LABSTRACT – December 2020
Respiratory Virus Testing Update

**Audience**
Healthcare providers and public health practitioners.

**Overview**
This document provides an update to the Public Health Ontario (PHO) Laboratory respiratory virus testing algorithm and associated Labstract that was initially communicated in October 2020. For SARS-CoV-2 virus (the cause of COVID-19) PCR-specific information, see the [Coronavirus Disease 2019 (COVID-19)-PCR Test Information Sheet](#).

On November 2, 2020 PHO Laboratory implemented a new laboratory developed multiplex respiratory virus PCR panel, “FLUID”, which targets influenza A, influenza B, SARS-CoV-2, and respiratory syncytial virus (A + B). Use of the FLUID assay is coordinated with existing multiplex respiratory virus PCR (MRVP) capacity across PHO Laboratory sites to optimize the viral respiratory testing response.

Commencing January 4, 2021, PHO Laboratory will be altering the routine use of multiplex respiratory virus PCR (MRVP) and rapid influenza testing for symptomatic patients in institutional and outbreak settings, as outlined below.

The modified testing algorithm will enable shorter turnaround times for SARS-CoV-2 and influenza testing results for the laboratory network in Ontario, while also facilitating delivery of a standardized approach to seasonal respiratory virus testing.

**What has changed?**

**Changes to eligibility of MRVP testing for institutionalized persons and outbreaks:** Previously, MRVP testing was available for persons tested in institutions (non-outbreak) and the first four specimens from institutional and other outbreaks.

The FLUID assay will now be used for symptomatic persons tested in institutions and respiratory outbreaks (routinely up to 4 specimens on symptomatic persons). Requests for additional seasonal respiratory virus testing in institutions or outbreak settings (including MRVP testing for FLUID-negative outbreaks) will require collection and submission of new specimens. The additional specimens may be collected on previously tested, or as yet untested symptomatic patients, affected by the outbreak. MRVP will routinely be done on up to four specimens per FLUID-negative (or influenza/COVID-19 negative) outbreak.

**To request MRVP testing for influenza/COVID-19 negative institutionalized persons or outbreaks, document on the requisition:** “MRVP [FLUID-negative patient (or outbreak)]” or “MRVP”
[influenza/COVID-19 negative patient (or outbreak)], depending on what previous testing was done, to facilitate appropriate MRVP test assignment.

**Changes to rapid influenza testing:** Previously, rapid influenza testing was performed on the first four specimens submitted on symptomatic persons from respiratory outbreaks. Rapid influenza testing will now only be done on these specimens when commencement of PCR testing is anticipated to be delayed >24 hours from the time the specimen is received at PHO Laboratory.

**Laboratory testing algorithm**

The specific test being requested AND patient setting must appear on the requisition to help with appropriate test assignment and triaging of specimens. If patient setting is not provided, the specimen will only be tested for SARS-CoV-2. For outbreaks or investigations, the requisition must include the assigned outbreak or investigation number.

Table 1 outlines updated eligibility criteria for respiratory virus testing at PHO Laboratory.

To ensure earliest possible testing for SARS-CoV-2, influenza and RSV, PHO may transfer specimens from one PHO Laboratory site to another, with initial testing by SARS-CoV-2 or FLUID at one site, followed by FLUID and/or MRVP at another. See Table 2 for details.

**Table 1: Eligibility criteria for respiratory virus testing available at PHO’s laboratory by patient setting and outbreak status**

<table>
<thead>
<tr>
<th>Patient Status</th>
<th>Symptomatic Patients: Testing Dictated by Patient Setting and outbreak status</th>
<th>Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptomatic</td>
<td>ICU/CCU inpatients(^2) Remote communities</td>
<td>FLUID(^3,4) followed by MRVP(^4,5,6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>OR</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SARS-CoV-2 and MRVP(^4,5,6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>(Both combinations will provide testing for the same viruses.)</em></td>
</tr>
<tr>
<td>Symptomatic</td>
<td>Institutional and other public health unit declared respiratory infection outbreaks (including school outbreaks)</td>
<td><strong>Up to 4 outbreak specimens(^7):</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Influenza rapid testing(^8,9) (will be done if PCR testing is delayed &gt;24 hours)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FLUID(^3,4) <strong>OR</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SARS-CoV-2 followed by FLUID(^3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Additional specimens will be tested for SARS-CoV-2 only.(^7)</strong></td>
</tr>
<tr>
<td>Patient Status</td>
<td>Symptomatic Patients: Testing Dictated by Patient Setting and outbreak status</td>
<td>Testing</td>
</tr>
<tr>
<td>----------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>Ward (non-ICU/CCU) inpatients</td>
<td>FLVID³</td>
</tr>
<tr>
<td></td>
<td>Institutions (non-outbreak) (e.g. long-term care homes, retirement homes, correctional facilities, congregate living settings)</td>
<td>OR SARS-CoV-2 followed by FLVID³</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>Emergency room patients</td>
<td>SARS-CoV-2¹⁰</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>Ambulatory/outpatient settings, assessment centres, including ambulatory influenza high risk patients</td>
<td>SARS-CoV-2¹⁰</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>Not specified on requisition</td>
<td>SARS-CoV-2¹¹,¹⁰</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>All patient settings</td>
<td>SARS-CoV-2 only</td>
</tr>
</tbody>
</table>

**Table footnotes:**

1. **The preferred requisition for all respiratory virus testing is the PHO Laboratory COVID-19 Virus Test Requisition.** The specific test being requested AND patient setting must appear on the requisition to help with appropriate test assignment and triaging of specimens. If patient setting is not provided, the specimen will only be tested for SARS-CoV-2. For outbreaks or investigations, the requisition must include the assigned outbreak or investigational number.

2. ICU - Intensive Care Unit; CCU - Critical Care Unit.


4. Specimens received at PHO Laboratory sites in Hamilton, Kingston, or Thunder Bay for MRVP testing will first undergo SARS-CoV-2 (Hamilton) or FLUID (Kingston, Thunder Bay) testing, and will be forwarded to one of the MRVP testing sites (Toronto, London, Ottawa and Timmins) for
MRVP testing. Specimens received at Orillia, Peterborough, Sault Ste Marie or Sudbury sites will be forwarded to another PHO Laboratory site for molecular (PCR) testing.

   **Note:** The assay detects the different RSV, parainfluenza and seasonal human coronaviruses named above but does not differentiate between them. It does not detect or cross-react with SARS-CoV-2.

6. Cytomegalovirus (CMV) PCR is available upon request (indicate on the requisition that this test is requested) for bronchoalveolar lavage (BAL)/bronchial wash/pleural fluid.

7. A limit of four outbreak specimens are routinely accepted for seasonal respiratory virus testing using the FLUVID assay, which must be ordered on the PHO Laboratory requisition.
   **Requests for additional seasonal respiratory virus testing in institutions or outbreak settings (including MRVP testing for FLUVID-negative outbreaks) will require collection and submission of new specimens.** The additional specimens may be collected on previously tested, or as yet untested symptomatic patients, affected by an outbreak. MRVP will routinely be done on up to four specimens per FLUVID-negative (or influenza/COVID-19 negative) outbreak.
   Use the **PHO Laboratory COVID-19 Virus Test Requisition**. Under Section 5-Test(s) Requested on the requisition, check the “Respiratory viruses” box and document on the requisition: “MRVP [FLUVID-negative patient (or outbreak)]” or “MRVP [influenza/COVID-19 negative patient (or outbreak)]”, depending on what previous testing was done, to facilitate appropriate MRVP test assignment.
   The **Respiratory Outbreak Testing Prioritization Protocol** should be followed for all outbreak submissions.

8. Influenza rapid antigen testing will be performed on the first four outbreak specimens from symptomatic patients if influenza PCR testing (e.g. FLUVID) commencement is delayed to >24 hours from specimen receipt at PHO Laboratory.

9. Caution: Influenza rapid testing can only be performed if specimen is received in suitable media. See Specimen Collection and Handling below.

10. To assist with patient management when seasonal respiratory virus testing is not available, healthcare providers are encouraged to refer to PHO’s respiratory surveillance reports for information on respiratory pathogen activity in Ontario (see Section 5 below).
Table 2: Respiratory virus testing conducted at PHO Laboratory sites

<table>
<thead>
<tr>
<th>Receiving PHO Laboratory Sites</th>
<th>Testing conducted</th>
<th>Management of specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toronto, London, Ottawa, Timmins</td>
<td>Rapid Flu, SARS-CoV-2, FLUVID, MRVP</td>
<td>Specimens will be tested at receiving site</td>
</tr>
<tr>
<td>Hamilton</td>
<td>Rapid Flu, SARS-CoV-2</td>
<td>Specimens transferred to Toronto or London if FLUVID or MRVP needed</td>
</tr>
<tr>
<td>Kingston, Thunder Bay</td>
<td>Rapid Flu, SARS-CoV-2, FLUVID</td>
<td>Specimens transferred to Toronto, London, Ottawa or Timmins if MRVP needed</td>
</tr>
<tr>
<td>Orillia, Peterborough, Sault Ste Marie, Sudbury</td>
<td>Rapid Flu, no molecular testing</td>
<td>Specimens transferred to one of the above laboratory sites</td>
</tr>
</tbody>
</table>

When influenza is circulating, laboratory confirmation of influenza is not needed before initiating treatment, as waiting until influenza is confirmed will delay initiation of therapy. Current clinical guidelines recommend that during influenza season, influenza antiviral therapy (e.g., oseltamivir or zanamivir) be started empirically for patients with:

- Moderate, progressive, severe or complicated influenza, such as individuals who are hospitalized with influenza-like illness; OR
- Compatible symptoms who are at high risk of influenza complications/severe disease.

For more information, please see the references provided with this document.

To understand which seasonal respiratory pathogens are circulating and to assist with influenza antiviral treatment decisions, healthcare providers are reminded to regularly review PHO’s Ontario Respiratory Pathogen Bulletin (ORPB). The ORPB is updated weekly and provides an overview of influenza and other respiratory pathogens circulating in the province. Data on influenza positivity is also presented at the local public health unit level to provide jurisdiction-specific information.

For epidemiological information on COVID-19 activity in Ontario see PHO’s Ontario COVID-19 Data Tool.

Specimen collection and handling

Refer to the Coronavirus Disease 2019 (COVID-19)-PCR Test Information Sheet Specimen Collection and Handling instructions for directions and information, including a guide to which swabs and media are suitable for influenza rapid testing, SARS-CoV-2 testing and MRVP.

CAUTION: “molecular transport media” (i.e. media containing guanidine) is unsuitable for influenza rapid testing. Specimens which are not suitable for rapid testing will be sent directly for FLUVID testing, which may result in a delay if the specimen needs to be transported to a different testing site.

Check the expiry dates of both the collection swabs and transport media (tube), as specimens collected using expired swabs or tubes will be rejected. See Criteria for Acceptance of Patient Specimens.
To maintain optimum viability, specimens should be stored at 2-8°C following collection and shipped to PHO on ice packs. If transport of specimens to the testing laboratory will be delayed more than 72 hours, specimens should be frozen at -70°C or below and shipped on dry ice.

**Additional respiratory virus testing services at PHO’s laboratory**

1. **Influenza Antiviral Susceptibility Testing**

Currently circulating influenza A subtypes (H1N1pdm09 and H3N2) are universally amantadine resistant. Almost all currently circulating influenza A and B viruses are oseltamivir susceptible; however, resistance has been documented on rare occasions. Conversely, pre-pandemic seasonal influenza A (H1N1) was known to be amantadine susceptible, and almost universally oseltamivir resistant, but it has not been detected in Ontario since mid-2009.

Routine susceptibility testing is not required for clinical care; however, a proportion of influenza-positive samples will be forwarded to the National Microbiology Laboratory (NML) for strain typing and antiviral susceptibility testing. Limited susceptibility testing is also available at PHO.

Recommended indications for antiviral susceptibility testing in Ontario include:

- Influenza developing during or soon after completion of influenza antiviral prophylaxis (e.g., oseltamivir or zanamivir).
- Severely ill patients such as those admitted to an ICU with laboratory-confirmed influenza not responding to influenza antiviral therapy.
- Fatalities in patients with laboratory-confirmed influenza being treated with influenza antiviral therapy.
- Persistent influenza viral shedding, defined as a repeat PCR test positive after seven days or more of treatment. Repeat PCR testing could be undertaken for patients who are not responding to antiviral therapy. Immunocompromised patients are at greater risk for more severe disease, persistent viral shedding and development of antiviral resistance.
- Positive test for influenza A in a traveller returning from an area where resistance is endemic.

To request influenza susceptibility testing for a patient who meets any of the above criteria, please document the request on the laboratory requisition along with any relevant information. To make a request on a sample already submitted to PHO, please contact our Customer Service Centre at 1-877-604-4567 or 416-235-6556 or your local PHO laboratory.

2. **Testing for Middle East Respiratory Syndrome Coronavirus (MERS-CoV)**

MERS-CoV is a coronavirus that emerged in the Middle East in 2012. Patients with relevant clinical presentation and epidemiological risk factors for MERS-CoV infection, such as travel to certain countries in the Middle East or contact with a confirmed or probable MERS-CoV case, should be considered for MERS-CoV testing. For further information, please see the [MERS-CoV Test Information Sheet](#).

**Note:** MRVP detects seasonal human coronavirus but does not detect MERS-CoV.
3. Testing for Novel Influenza Viruses

a) Avian influenza viruses

PHO’s laboratory conducts testing for avian influenza (e.g., H5N1, H5N2, and H7N9) as required based on the information provided on the test requisition. Samples indicating travel to affected areas or with exposure to known cases of avian influenza, will be tested by real-time influenza A PCR (US CDC protocol) and if positive will be tested first for seasonal subtypes (H3N2, H1N1pdm09). If no seasonal subtype is detected, further testing for avian influenza subtypes will be conducted.

If you suspect avian Influenza, contact your local health unit and PHO’s Laboratory Customer Service Centre at 1-877-604-4567 prior to submitting specimens.

In April 2013, the World Health Organization (WHO) confirmed emergence of new avian influenza A (H7N9) virus in China. For further information, including information on laboratory testing, see the Ontario Ministry of Health’s Guidance for Health Care Workers and Health Sector Employers on Asian lineage Avian Influenza A(H7N9).

Influenza A (H5N1) infections in humans have been infrequently reported since 2003 with cases occurring in Asia, and later in Africa, Europe, and the Middle East, mostly due to exposure to infected birds or their environment. For further information see the Ministry of Health’s H5N1 Flu Virus Fact Sheet.

b) Variant (swine origin) influenza viruses in humans

Testing is available on request for variant influenza viruses, such as H3N2v, in persons who develop acute respiratory illness following direct contact with swine or their environment. Only limited human-to-human transmission has been documented.

NOTE: Samples that do not subtype for seasonal influenza will be tested for a panel of avian and/or swine influenza viruses.

4. Testing for Enterovirus (including EV-D68 and other serotypes)

EV-D68 circulated in Ontario in the summer and fall of 2014, and to a lesser extent in 2016. More recently several cases of EV-D68 were detected at PHO Laboratory up to November 2018. For information about non-polio enterovirus, including D68, see PHO’s Infectious Disease page for Enterovirus D68.

For information on specimen collection and testing for enterovirus, see the Enterovirus Test Information Sheet.

5. PHO Surveillance Reports and Network:

PHO routine surveillance reports are available on the PHO website:

- Ontario Respiratory Pathogen Bulletin
- Laboratory-Based Respiratory Pathogen Surveillance Report summarizes all respiratory pathogen testing done at PHO
- Ontario COVID-19 Data Tool
6. Sentinel Practitioner Surveillance Network (SPSN)

For general information on Ontario’s SPSN and information for practitioners interested in contributing to the network, see Sentinel Practitioner Surveillance Network (SPSN) — Influenza Vaccine Effectiveness Program.

The SPSN performs molecular respiratory viral testing on patients with influenza-like-illness visiting community-based sentinel health care providers or designated COVID-19 Assessment Centres, across Ontario. Other provinces also participate in the SPSN. SPSN sentinel heath care providers in Ontario will be able to offer influenza, other seasonal respiratory virus (by MRVP) and COVID-19 testing to patients meeting SPSN testing criteria, which includes outpatients who would not normally be eligible for influenza and other seasonal respiratory virus testing.

References


2. AMMI Canada Guidelines. Available at: ammi.ca/?ID=122


For further information

- Specimen collection, submission, testing, and reporting information is available in detail on the Respiratory Viruses (including influenza) Test Information Sheet (TIS), located on our website at: Respiratory Viruses Virus Detection TIS
- Contact PHO’s Laboratory Customer Service Centre at 416-235-6556 or 1-877-604-4567 (toll-free), or by email at customerservicecentre@oahpp.ca
- For PHO’s Laboratory specimen collection information and previous Labstracts, refer to publichealthontario.ca/test directory
- The current versions of PHO Test Requisitions and other forms are available at publichealthontario.ca/Requisitions
- To subscribe to receive future Labstracts, register on our website
- To register for Autofax and receive laboratory reports by fax directly from our laboratory information system as soon as they are released, contact the PHO Laboratory Customer Service Centre.
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