distinguishing the viruses of these infections, a wide range of diagnostic tests are available: rapid influenza diagnostic tests, rapid molecular assays, immunofluorescence and RT-PCR.

Rapid diagnostic tests are based on the immunologic identification of viral nucleoprotein antigens in respiratory secretions. Currently, rapid influenza diagnostic tests must achieve minimum standards of 80% sensitivity for Influenza A and B compared to RT-PCR. Compared to viral cultures, a 90% sensitivity for influenza A and 80% for influenza B must be reached [6-8].

Results and Discussion

Evaluation of CITEST COVID-19 and flu A/B multiplex panel for home use (nasal swab)

Materials and directions for use: Materials provided included a test cassette, extraction buffer, sterile swab, tube holder, biosafety bag and a package insert.

The COVID-19 and flu A/B multiplex panel for home use (Nasal swab) is a qualitative membrane-based immunoassay for the detection of SARS-CoV-2 nucleocapsid protein, Influenza A and Influenza B nucleoproteins antigens in human nasal swab specimen.

The sample used in this test is human nasal swab specimen. The biosafety bag is brought to room temperature (15°C-30°C) before opening. Next, the test cassette is removed from the sealed bag and should be used within one hour.

For nasal swab use: First wash your hands thoroughly before testing. Remove the cover of the tube containing the extraction buffer and place the tube in the tube holder. Remove the swab from the bag, taking care not to touch the "cotton" tip. Then Insert the swab into your nostril. Do not exceed 2 cm, and firmly rub the swab in a circular motion around the inside wall of each nostril 5 times-10 times. After sampling, close the cap or fit the tube tip onto the tube. Remove the test cassette from the sealed foil pouch and use it within one hour, and placing the test cassette on a clean and level surface. Then invert the tube and add 3 drops of solution to each Specimen well (S) of the test cassette and start the timer. Take care not to move the test cassette during test development, and read the results after 10 minutes.

Performance characteristics: The COVID-19 and flu A/B multiplex panel for home use (Nasal swab) has been evaluated with

specimens obtained from patients. RT-PCR is used as the reference method for the COVID-19 and flu A/B multiplex panel for home use (Nasal swab). Specimens were considered positive if RT-PCR indicated a positive result. Specimens were considered negative if RT-PCR indicated a negative result (Table 1).

The COVID-19 and Flu A/B multiplex panel for home use		RT-PCR (Nasopharyngeal Swab)		Total
		Positive	Negative	
SARS-CoV-2 Antigen	Positive	375	3	378
	Negative	11	681	692
Total		386	684	1070
Relative sensitivity		97.15%(95%CI:94.96%~98.57%)		
Relative Specificity		99.56%(95%CI:98.72%~99.91%)		
Accuracy		98.69%(95%CI:97.81%~99.28%)		

Table 1: SARS-CoV-2 antigen test by using nasal swab and RT-PCR.

1070 specimens were used in the clinical trial to detect the SARS-CoV-2 antigen. The results showed that the specificity was 99.56%, the sensitivity was 97.15% and the overall accuracy was 98.69%(Table 2).

The COVID-19 and Flu A/B multiplex panel for home use		RT-PCR		Total
		Positive	Negative	
Influenza A Antigen	Positive	68	2	70
	Negative	3	485	488
Total		71	487	558
Relative Sensitivity		95.77% (95%CI: 88.14%~99.12%)		
Relative Specificity		99.59% (95%CI: 98.52%~99.95%)		
Accuracy		99.10% (95%CI: 97.92%~99.71%)		

Table 2: Influenza A antigen test by using nasal swab and RT-PCR.

In the clinical experiment of detecting Influenza A antigen, the clinical experiment included 558 samples, and the results showed that the specificity was 99.59%, the sensitivity was 95.77% and the overall accuracy rate was 99.10% (Table 3).

The COVID-19 and Flu A/B multiplex panel for home use		RT-PCR		Total
		Positive	Negative	
Influenza B Antigen	Positive	48	3	51
	Negative	3	504	507
Total		51	507	558
Relative Sensitivity		94.12% (95%CI: 83.76%~98.77%)		
Relative Specificity		99.41% (95%CI: 98.28%~99.88%)		
Accuracy		98.92% (95%CI: 97.67%~99.60%)		

Table 3: Influenza B antigen test by using nasal swab and RT-PCR.

In the clinical experiment of detecting Influenza A antigen, 558 samples were included. The results showed that the specificity was 99.41%, the sensitivity was 94.12% and the overall accuracy rate was 98.92%

Conclusion

During the SARS-CoV-2 pandemic, lockdowns, restrictions and widespread use of masks did limit the spread of seasonal influenza. However, with the removal of restrictions, influenza may overlap with COVID-19, thus posing a diagnostic challenge for infectious viruses. RT-PCR remains the gold standard for diagnosis of both infections, but there are limitations including laboratory equipment requirements and reagent costs limiting their use in point-of-care settings and low-income countries. Therefore, the rapid test kit can make a rapid diagnosis of SARS-CoV-2 and influenza virus infections under these restricted conditions.

The CITEST COVID-19 and flu A/B multiplex panel for home use (Nasal swab) in this evaluation performed well in standard experimental conditions. The tests showed both excellent accuracy and specificity. The results of tested samples demonstrate that the COVID-19 and flu A/B multiplex panel for home use (Nasal swab) developed by CITEST Diagnostics Inc. meets the requirements of professional *in vitro* diagnostic intended use. As a result, the CITEST COVID-19 and flu A/B multiplex panel can be used for the rapid and accurate detection of infectious viruses in cases of overlap between COVID-19 and influenza A/B viruses.

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