COVID-19 CADTH Horizon Scan

Combined Testing for Severe Acute Respiratory Syndrome Coronavirus 2 and Influenza

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Key Message

When severe acute respiratory syndrome coronavirus 2, or SARS-CoV-2 — the virus causing coronavirus disease 2019, or COVID-19 — and influenza are co-circulating in the winter months, combined diagnostic tests can be used to detect both viruses from 1 respiratory specimen in a single reaction. Using nucleic acid amplification in lab or point-of-care settings, most of these combined tests are based on reverse-transcription polymerase chain reaction, the current standard in detecting SARS-CoV-2 and influenza. These tests may be useful for patients with risk factors for severe reactions to influenza and coronavirus disease 2019, and in allowing labs to process more samples, thus helping to prevent backlogs.

How It Works

With the implementation of public health measures to mitigate the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes coronavirus disease 2019 (COVID-19), there has been a considerable reduction in the incidence of other respiratory viruses such as influenza. According to a recent FluWatch report published in May 2021 by the Public Health Agency of Canada (PHAC), there were 72 influenza detections to date for the 2020–2021 influenza season. In contrast, influenza numbers for the same periods in the past 6 seasons averaged 51,508 detections. Due to similar clinical manifestations of SARS-CoV-2 and influenza, there is a need for testing and treating potential viral co-infections.

The standard method of testing and diagnosing active infections of influenza (types A and B) and SARS-CoV-2 is with the use of molecular assays, also known as nucleic acid amplification tests. To help reduce sample processing time, combined molecular assays have been developed to simultaneously detect influenza and SARS-CoV-2 in a single reaction. Test results from a single specimen are available in 15 minutes to 8 hours. These tests detect viral genetic material (i.e., ribonucleic acid [RNA]) contained in specimens collected from the upper respiratory tract with nasopharyngeal or nasal swabs. To detect small quantities of viral RNA in collected specimens, these tests rely on the amplification of nucleic acids to produce multiple copies of the viral genetic material. This process ensures high test sensitivity — meaning, the test accurately detects the virus when present in a specimen and helps reduce the likelihood of false-negative test results.

There are 2 main types of nucleic acid amplification methods: reverse-transcription polymerase chain reaction (RT-PCR) and isothermal amplification (e.g., transcription-mediated amplification [TMA], loop-mediated isothermal amplification [LAMP]). Considered as the standard test for viral detection, lab-based RT-PCR takes up to 12 hours and requires the use of expensive thermal cyclers to create alternating temperatures. In contrast, the process of isothermal amplification is quicker and is conducted under constant temperatures, thus negating the need for thermal cyclers for RNA amplification. Currently, the following combined molecular assays testing for SARS-CoV-2 and influenza (types A and B) developed and marketed in North America are based on RT-PCR or TMA.
Tests based on RT-PCR:

- Roche cobas SARS-CoV-2 & Influenza A/B (for lab use on cobas 6800/8800 platforms)
- Roche cobas SARS-CoV-2 & Influenza A/B on Liat System (for lab and point-of-care use)
- Cepheid Xpert Xpress SARS-CoV-2/Flu/RSV
- Becton, Dickinson and Company SARS-CoV-2/Flu for BD MAX System
- NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay
- Bio-Rad Reliance SARS-CoV-2/FluA/FluB Assay Kit
- Thermo Fisher Scientific TaqPath COVID-19, FluA, FluB Combo Kit
- CDC Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay
- Quest Diagnostics RC COVID-19+Flu

Test based on TMA:

- Hologic Aptima SARS-CoV-2/Flu Assay

Who Might Benefit?

Combined molecular assays for SARS-CoV-2 and influenza (types A and B) could have beneficial implications for lab resources and patient health outcomes. Findings from a recent systematic review suggested that as high as 10% of patients who tested positive for SARS-CoV-2 were co-infected with another virus such as influenza. As some of the signs and symptoms of COVID-19 overlap with those of other respiratory viruses, the correct diagnosis of SARS-CoV-2 infection, either alone or as a co-infection, is important in informing appropriate public health measures and optimal treatment.

The simultaneous testing for SARS-CoV-2 and influenza infection is especially crucial for patients with risk factors for severe disease such as chronic conditions (e.g., high blood pressure, diabetes) and obesity, and for patients who are older. The need to distinguish between SARS-CoV-2 and influenza is particularly important during the winter months, when both viruses have the potential to be co-circulating. The COVID-19 Treatment Guidelines published by the National Institutes of Health recommend testing for SARS-CoV-2 and influenza in all hospitalized patients with suspected COVID-19 or influenza when both viruses are present in the community. Furthermore, these tests may help inform the need and urgency for strict patient isolation and contact tracing measures.

In addition to benefits to patient health outcomes, SARS-CoV-2 and influenza co-tests also have the potential to increase community viral surveillance and improve lab efficiencies. By simultaneously testing for SARS-CoV-2 and influenza from one respiratory specimen in a single reaction, labs could process more samples to help prevent backlogs and conserve test materials (e.g., reagents) that may be in short supply. Finally, combined molecular testing for SARS-CoV-2 and influenza provides public health organizations with ongoing surveillance of the spread of both viruses in the community.
Availability in Canada

Combined molecular assays targeting SARS-CoV-2 and influenza with market authorization in Canada through Interim Order are listed on the Health Canada website. In November 2020, Roche obtained authorization for 2 tests: SARS-CoV-2 & Influenza A/B test that runs on lab-based platforms (i.e., cobas 6800/8800) and SARS-CoV-2 & Influenza A/B test that is processed through the cobas Liat molecular point-of-care (POC) instrument. In December 2020, Cepheid obtained authorization for its Xpert Xpress SARS-CoV-2/Flu test.

In the US, combined molecular assays with Emergency Use Authorization are posted on the FDA website. In addition to the Roche and Cepheid tests, several other combined molecular assays targeting SARS-CoV-2 and influenza have obtained authorization in the US market: BD SARS-CoV-2/Flu for BD MAX System; NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay; Bio-Rad Reliance SARS-CoV-2/FluA/FluB Assay Kit; Thermo Fisher Scientific TaqPath COVID-19, FluA, FluB Combo Kit; CDC Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay; Quest Diagnostics RC COVID-19+Flu; and Hologic Aptima SARS-CoV-2/Flu Assay.

What Does It Cost?

The manufacturer’s list prices (per test) for the Roche lab-based and POC SARS-CoV-2 & Influenza A/B tests are $40.56 and $59.18 in Canadian dollars, respectively. The manufacturer’s list price for the Cepheid Xpert Xpress SARS-CoV-2/Flu/RSV test is not available.

As most SARS-CoV-2 and influenza diagnostic tests are publicly funded in Canada, the breakdown of costs associated with test procurement, administration, and processing are not clearly reported and/or readily available. Proper accounting of the total cost of performing combined molecular assays would have to take into consideration various components such as testing devices and associated lab supplies (e.g., reagents), health care providers collecting samples, shipping and storing of samples, and lab technicians processing the tests.

Current Practice

There are 2 broad categories of tests for the detection of SARS-CoV-2 and influenza (types A and B): diagnostic tests and antibody tests. Molecular assays and antigen tests are types of diagnostic tests used to detect active viral infections by taking samples with nasopharyngeal (or nasal) swabs. Molecular assays use nucleic acid amplification methods such as RT-PCR to detect viral genetic material, whereas antigen tests use methods such as lateral flow assays and chromatographic assays to detect viral antigen proteins. Antibody tests, also known as serology tests, requires blood samples drawn from a fingerstick or vein. These tests detect antibodies that are produced as part of an immune response to SARS-CoV-2 or influenza.

Currently, lab-based RT-PCR is considered the standard test for both SARS-CoV-2 and influenza diagnosis. The interim guidance provided by PHAC recommends confirmatory RT-PCR testing for positive SARS-CoV-2 results obtained from antigen tests. Since antibodies develop days to weeks after an infection, antibody tests are not used for the diagnosis of an active SARS-CoV-2 infection. Because antibodies remain in the blood for
an undetermined time after symptom resolution, PHAC recommends caution when interpreting antibody test results for SARS-CoV-2.20 Furthermore, the Centers for Disease Control and Prevention does not currently recommend the use of antibody testing in determining the need for COVID-19 vaccination or in evaluating immunity after COVID-19 vaccination.21

Regarding influenza surveillance in Canada, FluWatch is a national network of labs, hospitals, physician clinics, and ministries of health.22 In Ontario, molecular testing for influenza with RT-PCR is indicated for symptomatic hospitalized patients, outbreak investigations, and remote community residents.23 Antigen testing for influenza is used if RT-PCR testing is delayed for more than 24 hours.24 Compared to RT-PCR testing for influenza, antigen tests have lower sensitivities and specificities.4 Antibody testing is not recommended for influenza diagnosis and is not available for clinical testing in Canada.25

What Is the Evidence?

The diagnostic accuracy of combined molecular assays for SARS-CoV-2 and influenza (types A and B) is determined by positive percent agreement (PPA, also known as sensitivity) and negative percent agreement (NPA, also known as specificity).26,27 According to guidance provided by the FDA, clinical agreement studies comparing new combined molecular assays versus the current standard (i.e., individual molecular assays based on RT-PCR) should use positive clinical specimens for test validation.26 PPA describes a test’s ability to detect SARS-CoV-2 and/or influenza when present in a specimen (i.e., true-positive), whereas NPA describes its ability to not detect the viruses when they are not present in the specimen (i.e., true-negative).27 Tests with higher PPA or NPA have better diagnostic accuracy.27 PPA and NPA values for combined molecular assays with Emergency Use Authorization are available on the FDA website11 and are listed in Table 1. Sensitivities for tests authorized in Canada through Interim Order are available on the Health Canada website.12
## Table 1: Accuracy of Combined Testing for SARS-CoV-2 and Influenza

<table>
<thead>
<tr>
<th>Test name</th>
<th>Type of test</th>
<th>Currently authorized in Canada</th>
<th>Lab-based or POC</th>
<th>Positive percent agreement</th>
<th>Negative percent agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roche cobas SARS-CoV-2 &amp; Influenza A/B</td>
<td>RT-PCR (specimen collected by HCP)</td>
<td>Yes</td>
<td>Lab(^{28})</td>
<td>SARS-CoV-2: 96.4% Influenza A: 100.0% Influenza B: 100.0%</td>
<td>SARS-CoV-2: 98.0% Influenza A: 99.6% Influenza B: 99.7%</td>
</tr>
<tr>
<td>Roche cobas Liat SARS-CoV-2 &amp; Influenza A/B</td>
<td>RT-PCR (specimen collected by HCP or self-collected nasal swab in a health care setting with instructions by an HCP)</td>
<td>Yes</td>
<td>Lab(^{29}) and POC(^{29})</td>
<td>SARS-CoV-2: 100% Influenza A: 98.3% Influenza B: 95.2%</td>
<td>SARS-CoV-2: 100% Influenza A: 96.0% Influenza B: 99.4%</td>
</tr>
<tr>
<td>Cepheid Xpert Xpress SARS-CoV-2/Flu/RSV</td>
<td>RT-PCR (specimen collected by HCP)</td>
<td>Yes</td>
<td>Lab(^{30}) and POC(^{31})</td>
<td>SARS-CoV-2: 97.9% Influenza A: 100.0% Influenza B: 100.0%</td>
<td>SARS-CoV-2: 100.0% Influenza A: 100.0% Influenza B: 99.0%</td>
</tr>
<tr>
<td>Hologic Aptima SARS-CoV-2/Flu Assay</td>
<td>RT-TMA (specimen collected by HCP)</td>
<td>No</td>
<td>Lab(^{32})</td>
<td>SARS-CoV-2: 96.1% Influenza A: 100.0% Influenza B: 100.0%</td>
<td>SARS-CoV-2: 99.6% Influenza A: 99.2% Influenza B: 100.0%</td>
</tr>
<tr>
<td>BD SARS-CoV-2/Flu for BD MAX System</td>
<td>RT-PCR (specimen collected by HCP)</td>
<td>No</td>
<td>Lab(^{33})</td>
<td>SARS-CoV-2: 96.2% Influenza A: 100.0% Influenza B: 98.3%</td>
<td>SARS-CoV-2: 100% Influenza A: 98.9% Influenza B: 100.0%</td>
</tr>
<tr>
<td>NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay</td>
<td>RT-PCR (specimen collected by HCP)</td>
<td>No</td>
<td>Lab(^{34})</td>
<td>SARS-CoV-2: 100% Influenza A: 100.0% Influenza B: 96.7%</td>
<td>SARS-CoV-2: 98.0% Influenza A: 100.0% Influenza B: 98.0%</td>
</tr>
<tr>
<td>Bio-Rad Reliance SARS-CoV-2/FluA/FluB Assay Kit</td>
<td>RT-PCR (specimen collected by HCP)</td>
<td>No</td>
<td>Lab(^{35})</td>
<td>SARS-CoV-2: 97.6% Influenza A: 100.0% Influenza B: 100%</td>
<td>SARS-CoV-2: 100% Influenza A: 100.0% Influenza B: 100%</td>
</tr>
<tr>
<td>Thermo Fisher Scientific TaqPath COVID-19, FluA, FluB Combo Kit</td>
<td>RT-PCR (specimen collected by HCP)</td>
<td>No</td>
<td>Lab(^{36})</td>
<td>SARS-CoV-2: 96.1% Influenza A: 96.4% Influenza B: 91.7%</td>
<td>SARS-CoV-2: 100% Influenza A: 99.0% Influenza B: 96.8%</td>
</tr>
<tr>
<td>CDC Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay(^a)</td>
<td>RT-PCR (specimen collected by HCP)</td>
<td>No</td>
<td>Lab(^{37})</td>
<td>SARS-CoV-2: 100.0% Influenza A: 100.0% Influenza B: 100.0%</td>
<td>SARS-CoV-2: 100.0% Influenza A: 100.0% Influenza B: 100.0%</td>
</tr>
<tr>
<td>Quest Diagnostics RC COVID-19+Flu</td>
<td>RT-PCR (specimen self-collected at home if deemed appropriate by HCP)</td>
<td>No</td>
<td>Lab(^{38})</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

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\(^a\) Positive and negative percent results obtained using the QIAGEN QIAcube HT test platform.
Safety

Specimen collection with nasopharyngeal swabs and sample processing with molecular assay instruments have safety implications for health care professionals and lab technicians. Because aerosols can be generated during sample collection, those involved in this process need to adhere to infection control measures including wearing personal protective equipment (e.g., masks, face shields, and gowns). Additionally, sample processing with molecular assay instruments should be performed in negative pressure isolation rooms.

Issues to Consider

As combined molecular assays targeting SARS-CoV-2 and influenza are designed to detect active infections, test results can only indicate if a patient is currently infected with these specific viruses. Therefore, these tests do not provide information on potential co-infections with bacteria and/or viruses other than SARS-CoV-2 and influenza, with the exception of tests developed by Cepheid and NeuMoDx that can also detect respiratory syncytial virus. A systematic review suggested that as high as 8% and 4% of patients who tested positive for SARS-CoV-2 had a bacterial and fungal co-infection, respectively.

The National Institutes of Health COVID-19 Guidelines recommend that clinicians consider testing for other pathogens based on clinical circumstances (e.g., influenza-positive patients with a likelihood of developing bacterial superinfection). Additionally, it is important to note that the treatment of influenza remains the same irrespective of co-infection with SARS-CoV-2. Specifically, when caring for hospitalized patients, clinicians should not wait for influenza test results and should initiate treatment for influenza with oseltamivir as soon as possible. For non-hospitalized patients with suspected or confirmed influenza, oseltamivir should be initiated as soon as possible only for individuals with complications or chronic health conditions. Upon obtaining negative molecular assay (i.e., nucleic acid amplification test) results for influenza, antiviral therapy for influenza can be discontinued in hospitalized and non-hospitalized patients. Finally, patients who test negative for SARS-CoV-2 would not need to be put under strict isolation.

Related Developments

Currently, none of the approved combined molecular assays are designed to be used at home without the need to send the specimen to a lab. However, Lucira Check It Test Kit, an at-home SARS-CoV-2 molecular test based on nucleic acid amplification, has received FDA Emergency Use Authorization and Health Canada’s Interim Order approval. At a cost of approximately CA$75 per test, this test uses isothermal amplification — specifically, loop-mediated isothermal amplification, or LAMP — and can yield results in 30 minutes right at home. A recently published CADTH newsletter on at-home SARS-CoV-2 tests provided details on LAMP and antigen-based SARS-CoV-2 tests.

To help eliminate a biosafety risk to those involved in sample collections and overcome barriers for those who cannot get tested in person by a health care professional, self-collection kits have been developed for multiplex molecular assays. When deemed appropriate by a health care professional, the Quest Diagnostics RC COVID-19+Flu RT-PCR self-collection kit can be used without the need for medical supervision. To promote test accuracy, end-users need to closely follow the manufacturer’s self-collection
instructions: a sample that is improperly self-collected may lead to a false-negative test result, which is a negative finding in an individual who is actually positive. This may lead to inadvertent viral spread, as public health measures such as contact tracing would not be taken.

Looking Ahead

Various RT-PCR and antigen-based POC rapid tests for influenza have been available in Canada for some time. However, findings from a recent Canadian cost-effectiveness modelling study on POC tests for influenza in the emergency department suggested that treating high-risk patients with influenza-like illness without the use of rapid influenza tests was more cost-effectiveness and resulted in reduced mortality. A systematic review study protocol has been registered with PROSPERO to investigate the diagnostic accuracy of various combined molecular assays based on RT-PCR. With increased availability of lab and POC-based combined molecular tests detecting SARS-CoV-2 and influenza in the Canadian market, further studies comparing multiplex molecular tests versus tests that only detect SARS-CoV-2 would provide an additional knowledge base on the clinical utility and cost-effectiveness of co-testing. Additionally, by distinguishing between SARS-CoV-2 and influenza, the correct public health measures can be implemented promptly to help contain COVID-19 breakthrough cases.
References


