CANADIAN PANDEMIC INFLUENZA PREPAREDNESS:
Planning Guidance for the Health Sector

Laboratory Annex

December 3, 2015
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APPENDIX A – PILPN RECOMMENDATIONS FOR THE PROVISION OF PUBLIC HEALTH LABORATORY SERVICES DURING PANDEMIC INFLUENZA .... 32
1.0 INTRODUCTION

1.1 Background
The Pandemic Influenza Laboratory Preparedness Network (PILPN) of the Canadian Public Health Laboratory Network (CPHLN) has developed this document, which is based on current best practices for the laboratory detection of influenza as well as the lessons learned from the 2009 H1N1 pandemic. Specifically, this Laboratory Annex to the Canadian Pandemic Influenza Preparedness: Planning Guidance for the Health Sector (CPIP) addresses issues related to appropriate sample type and specimen collection; laboratory testing; laboratory-based surveillance and data collection; communication issues; and pandemic preparedness.

1.2 Scope and Audience
In the context of the CPIP, “laboratory” refers to clinical laboratories. While the primary audience is clinical laboratory professionals, the document may serve as a practical reference for other stakeholders, such as front-line clinicians and epidemiologists. In addition, the Laboratory Annex highlights important aspects of the laboratory response, so that pandemic planners, decision-makers and other relevant stakeholders can be fully aware of the resources required to support that response. It is expected that this guidance will be adaptable to different situational and regional/jurisdictional contexts.

1.3 Changes to This Version
Consistent with the main body of the CPIP, this version of the Laboratory Annex incorporates lessons learned during and since the 2009 pandemic and provides a risk management approach to support a flexible and proportionate response.

As with the previous CPIP Laboratory Guidelines, nucleic acid amplification tests (NAAT) remain the pillar of the laboratory testing strategy because of their sensitivity, scalability and capacity to rapidly diagnose influenza.

Changes in the current version of the Laboratory Annex include the following:
- The structure, organization and overarching direction of the annex parallel those of the main body of the updated CPIP.
- The key elements of the annex reflect the continuum of testing activities that laboratories must incorporate into their pandemic preparation and response. At the pre-analytical stage, this involves
specimen collection, transport and accessioning (entry into the laboratory information system); at the analytical stage, it comprises the testing process itself; and at the post-analytical stage, it includes the analysis and reporting of specific individual results as well as the aggregation and summarization of results. This is often referred to as “lab-based epidemiology”, which is used to inform epidemiology partners and planners.

- Testing volumes have been estimated on the basis of the four planning scenarios outlined in the main body of the CPIP.
- The triggers outlined in the annex are consistent with the main body of the CPIP and reflect a scalable range of options to be considered as the novel virus spreads in this country.
- The ethical considerations used in developing the annex are described.
- The annex has been adjusted to reflect the current consensus on roles and responsibilities, as outlined in the main body of the CPIP.
2.0 CONTEXT FOR PLANNING

2.1 Role of Laboratories in Prevention and Treatment of Pandemic Influenza

In the event of pandemic influenza or the emergence of a novel subtype of influenza virus, laboratories will be instrumental in facilitating the delivery of rapid and appropriate public health responses. During a pandemic, laboratory testing will accomplish the following:

- Identify the earliest Canadian cases of a novel influenza strain;
- Support public health surveillance by monitoring the geographic spread of disease and the impact of interventions;
- Facilitate clinical management by distinguishing patients infected with the pandemic influenza virus from those with other respiratory diseases;
- Determine the strengths and weaknesses of different testing methods;
- Monitor circulating influenza viruses for antiviral resistance (AVR); and
- Contribute to the assessment of influenza vaccine effectiveness through the subtyping and characterization of influenza virus strains, which can help determine potential mismatch.

Primary detection assays to help with patient management and the public health response are provided by provincial public health laboratories (PHL) and many front-line hospital laboratories. While some hospital laboratories may also have the capacity for subtyping, this function is primarily the responsibility of the PHL.

Surveillance will require a coordinated approach from all levels – local, regional, provincial/territorial (PT) and national – to ensure that the data are adequately captured and interpreted. A statistically appropriate number of specimens should flow through the PHL to the National Microbiology Laboratory (NML) for further viral characterization, including AVR testing for those PHLs that do not have the capability or capacity.

2.2 Uncertainties and Unpredictability

There remain many uncertainties that influence how laboratories can prepare for the next pandemic:

- When will a pandemic happen and when will it be identified in Canada? As illustrated during the 2009 pandemic, the lead time for test development and validation may be very short, and competing requests from multiple users may overextend commercial suppliers of test materials.
• **What specimen will be needed to identify the novel virus?** The ideal specimen for detecting the virus may not be clear at the time the novel virus is identified.

• **What will the subtype be and will current methods identify the novel virus?** NAATs methods require the use of primers directed at a conserved region within the viral genome. Real-time assays also have probes that must bind within this conserved region. If there is mutation within the binding sites of these primers and probes, the assay may not detect the new virus or may detect it only with suboptimal sensitivity. In-house assays allow greater flexibility in modifying the primers and probes more rapidly, whereas commercially available kits may take significantly longer to adjust to these changes.

• **Which animal species will be the source of the virus?** Viruses of zoonotic origin may have further enhanced containment level (CL) requirements. In such instances, this will require coordination and collaboration between the Canadian Food Inspection Agency and the Public Health Agency of Canada (PHAC), which may influence some aspects of laboratory testing.

• **Who will be the most severely affected?** The target demographic cannot be predicted with certainty. This may have an impact on laboratory staffing if the demographic most affected coincides with that of the laboratory staff.

• **How will existing laboratory capacity meet the increased demands of a pandemic?** In response to budget restraints, many laboratories have already implemented measures to streamline operations and improve testing efficiency. Consequently, there is little flexibility in the system to meet the human resource imperatives that may be needed for increasing testing. Although rapid advances in diagnostic technologies have and will continue to improve diagnostic and typing capacity, there remains a need to apply a more systematic approach to identifying the circulating viruses in the community and their health impact. Ideally, a coordinated sentinel system to detect influenza and influenza-like illness (ILI) in the community should be in place to track the spread of an influenza pandemic in close to real time.

• **Will the virus be resistant to antivirals?** Resistance of influenza viruses to antivirals can occur spontaneously or arise in the course of treatment, especially in people with immune compromising conditions or during antiviral prophylaxis. AVR would significantly increase testing demands, as each specimen would require virus diagnosis followed by resistance testing.

### 2.3 Lessons Learned from the 2009 Pandemic

Canada's public health response to the 2009 H1N1 pandemic provides some valuable lessons learned vis-à-vis the potential role and contribution of laboratories in future outbreaks:

**General:**

• **Epidemiology and laboratory surveillance requirements need to be identified and defined at the onset of the pandemic.**

• **Laboratories should prepare for an increase in testing of up to 10 times their highest seasonal demand during the peak of the pandemic wave, which may last for 3 weeks in their geographic location.** The increased demand for testing over the duration of the 2009 pandemic was approximately 5 to 7 times the historical demand. However, at the peak of the pandemic, demand in many laboratory jurisdictions actually reached 10 times their normal levels.

• **Influenza case definitions developed for clinical management purposes need to evolve in real time.** During the 2009 pandemic, the ILLI case definition was a poor predictor of influenza in hospitalized patients. This led physicians and infection control personnel to rely on diagnostic testing to help with patient management.
• **Reassessment of the risk group (RG) designation of the virus needs to be monitored and timely, as delays in appropriate designation can hinder laboratory testing efficiency.** In 2009, even after the virus was widespread in the community, enhanced containment levels were required for propagation of the virus, which was restrictive to some laboratories’ diagnostic efforts.

• **PILPN linkages need to be maintained during the interpandemic period.** The prior existence and ongoing functioning of the PILPN facilitated the rapid response and dissemination of information. It allowed for a coordinated national approach to testing and sharing of the reagents and protocols necessary for the validation and optimization of the molecular platforms.

• **Laboratories should review their human resource policies and facilitate proactive/early cross-training of staff to help meet demand.** The ability to hire staff to meet the surge in demand may be limited by labour laws and accreditation requirements.

• **Laboratories should anticipate intellectual property and copyright issues regarding data sharing and research papers, and should develop potential solutions.** Research demands often put unrealistic pressure on the PHLs. During the 2009 pandemic, issues regarding data sharing, intellectual property, copyright and other publication issues were often unclear. Laboratories should anticipate these types of issues and develop potential solutions, such as establishing formal data-sharing agreements and Material Transfer Agreements (MTAs), in advance so that, should these issues arise, they can be addressed early in an outbreak.

### Pre-analytical:

• **Laboratories should have plans to meet surge demands through access to laboratory supplies, such as appropriate collection kits.** At the early stages of the 2009 pandemic, clinical supplies, such as swabs and viral transport media, were scarce.

• **Laboratories should consider how the additional volume of specimens and the potential complexity associated with the receipt and processing of novel specimens will affect the pre-analytical process.** Many laboratories dramatically underestimated the impact of pre-analytical challenges, including specimen type, accessioning and the need for aliquoting (dividing) specimens. In addition, lack of front-end automation challenged the ability of laboratories to meet demand.

• **Laboratory requisition forms should be formatted to clearly identify priority (i.e. stat) requests and accommodate unique testing/data set requirements.** The information supplied with the laboratory requisition was often inadequate in helping with triage. Some laboratories developed a modified or new requisition, or achieved better coordination with clinical partners.

• **Nasopharyngeal swabs (NPS) may prove inadequate in detecting infections associated with the lower respiratory tract, and this should be reflected in testing protocols.** NPS can produce false-negative results in patients with severe disease. Lower respiratory tract specimens, such as endotracheal (ET) secretions or bronchoalveolar lavage (BAL) specimens, may be required to verify the diagnosis.

### Analytical:

• **NAATs were the primary method of detection for the novel virus early in the pandemic, although a variety of tests were required to fulfil the entire range of testing.** The approach using the M gene sequences as a universal target was effective. Additional testing was necessary to differentiate the pandemic virus strain from other seasonal strains in order to meet federal/provincial/territorial (FPT) reporting requirements. A second assay targeting the haemagglutinin or neuraminidase gene was necessary to differentiate pandemic strains from other seasonal strains. In addition, many jurisdictions were interested in identifying all circulating influenza strains.
• The requirement for CL3 handling needs to be rapidly reassessed early in the pandemic to reflect whether the virus is being rapidly transmitted in the community. As per the Human Pathogens and Toxins Act, laboratories should expect that manipulations of virus culture could be restricted to CL3 facilities and that the processing of diagnostic specimens for NAATs would require CL2 with enhanced precautions. Reassessment of the risks associated with work activities and the agent itself needs to be conducted regularly and in a timely fashion as new information on the agent becomes available.

• Laboratories should anticipate increased demand for NAAT reagents. Early in the 2009 pandemic, demand for reagents from commercial suppliers exceeded supply and so slowed the ability to develop new assays.

• Laboratories will need to define when multiplex testing should be used. Multiple respiratory viruses can and do circulate at any given time. Although multiplex testing is becoming increasingly available, it is expensive. Consequently, it is recommended that laboratories define when multiplex testing should be used so that resources can be appropriately directed at detecting the pandemic strain.

• The role of rapid influenza diagnostic tests (RIDTs) is limited to detecting outbreaks in remote settings. The performance of RIDTs, which rely on antigen detection, shows poor sensitivity, and negative tests were of limited value for individual patient management. RIDTs may have a role in detecting outbreaks in remote settings where there is limited access to NAAT or in rapidly confirming influenza in an outbreak setting (this is based on the principle that, even with a test of low sensitivity, multiple tests performed on symptomatic patient populations can yield overall sensitivities of 95% to 100% when 4-6 individuals are tested).

• During the interpandemic period, the diagnostic capacity of front-line hospital laboratories should be strengthened. Since the pandemic, NAATs have become a common diagnostic option in front-line hospital laboratories. However, quality control and proficiency testing are paramount and can be a challenge when new sites are brought on line in a rapid fashion. Enhancing the diagnostic capacity of front-line hospital laboratories during the interpandemic period would further strengthen Canada’s ability to respond to a pandemic.

• A coordinated approach to testing and reporting at the PT level should be developed to help achieve consistent reporting at the national level. During the 2009 pandemic, it was readily apparent that testing criteria were different in each jurisdiction. Some tested all specimens, others limited testing to patients being admitted to hospitals, and many jurisdictions suspended some services to free up resources. It is important that public health practitioners understand the different testing strategies used in different jurisdictions.

Post-analytical:

• Effective communication strategies at all levels of laboratory testing are required for better and more timely data exchange. The 2009 pandemic demonstrated the need to improve communication among front-line laboratories and PHLs for a more coordinated response, particularly with regard to the flow of data for surveillance. Communications with community physicians were also challenging. There was an increase in the number of calls to the PHLs, which required laboratories to modify communication strategies so that highly trained personnel were used appropriately.

• Strengthened realtime linkages with epidemiological and statistical partners are necessary. These would help to analyze trends and determine whether laboratories could better utilize processes for greater efficiency.

• Improved planning for storing and tracking specimens is needed. Challenges regarding storage and tracking of specimens were underestimated in many PHLs during the 2009 pandemic.
2.4 Program Delivery in the Canadian Context

As noted in the main body of the CPIP, it is assumed that there will be geographic variability with regard to the timing and intensity of waves, although multiple jurisdictions will likely be affected simultaneously. The Canadian population is geographically dispersed, with large areas of rural or remote populations. Consequently, access to laboratory services (particularly NAATs) may be an issue in some communities within such regions, leading to delayed turnaround time. This may necessitate a collaborative response among different jurisdictions to help ensure that optimal laboratory testing is in place to guide both individual and community responses. For example, the Territories routinely rely on British Columbia and Alberta for influenza testing; therefore, testing strategies need to be tailored in these regions to encompass the logistics of transporting specimens in a timely fashion in order to obtain results that are useful for patient or outbreak management. It is also important that the samples transported be appropriate and sample integrity maintained to avoid even further delays due to laboratory rejection of samples deemed unfit for testing. The implications for laboratory testing need to be considered in any novel care delivery models.

2.5 Ethical and Legal Considerations

Pandemic preparedness and response are intimately tied to public health and epidemiology objectives, together with the often competing patient care imperative. While front-line laboratories focus primarily on patient care, it is important that PHLs balance the needs of patient care with public health/epidemiology objectives.

In this context, ethical considerations promote a social justice approach that is based on trust, solidarity, reciprocity, stewardship, equity and fairness. The Laboratory Annex was developed through consideration of the guiding principles and approaches outlined in the main body of the CPIP, particularly focusing on the following:

- **Collaboration** – By its very nature, the PHL system response from the front-line laboratory to the NML and its connections to the World Health Organization (WHO) requires a collaborative approach. Collaboration between epidemiologists and laboratory professionals is paramount to achieving an effective surveillance system that will underpin the pandemic response. The CPHLN has developed this annex using FPT and stakeholder input.

- **Laboratory stewardship** – Laboratory stewardship is a key characteristic of PHL systems, and the appropriate use of limited resources is under constant consideration. Key ethical considerations used in the development of this annex include flexibility, proportionality and evidence-informed decision-making. During a pandemic, constant communication is essential to form consensus and to facilitate understanding of the necessary decisions.

- **Use of established practices and systems** – While it is understood that established influenza surveillance and response systems exist to detect both pandemic and annual epidemic influenza, the response required to detect pandemic influenza at its peak of activity requires moderately different laboratory workflows, prioritization and reporting processes to guarantee responsiveness. Building on established practices and systems will facilitate the ramping up required to meet the pandemic demands.

As noted in the main body of the CPIP, one of the supporting objectives for Canada’s goals for pandemic preparedness and response is maintaining trust and confidence through support of evidence-informed decision-making by collection, analysis and sharing of surveillance and other scientific information. Having data-sharing agreements in place before the next pandemic will help achieve a coordinated and efficient flow of information through the laboratory system. It is important that FPT governments work together to develop and agree upon common sets of minimum data requirements needed to effectively support the surveillance systems required to guide the pandemic response and that laboratories are an intimate partner in these discussions.
3.0 CANADA’S PANDEMIC LABORATORY STRATEGY

3.1 Objectives

The purpose of testing for influenza virus falls into two broad categories:

1) Population-based surveillance: Surveillance is essential in identifying the novel strain in Canada and monitoring its progress upon arrival. The data generated by laboratory surveillance activities will be important components of evidence-informed decision-making for key public health decision-makers. Population-based surveillance requires that laboratory networks have the capacity to detect and subtype the novel virus in order to differentiate it from common circulating influenza strains. Subtyping and characterization of strains may be particularly relevant if the emerging virus has a different antiviral susceptibility pattern and can help determine vaccine effectiveness and potential mismatch. To this end, it is recommended that a proportion of influenza isolates representing community-based cases, as well as isolates from hospitalized patients, be isolated in cell culture and submitted to the NML for further characterization, including antiviral resistance and antigenic variation. Laboratories equipped to readily determine the genome sequence will be able to characterize the virus in greater detail and identify the appearance of genotypes associated with an enhanced risk of mortality. It is anticipated that laboratories will also provide diagnostic support to research initiatives as required.

2) Diagnostic testing of patients presenting with ILI: Once the novel strain becomes widespread1 in the community, testing may not be indicated for the clinical management of people with uncomplicated ILI; rather, treatment would be based on clinical presentation. The remainder of testing could then focus on community-based surveillance programs; outbreaks; hospitalized patients and those with risk factors for severe disease for whom test results may influence decisions regarding care and treatment; infection control; and management of close contacts. In such circumstances, it is important that these treatment and patient management decisions, as well as infection control practices, are not delayed pending the availability of testing results.

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1 Influenza activity levels are defined by FluWatch at the following link: phac-aspc.gc.ca/fluwatch/13-14/def13-14-eng.php. (Please note that testing and surveillance protocols/thresholds are determined independently by each province/territory.)
3.2 Planning Assumptions

As noted in the main body of the CPIP, identifying planning assumptions is a way to deal with uncertainty. Although planning assumptions provide a useful framework for planning, they should not be regarded as predictions. In the absence of certainty, planning assumptions help to inform planning and decisions regarding the establishment of laboratory protocols and procedures, and stockpiling of materials and supplies that will be necessary during the pandemic response. Planning assumptions related to pandemic preparedness are found in the main body of the CPIP.

Several laboratory-specific planning assumptions are identified below:

- Vaccine will not be available for the first wave, and thus demand for testing will remain high.
- Timely and accurate laboratory surveillance and diagnostics will be critical to appropriate clinical and public health management.
- Over the course of the pandemic there will be at least a 5- to 10-fold increase in influenza diagnostic testing.
- Resources, including human resources, will be constrained, and surveillance and clinical testing priorities will need to be re-evaluated as the pandemic progresses.
- Demand for testing will remain elevated between pandemic waves.
- Depending on the severity of the pandemic, workplace absenteeism will likely be spread over several weeks and will include laboratory personnel.
- Laboratories will see an increase in testing requests for non-influenza infections, including nosocomial infection testing, because of increased numbers of hospital admissions.
- There will be shortages of the materials and supplies needed during the pandemic period because of supply chain interruptions (e.g. mail and courier disruptions, border closures or supply limitations). Therefore, plans are required to allow for a consistent 8-week supply (assumed time frame of peak period for two pandemic waves) of both influenza and non-influenza related materials.
- There will be a great increase in the demand for information. Appropriate and timely communication strategies and resources will be required to meet this need.
### 3.3 Key Triggers and Typical Accompanying Actions

Key triggers and typical accompanying actions from a laboratory perspective are identified in Table 1 below.

<table>
<thead>
<tr>
<th>KEY TRIGGERS</th>
<th>TYPICAL ACCOMPANYING ACTIONS</th>
</tr>
</thead>
</table>
| **NOVEL VIRUS CAUSING HUMAN CASES DETECTED ANYWHERE IN THE WORLD** | - Confirm the ability to detect the virus:  
  - Review protocols and evaluate the performance of various platforms through the use of plasmids/nucleic acid extraction of the novel virus.  
  - Develop new processes if the virus cannot be identified or subtyped by current assays.  
  - Confirm the ability to detect the virus through PHL participation in proficiency panels.  
  - NML to increase communications with PHLs, which can relay information to front-line laboratories.  
- Define and communicate biosafety concerns.  
- Enhance surveillance for the novel virus.  
- Establish guidance on whom and how to test, i.e. testing strategies (both surveillance and clinical)  
  - Whom and what to test will be defined collaboratively with the epidemiologists. |
| **NOVEL/PANDEMIC VIRUS (WITH SUSTAINED HUMAN TRANSMISSION) FIRST DETECTED IN CANADA** | - NML Operations Centre to be activated to coordinate FPT communications with PHLs and international stakeholders.  
- NML Operations Centre to liaise with Health Portfolio Operations Centre as and when required.  
- Evaluate the performance of the various platforms through the use of plasmids/nucleic acid extraction of novel virus.  
- Confirm the ability to detect the virus through PHL participation in proficiency panels.  
- Activate and communicate new processes (i.e. identification and antiviral susceptibility) to stakeholders.  
- Review stockpiles and pandemic plan(s) to identify and address gaps that require remediation.  
- Ensure that arrangements for restocking of reagents and supplies are in place.  
- Expect increased testing and demand while maintaining key surveillance activities (i.e. antiviral susceptibility and strain characterization). |
**3.4 Key Elements of the Response**

**3.4.1 PRE-ANALYTICAL**

**Specimen type and collection:** The ability to detect influenza virus depends on many factors:

- Illness state and location of the virus in different anatomical compartments;
- Timing of specimen collection with respect to onset of symptoms;
- Age of the patient;
- Type of specimen (preferred type outlined in Table 2);
- Collection swab;
- Specimen transport; and
- Diagnostic test.

While the shedding patterns of a novel influenza virus may be variable, because the population lacks immunity it is likely that the novel virus will behave in a way that is similar to what is seen in seasonal influenza with children. Specimens should be collected within 5 days of onset of symptoms and preferably within 48 hours. Sampling beyond 5 days may be considered in young children or the elderly, in the immunocompromised and in patients with persisting or worsening symptoms regardless of age. Specimens should be collected from patients admitted to the hospital with suspected influenza regardless of symptom duration. While the ideal specimen will need to be defined as the pandemic progresses, suggested specimen types are outlined in Table 2.

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**TABLE 2 – SPECIMEN TYPES FOR THE DETECTION OF INFLUENZA VIRUS ACCORDING TO PRESENTATION**

<table>
<thead>
<tr>
<th>NATURE OF ILLNESS</th>
<th>SPECIMEN OF CHOICE</th>
<th>ALTERNATIVE SPECIMENS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASYMPTOMATIC</td>
<td>Do not test.</td>
<td></td>
</tr>
</tbody>
</table>
| MILD/MODERATE ILI                              | Nasopharyngeal swab (NPS)/nasopharyngeal aspirate (NPA)                           | Deep nasal swab WITH a throat swab or mid-turbinate swab
                                                                                             | Throat swab and sputum                                                             |
| Video demonstration of NPS/NPA collection can  |                                                                                   |                                                                                      |
| be accessed at: youtube.com/watch?v=TFw$efezIHU|                                                                                   |                                                                                      |
| SEVERE RESPIRATORY ILLNESS OR LOWER RESPIRATORY | NPS AND endotracheal (ET) secretions or bronchoalveolar lavage (BAL) specimens   |                                                                                      |
| TRACT INFECTION                                |                                                                                   |                                                                                      |
| AUTOPSY                                        | Lung tissue or other tissues from suspected organ involvement. Specimens should be|                                                                                      |
|                                                 | fresh or frozen at –70°C. DO NOT put into formalin fixative.                       |                                                                                      |

*a* Limited data on the use of these specimens compared with NPS suggest that there is a reduction in sensitivity.

*b* Throat swabs and sputum should be considered in addition to an NPS if the novel virus is of avian origin, as limited data from the H5N1 and H7N9 outbreaks indicate that the avian viruses can be identified using these specimens. Note: many laboratories may not have protocols for the detection of respiratory viral pathogens from sputum.

*c* National experience with intensive care unit (ICU) patients suggests that in some patients NPS may be negative whereas ET intubation aspirates or BAL specimens collected simultaneously are likely to be positive.

*d* There are no studies currently available that compare test performance of ET aspiration specimens with that of BAL specimens to determine which is the preferred specimen.

Flocked swabs should be used to collect nasopharyngeal or nasal/throat combination specimens. Wooden shaft swabs are inhibitory to nucleic acid-based testing and are therefore not recommended.³

Appropriate personal protective equipment (PPE) is recommended for collection of specimens. The infection control/occupational health guidelines may differ among PTs. For relevant PPE guidance, it is recommended that laboratories check with their infection prevention and control guidelines and with their local public health agencies, as well as the Canadian Biosafety Standards and Guidelines. These guidelines are available at: canadianbiosafetystandards.collaboration.gc.ca/.

**Specimen transport:** Specimens should be collected and transported to the laboratory as soon as possible, preferably within 72 hours, on cold packs (+4°C). If a longer delay is anticipated, specimens should be frozen at –70°C or lower and transported on dry ice. However, freezing may affect the recovery of the virus if culture is required. Specimens should not be frozen at –20°C.

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If $-70^\circ C$/dry ice is not available, the specimens should remain at $+4^\circ C$ and shipped as soon as possible. Recent data suggest that when extracted RNA is being transported alone, it will remain stable at $+4^\circ C$. Specimens should be transported as diagnostic specimens as per the usual practice for seasonal influenza specimens, and no enhanced precautions are necessary.

It is important to ensure that the specimen tube and requisition are completed correctly and fully, with matching patient names and unique identifiers, and relevant clinical and epidemiological information.

**Surge Capacity:** Many laboratories underestimated the pressures that the increase in testing demand during the 2009 pandemic would put on the pre-analytical process within the laboratory. The following issues should be considered in planning for the next pandemic:

- **Accessioning:** Increased resources (e.g. clerical) and alternative processes, which could include expansion of the accessioning area, need to be considered to mitigate potential delays in this critical function.

- **Prioritization:** It is important that laboratories have processes established to prioritize specimens when capacity is exceeded. Such processes might include the development of special requisitions and online order entry to help standardize the information required for triaging specimens. As previously noted, it is extremely difficult to implement novel approaches during an emergency. Therefore, building on established practices and systems to the extent possible and piloting new processes prior to an emergency are recommended.

- **Aliquoting:** During the 2009 pandemic, the aliquoting of specimens was often necessary to ensure that there was sufficient specimen available to re-test and submit to the NML for further characterization or to be able to respond to research-related demands. It is recommended that laboratories have a process established to help achieve this aliquoting step without affecting the turnaround time of the specimen processing.

### 3.4.2 ANALYTICAL

A number of methods are available for the detection of influenza, each of which has varying abilities. NAAT protocols, such as conventional reverse transcriptase polymerase chain reaction (RT-PCR) or real-time RT-PCR (rRT-PCR), with their high sensitivity, rapid turnaround time and potential strain characterization capability, together with high throughput and the ability for automation, are the method of choice for pandemic influenza testing. Table 3 below summarizes the testing options available for detection and characterization of influenza; additional detail regarding the various testing methods is provided after the table.

---

TABLE 3 – METHODS OF DETECTING AND CHARACTERIZING INFLUENZA

<table>
<thead>
<tr>
<th>TEST</th>
<th>METHOD</th>
<th>TIME TO PERFORM THE TEST</th>
<th>SENSITIVITY(^a)</th>
<th>SUB-TYPING</th>
<th>COST</th>
<th>THROUGH-PUT</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAAT (RT-PCR(^b))</td>
<td>RNA detection</td>
<td>4-8 h</td>
<td>86%-100%</td>
<td>Yes</td>
<td>$$-$$$</td>
<td>++++</td>
</tr>
<tr>
<td>Viral culture</td>
<td>Virus isolation</td>
<td>2-10 days</td>
<td>About 30%</td>
<td>Yes(^c)</td>
<td>$$</td>
<td>+</td>
</tr>
<tr>
<td>Direct immuno-fluorescence assays (DIFA) or indirect immuno-fluorescence assays (IIFA)</td>
<td>Antigen detection</td>
<td>2-4 h</td>
<td>47%-93%</td>
<td>No</td>
<td>$$</td>
<td>++</td>
</tr>
<tr>
<td>Rapid influenza detection tests</td>
<td>Antigen detection</td>
<td>0.5 h</td>
<td>10%-69%</td>
<td>No</td>
<td>$$$</td>
<td>++</td>
</tr>
</tbody>
</table>

\(^a\) Compared with RT-PCR tests; RT-PCR tests are compared with other NAAT methods.
\(^b\) RT-PCR is the most widely used NAAT.
\(^c\) Requires further characterization by nucleic acid-based testing or antigen characterization.

TESTING METHODS

1. **NAATs**: NAATs, such as RT-PCR, are the recommended method of choice for detection and characterization of influenza because of their ideal test performance, automation and scalability.\(^5\),\(^6\) NAATs can be performed in CL2 clinical diagnostic laboratories. Many commercially available kits and methods developed “in-house” are currently being used to diagnose and differentiate influenza types and subtypes. While sensitivity is high, it can vary among assays.\(^7\)\(^8\)\(^9\)\(^10\) When a laboratory in Canada identifies a novel strain with pandemic potential, the NML, in consultation with the WHO and its collaborating centres on influenza, will share sequence data so that the PHLs can optimize the protocols used in NAATs to identify the novel subtype. The PHLs and designated diagnostic laboratories will in turn share information with their local laboratories.

It is recommended that provincial laboratories have procedures in place for both the detection and subtyping of influenza viruses. Specimens positive for influenza A from patients with epidemiological and clinical features that suggest a novel subtype of influenza should be subtyped for common seasonal influenza viruses using RT-PCR. PHLs or designated laboratories should perform rapid subtyping of positive specimens. Specimens that are repeatedly positive for influenza but cannot be subtyped or have a unique genome sequence should be forwarded to the NML for further

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characterization. As the pandemic evolves, subtyping may remain an important aspect of testing to support ongoing surveillance requirements and may be important in patient management if the antiviral susceptibility profile is different from that of seasonal strains. However, if the pandemic virus becomes the dominant circulating strain, subtyping may have limited value.

It is recommended that a continued effort be made to decentralize NAATs and establish additional capacity in hospital laboratories. To support this effort, it is recommended that PHLs take appropriate initiatives and help establish additional testing sites in the respective jurisdictions, as well as mechanisms to help ensure that central reporting of results takes place. It is further recommended that laboratories should optimize reporting strategies such that both positive and negative results are reported expeditiously.

While NAAT assays are the most sensitive detection method, there are many commercially available kits for the detection of influenza, including a number of multiplex assays, with varying degrees of analytical sensitivity. Recent data show that the analytical sensitivity of a number of commercial assays for the detection of H7N9 virus is poor compared with the in-house assays used at most PHLs, which are based on the Centers for Disease Prevention and Control kit. It is imperative that the performance of commercial assays in their ability to detect novel pathogens be assessed when novel influenza strains are identified.

2. Virus culture: Maintaining culture capacity is important to support national and international (WHO) surveillance programs, as viral isolates are required for antigenic characterization to monitor for potential antigenic drift and antiviral resistance. Influenza can be isolated in a number of different cell cultures, including Madin-Darby canine kidney (MDCK), primary rhesus monkey cell lines and commercially available co-culture preparations (MDCK and mink lung or MDCK and A549). The effectiveness of these cell lines for culturing a novel virus of pandemic potential is not known, and other cell lines may be assessed for optimal viral growth. Because a novel influenza virus would potentially pose a high risk to the health of individuals, it is expected that novel influenza viruses will be considered RG 3 agents and, as such, restricted to PHLs with CL3 capacity. As discussed earlier in the document there needs to be a rapid risk assessment of where a pandemic virus could be handled from a viral isolation perspective.

Because the NML has the technical capacity for antigenic characterization and is required to conduct surveillance reporting to the WHO, the NML will be the primary laboratory for this type of testing. However, once reference antisera become available, there will be an option for subtyping and antigenic characterization, using haemagglutination inhibition assays and neutralization assays, to be carried out by laboratories with the appropriate containment facilities, as dictated by the containment level requirements of the novel strain (see Viral Characterization below).

12 Chan KH, To KK, Chan JF, Li CP, Chen H, Yuen KY. Analytical sensitivity of seven point-of-care influenza virus detection tests and two molecular tests for detection of avian origin H7N9 and swine origin H3N2 variant influenza A viruses. J Clin Microbiol 2013 Sep;51(9):3160-1.
3. **DIFA and IIFA:** Although the sensitivity of DIFA for detection of influenza A virus in one study was 93% (compared with RT-PCR),\(^{15}\) others have reported the sensitivity to be as low as 47%,\(^{16}\) suggesting that these assays may not be sufficiently sensitive to rule out influenza A infection.\(^{17}\) Also, additional testing is necessary for strain identification. If DIFA methods are to be used, experience with the previous pandemic suggests that an adequate sample must contain ≥60 columnar epithelial cells per test well.\(^{18}\) This assay is time-consuming, requires fluorescence microscopes and is subjective, making it a low throughput assay – all of which limit its scalability.

4. **Rapid influenza detection tests:** A number of RIDTs are commercially available and are in routine use. Although the specificity of RIDTs is reasonable, their poor sensitivity limits their usefulness in the management of individual patients. Data suggest that the clinical sensitivity of these assays is widely variable, ranging from 10% to 69%.\(^{19,20,21,22,23,24}\) In addition, their analytical sensitivity for swine variant influenza and avian strains is poor.\(^{25,26,27,28,29,30}\) Therefore, a negative RIDT result does not rule out influenza, especially in the case of novel influenza subtypes. Moreover, the potential exists for false-negative test results, particularly at times of low disease prevalence. Therefore, RIDTs should generally not be used to inform clinical decisions about diagnosis and treatment in individual patients.

RIDT-based testing may have a role in monitoring outbreaks in some settings and may be the only option for timely determination of the presence of influenza in remote communities. Data from Ontario during the 2011-2012 influenza season suggest that although RIDTs have a sensitivity of 59% and 35% for the detection of H3 and influenza B viruses respectively using individual specimens, they have an overall sensitivity of 78.9% for detecting H3 outbreaks when testing up to four samples per outbreak.\(^{31}\)

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18 Chan KH et al. Op cit.
If RIDTs are used to assess influenza activity, the test limitations must be clearly understood and testing sites should train health care professionals in optimal specimen collection and testing procedures. The local PHL could provide assistance in validating RIDT assays and provide confirmatory testing by NAAT for positive RIDT results early in the pandemic and in outbreaks with negative RIDT results. If these tests are to be used, it is recommended that the relevant communities ensure that they have a stockpile of the test kits and the appropriate collection swabs.

5. **Serology:** Serologic tests are not routinely used for diagnosis because the need to obtain convalescent sera results in an inherently long turnaround time. Moreover, the current serological methods of haemagglutination inhibition and microneutralization are labour intensive. Serology has been successfully applied to seroprevalence studies and to surveillance. However, the identification of novel viruses will require the development and validation of new methods. Because of possible cross-reactivity and false positives, caution is advised in interpreting positive results early in the pandemic, when the necessary reagents for validation may be limited.

**VIRAL CHARACTERIZATION**

1. **Antigenic characterization:** Monitoring antigenic variation as the influenza season progresses is an important part of the surveillance program. It is recommended that jurisdictions with the ability to culture the novel virus send approximately 10% of respiratory specimens to the NML for antigenic and genetic characterization, and phenotypic AVR testing.

2. **Antiviral resistance monitoring:** AVR testing will be done primarily for surveillance purposes. There are two important criteria for the submission of specimens for testing: routine surveillance, in which all specimens submitted for antigenic characterization as outlined above are tested, and targeted testing of specimens from patients with features suggesting that they have a resistant virus (Table 4). If resistance is identified and the degree of resistance increases, AVR tests may play an important role in the clinical management of patients. Testing isolates for AVR can be accomplished using phenotypic and genotypic platforms, including sequence analysis of the neuraminidase gene or single nucleotide polymorphism assays directed at regions of known resistance that encode mutations such as H275Y. Once a mutation has been identified, the NML will develop or modify appropriate assays to identify the mutation and distribute the protocols to the PHLs that request them.

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TABLE 4 – THE RECOMMENDED SELECTION CRITERIA FOR SPECIMENS AND/OR ISOLATES TO BE SUBMITTED FOR ANTIVIRAL RESISTANCE TESTING

<table>
<thead>
<tr>
<th>SURVEILLANCE</th>
<th>≤5% POSITIVITY&lt;sup&gt;a&lt;/sup&gt;</th>
<th>&gt;5% POSITIVITY&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temporal and geographic representation. PHLs are requested to submit to the NML 10% of positive isolates obtained from community-based sampling, such as the Sentinel Physician Network.</td>
<td>Temporal and geographic representation. PHLs are requested to submit to the NML two random positive specimens per week obtained from community-based sampling. Outbreak of influenza A in a new jurisdiction or institution.</td>
<td></td>
</tr>
<tr>
<td>CLINICAL APPLICATION/CRITERIA</td>
<td>Failed therapy – ICU patient, 10 days post-treatment&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive test of patient with ILI while receiving or after having received prophylaxis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive test in a traveler returning from an area where resistance is endemic</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Persistent infection in people with immune compromising conditions</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nosocomial transmission in clinical areas with people with immune compromising conditions</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive test from a case in contact with an infected person with an immune compromising condition</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Early and late in the influenza season when the positivity rate for influenza is less than 5%.
<sup>b</sup> During peak influenza activity when the positivity for influenza exceeds 5%.
<sup>c</sup> Additional clinical criteria for phenotypic testing include ongoing deterioration in a patient with wild-type genotype.

CLINICAL FAILURE IN A PATIENT BEING TREATED WITH ANTIVIRALS

The definition of clinical failure in the treatment of influenza infection has not been established. A study of treatment outcomes of patients infected with the H5N1 virus showed that treatment failure was associated with persistent high viral load after 48 hours of therapy.<sup>34</sup> Laboratories performing real-time RT-PCR (rRT-PCR) for influenza have the potential to assess viral loads in patient specimens obtained after antiviral therapy, but in most cases this approach has not been adequately validated and is not routinely available.

Routine repeat RT-PCR is not recommended. While there are data outlining the shedding patterns of influenza in infected patients, the clinical implication of a positive RT-PCR in patients receiving antivirals is not clear. Canadian data from a study of household contacts during the first wave of the 2009 pandemic suggest that although only 13% of pH1N1-positive patients had live virus isolated in cell culture at 8 days after infection, the virus could be detected by RT-PCR in 74% of patients.<sup>35</sup> A similar German study over a 4-year period (2007-2011) revealed that patients can shed live virus (isolated in cell culture) for 4-6 days after infection, and RT-PCR can be positive for up to 9 days.<sup>36</sup> In a Vietnam study, the median time to a negative RT-PCR in oseltamivir-treated patients was 2.6 days, and less than 7% of treated patients had a positive RT-PCR at day 8 after infection. These findings highlight the limitations of using RT-PCR to monitor viral shedding in antiviral-treated patients.

individuals were RT-PCR positive 5 days after treatment. No specimens were culture positive after 5 days of treatment. These cases were considered clinically mild, suggesting that for those with an uncomplicated course of illness, the virus will have cleared in the majority of patients by 5 days. Therefore, in patients whose follow-up respiratory specimens have no detectable virus, the treatment can be deemed successful. However, the significance of positive results is not well understood.

Although routine repeat RT-PCR testing is NOT recommended, repeat testing would be appropriate if suspected failure of treatment, based on the clinical response to treatment (e.g. someone with worsening disease despite 10 days of antivirals and no other obvious cause, such as bacterial superinfection), occurs. In such cases, it is recommended that follow-up specimens, including ET suction and BAL specimens, be collected for testing by RT-PCR, and specimens showing substantial concentrations of virus be forwarded for AVR testing.

DETECTION OF OTHER RESPIRATORY VIRUSES

Canadian experience during the 2009 pandemic and in previous influenza seasons has demonstrated that a number of other respiratory viruses, such as parainfluenza and rhinovirus, can co-circulate with influenza virus, causing considerable morbidity. To avoid inappropriate assignment of morbidity and mortality to influenza, some effort directed at detection of other respiratory viral agents is warranted. Because resource issues may be a problem in many laboratories, broad routine testing for other viruses by all laboratories may not be feasible. Therefore, when influenza testing is negative, a prioritized sampling method is recommended, especially for patients with severe acute respiratory infection, people with immune compromising conditions, children under 5 years of age admitted with ILI, or ILI outbreaks in closed settings such as nursing homes.

3.4.3 POST-ANALYTICAL

It is recommended that laboratories work with their laboratory information system to ensure that newly developed assays can be appropriately reported. It is important that front-line laboratories coordinate with the provincial PHLs to make report data and specimens available for surveillance purposes. Changes in laboratory testing may be required to adapt to the increasing demand. To help ensure a timely communication of such changes, it is recommended that laboratories have a communication strategy to inform clinicians and other end-users of the changes and how these changes may affect surveillance or patient care. Although developing consistent messaging during an evolving health crisis is challenging, the dissemination of this information is a greater challenge if the infrastructure has not been developed beforehand. Achieving effective communication strategies during seasonal influenza is essential so that they can be drawn upon in times of crisis.

Laboratories should anticipate that industry may inquire about access to specimens and expertise in developing or assessing new assays. Consequently, it is recommended that policies regarding industry interactions, including MTA templates, be developed before the next pandemic to expedite the process. With increased laboratory test volumes, laboratories will also need to plan for the archiving/storage or removal of larger than normal numbers of specimens.

3.4.4 QUALITY ASSURANCE AND QUALITY CONTROL

Accreditation programs require participation in influenza proficiency programs by all laboratories performing any type of influenza diagnosis. The NML provides proficiency panels to assess the diagnostic sensitivity and specificity of tests available at PHLs and other viral diagnostic laboratories. The NML and PHLs share reagent lots designed to diagnose circulating or emerging influenza subtypes. The NML also provides at least one influenza proficiency panel per year to Canadian laboratories that wish to participate in NAAT identification of current influenza A strains.

As the 2009 pandemic highlighted, a novel virus may require new testing protocols. Appropriate validation and verification of these methods or of current commercially available assays are essential to achieve accurate test results. As a network, the provincial PHLs and the NML will continue to work collaboratively, sharing reagents and specimens to help laboratories meet this requirement.

As the pandemic evolves, it is anticipated that the diagnostic capabilities of laboratories will be strained. However, it will be important to continue quality assurance activities, such as participation in proficiency panels distributed by the NML. The NML will be responsible for providing the guidance and materials required.

The PILPN recommends participation in other accredited proficiency programs, such as those of the College of American Pathologists.

3.4.5 BIOSAFETY CONSIDERATIONS

International experience with the 2009 pandemic indicated that, from a laboratory perspective, the novel virus did not behave significantly differently from seasonal influenza strains. However, how the next novel influenza virus will behave remains unknown. In addition, at the beginning of the pandemic, when the virus is not widely circulating, there may be a greater chance of exposure to the virus in the laboratory setting than in the community. The Centre for Biosecurity (CB) at PHAC is responsible for providing guidance and the initial biosafety advisory on how diagnostic specimens and virus will be handled. The CB biosafety advisory may be revised as further information becomes available. Laboratories should expect that manipulation of virus culture will be restricted to CL3 facilities and that the processing of diagnostic specimens for NAATs will require CL2 with enhanced precautions. An additional biosafety consideration is influenza vaccination.  

39 It is recommended that all laboratory workers be vaccinated as per the recommendations established for use of the pandemic vaccine.

3.5 Laboratory Roles and Responsibilities

3.5.1 NML

The NML is responsible for the following:

- Fulfilling the relevant requirements of the Pandemic Influenza Preparedness Framework for the Sharing of Influenza Viruses and Access to Vaccines and Other Benefits, which was adopted by the World Health Assembly in 2011 (available at: who.int/influenza/pip/en/);
- Reporting laboratory results to the WHO and its collaborating partners;
- In consultation with the WHO and its collaborating centres on influenza (such as the United States Centers for Disease Control and Prevention), transferring sequence information to PHLs to help ensure that NAATs will be effective in identifying the novel subtype;

39 For more information, see PHAC’s Biosafety Advisories and Notifications website: phac-aspc.gc.ca/lab-bio/res/adv/avis/index-eng.php
• Giving priority to reagent preparation in anticipation of distribution to PHLs for the identification of the new strain when it has been identified somewhere in the world;
• Providing to the PHLs the information and support that will be essential in developing assays for newly emerging strains and establishing quality assurance. This support can include primers, probes, protocols, control reagents and proficiency panels;
• Antigenic characterization and antiviral phenotypic testing, and distribution of information to PHLs;
• Confirming, prior to the establishment of the novel virus in Canada, positive specimens suspected to contain a novel influenza virus that have been submitted from the PHLs; and
• Assisting with strain characterization.

3.5.2 PHLS
PHLs are responsible for the following:
• Having the capability and algorithms required to detect the emergence of a potential novel subtype;
• Along with designated diagnostic laboratories, ensuring that the primary subtyping results of viruses are submitted from front-line laboratories and reporting this information to the NML through the designated surveillance system;
• Sharing, along with designated diagnostic laboratories, information and reagents for identification of the novel strain and advice on cell lines, use of rapid test methodologies, required containment level, etc., with front-line laboratories and PT public health partners;
• Providing to the front-line laboratories the support that will be essential in developing assays or quality control of commercial assays, including protocols, control reagents and proficiency panels;
• Establishing MTAs with the NML and front-line laboratories before the pandemic to help ensure that transfer of materials and information is expedited; and
• Securing enough reagents to realistically cover 5 times the average seasonal testing levels.

3.5.3 FRONT-LINE LABORATORIES
Front-line laboratories are responsible for the following:
• Conducting, along with PHLs and designated diagnostic laboratories, the primary NAAT identification of influenza in patient specimens;
• Submitting diagnostic specimens to the PHL for further characterization according to local PT surveillance guidelines. As per standard practice, any laboratory with a suspected case of a novel influenza virus must forward the specimens immediately to its PHL; and
• Establishing MTAs with PHLs before the pandemic to help ensure that transfer of materials and information is expedited.

3.6 Risk Management Approach
This section discusses another important tool for pandemic planning: the use of multiple planning scenarios specifically intended to support the planning principles and approaches of evidence-informed decision-making, proportionality, flexibility and a precautionary/protective approach.

Planning scenarios provide a starting point to think through the implications and risks that would be associated with pandemics of varying population impact. Scenarios can also be used for exercises and training in support of pandemic plans. To help with risk identification, four pandemic planning scenarios have been developed that describe potential pandemic impacts varying from low to high.
When using these scenarios for pandemic planning purposes, laboratories would want to consider the full aspects of testing: pre-analytical, analytical and post-analytical components, including accessioning, reporting, laboratory information systems, storage of specimens, potential absenteeism and cross-training requirements.

Each laboratory would also define a testing volume baseline. While this may be simply an estimation equivalent to the volume that is expected in a routine influenza season, defining baseline volumes can be challenging. Laboratories may wish to consider the seasonal variation that occurs and the evolution of testing methodology, leading to changes in algorithms (who to test) and protocols. Many laboratories have changed their testing algorithms since the 2009 pandemic and therefore may not be able to provide a true baseline calculation. If laboratory testing algorithms and protocols are relatively consistent for a period of 3 years or more, the average or median of the test volumes over those years can be determined. However, if the testing algorithms and protocols have changed, it may be necessary for laboratories to model test volume estimates according to their historical low-, medium- and high-volume influenza seasons.

Using the pandemic planning scenarios framework, Table 5 outlines the impact related to influenza testing. However, it is recommended that laboratories consider the impact on other services, such as blood cultures, bacterial cultures and antimicrobial testing, all of which may increase with increasing hospitalization due to influenza. While these values are more difficult to quantify, it would be prudent for laboratories to prepare for an increase in these secondary tests.

**TABLE 5 – IMPACT BASED SCENARIOS FOR PANDEMIC INFLUENZA TESTING**

<table>
<thead>
<tr>
<th>NATURE OF IMPACT</th>
<th>A LOW IMPACT</th>
<th>B MODERATE IMPACT</th>
<th>C MODERATE IMPACT</th>
<th>D HIGH IMPACT</th>
</tr>
</thead>
<tbody>
<tr>
<td>BASIC VIRUS CHARACTERISTICS</td>
<td>Low transmission and virulence</td>
<td>High transmission/low virulence</td>
<td>High virulence/low transmission</td>
<td>High transmission and virulence</td>
</tr>
<tr>
<td>NATURE AND SCALE OF ILLNESS</td>
<td>Similar numbers to those in moderate or severe seasonal influenza outbreaks. Mild to moderate clinical features (in most cases).</td>
<td>Higher number of cases than in large seasonal outbreak but similar clinical profile. Overall increased numbers needing medical care and with severe disease.</td>
<td>Similar number of cases to large seasonal outbreak but illness is more severe. Overall increased numbers needing medical care and with severe disease.</td>
<td>Large numbers of people ill, high proportion with severe disease.</td>
</tr>
<tr>
<td>IMPACT ON TESTING</td>
<td>Baseline* (1×)</td>
<td>5×</td>
<td>7.5×</td>
<td>&gt;10×</td>
</tr>
</tbody>
</table>

* See above text for determining baseline volumes
4.0 INTEGRATION WITH OTHER RESPONSE COMPONENTS

4.1 Linkages

Collaboration is one of the guiding principles that underpin Canadian pandemic preparedness and response activities and decision-making. It is essential that the various aspects of pandemic preparedness and response are integrated where necessary to achieve an effective and coordinated response. To this end, the Laboratory Annex strives to identify the key linkages and interrelationships that will contribute to an effective and coordinated response.

It is important for laboratories and public health decision-makers to be engaged during the interpandemic period in order to establish awareness and understanding of laboratory functions, the requirements associated with influenza detection and the contextual relationship of the laboratory as one of the pillars of an effective pandemic response; this will, in turn, facilitate the rapid decision-making processes that are required during a pandemic.

On a practical level, data-sharing agreements among different laboratories in the PT, among the PTs, as well as between the PTs and PHAC, need to be clarified to achieve the seamless transfer of data that will support ongoing surveillance activities. It is imperative that these agreements address intellectual property, copyright issues and other publication issues.

Some key inter-relationships between the Laboratory Annex and other aspects of pandemic preparedness and response are outlined below.

4.1.1 SURVEILLANCE AND EPIDEMIOLOGY

To ensure that the data are comparable and interpreted correctly, it is imperative that epidemiologists understand the nuances of testing and how testing is delivered differently across the country. It is equally important that laboratories understand the needs of the epidemiologists and the data they require to conduct risk assessments and effectively analyze the progression of the pandemic.

Because surveillance is a cornerstone of pandemic planning and preparedness, surveillance during a health emergency should utilize and build upon pre-existing surveillance infrastructures and data-sharing agreements. Every year, ILI is an important cause of morbidity and mortality in the population, and significant resources are expended annually on influenza vaccination and response management. Other viruses also cause respiratory illness each year, and there is limited knowledge of the relative impact of these other viral agents on morbidity and mortality and how best to manage infected
individuals. Ensuring effective and functional surveillance for seasonal influenza and other respiratory viruses in Canada during the interpandemic period serves to optimize capacity for a pandemic response.

Given that rapid advances in diagnostic technologies will continue to improve the ability to accurately diagnose and identify influenza, novel influenza strains and other circulating viruses, the development and routine use of a broad-based network through which to understand the epidemiology of circulating respiratory viruses and to measure their health impact will also serve to build capacity within Canada. Such a network, ideally consisting of a coordinated sentinel network and hospital-based surveillance, would not only enhance capacity to evaluate existing interventions (e.g. vaccines, antivirals and cohorting practices) in the health outcomes of individuals infected with respiratory viruses but could also serve as the foundation for the surveillance response during a pandemic, providing strategic information to track and respond to the outbreak in near real time. Further information regarding the surveillance aspects of pandemic influenza planning can be found in the Surveillance Annex of the CPIP.

4.1.2 CLINICAL CARE AND INFECTION PREVENTION AND CONTROL

Understanding the performance characteristics, benefits and limitations of different testing methods is imperative to help patient management. Continued collaboration with relevant clinical groups will help to ensure that clinicians understand the different testing methods and any preferences for use.

Clinical and public health laboratories also support the clinical management of individuals through testing. However, as noted in the planning assumptions, it is anticipated that resources will be constrained, and consequently clinical testing priorities will need to be re-evaluated as the pandemic progresses. Once the novel strain becomes widespread in the community, testing may not be indicated for the clinical management of those with uncomplicated ILI. It will be important for clinicians in all health care settings to be aware of the guidelines for laboratory testing and, in a timely manner, of any changes to the guidelines (i.e. restrictions on testing) to prevent laboratories from being overwhelmed with requests for confirmation of diagnosis. Clinicians should be made aware of the appropriate requisitions to use, their labelling and the laboratories to which specimens should be sent.

Canada has stockpiles of antiviral drugs comprising oseltamivir and zanamivir, the latter included mainly as a hedge against resistance to oseltamivir. Laboratory testing should be undertaken when antiviral resistance is suspected (see the circumstances outlined in Table 4).

4.1.3 ABORIGINAL COMMUNITIES AND REMOTE OR ISOLATED COMMUNITIES

Unique challenges related to laboratory activities exist in First Nations and remote or isolated communities and must be considered in pandemic preparedness and response planning. It is critical, therefore, for community pandemic planners, along with laboratory experts and PT partners, to collaborate in order to promote seamless transfer of information and determine feasible options in providing communities with access to testing. Community pandemic planners in these communities need to be familiar with established testing guidelines and processes to ensure that laboratory specimens are dealt with appropriately.

One challenge that can be anticipated is obtaining timely access to diagnostics, which can often be difficult because of infrastructure limitations and delays in transportation to reference laboratories. Planners and front-line health care providers must consider geographic location and weather conditions when planning the transport of laboratory specimens, which are time- and temperature-sensitive. Extreme temperature fluctuations and time delays in transporting specimens may adversely affect laboratory test results (e.g. if a specimen is frozen and thawed, PCR testing may be falsely negative). It is advised that every effort be made to transport specimens without delay.
4.1.4 FRONT-LINE LABORATORIES
PHLs will be the gatekeepers to reference testing. To ensure that the flow of testing results information and specimens is appropriate, it is important that front-line laboratories are or can be integrated into the surveillance system. As stated previously, this includes prior development of data-sharing agreements and MTAs.

4.1.5 INDUSTRY AND PROCUREMENT
Any issues related to the performance of commercial assays and the availability of reagents must be communicated to the appropriate vendors. It is important that processes are in place for the rapid approval of necessary equipment and reagents to support the laboratory response. Collaboration between laboratories and their procurement departments will help meet laboratory needs.
5.0 RESEARCH NEEDS

Research plays a key role in addressing knowledge gaps about the influenza virus and effective influenza prevention, treatment and control strategies. Identification of key questions regarding the development of protocols before the next pandemic is important. To help available resources to be mobilized quickly, advance planning is required. Such advance planning considerations could include establishing mechanisms for rapid-response research prior to the pandemic being declared, leveraging existing partnerships between public health agencies, clinical and academic institutions, etc. It is important that laboratories be involved in this planning, as they can support research by providing data gathered from routine or reference testing or by performing additional testing to answer research questions. Strengthening the existing scientific capacity in the PHLs would also help to ensure that the infrastructure exists to address diagnostic questions and to support ongoing clinical studies and vaccine effectiveness.

It is recommended that laboratories consider advance planning for the infrastructure required to support research in collaboration with public health agencies, academic and clinical institutions, as well as for protocols for the use of specimens and the requirement for informed consent. This would include, but would not be limited to, the development of MTAs and data-sharing agreements, particularly regarding intellectual property, copyright and other publication issues, as well as processes to support the dissemination of information to end-users.
6.0 ASSESSMENT AND EVALUATION

With each health emergency, there is an opportunity to review and reassess planning. The development of the Laboratory Annex has allowed PILPN to review the experiences of the last pandemic in order to improve the guidance documents for the next one. When a novel influenza virus is detected in humans for the first time (such as the recent identification of H7N9), CPHLN and PILPN meet to review protocols and ensure that Canada can detect this novel pathogen should the need arise. CPHLN and PILPN will participate in any table top exercise led by PHAC to test the current preparedness and ability to respond, and PILPN will oversee the review of the Laboratory Annex every 2 years and incorporate new developments as they arise.
APPENDIX A – PILPN RECOMMENDATIONS FOR THE PROVISION OF PUBLIC HEALTH LABORATORY SERVICES DURING PANDEMIC INFLUENZA

Preamble

ASSUMPTIONS ABOUT DEMANDS FOR TESTING

• The peak demand for testing services, the duration of that peak period and the availability of testing supplies and reagents, as well as human resources, cannot be accurately predicted.

• The following recommendations are based on best estimates and assumptions of the PILPN as well as experience from the 2009 pandemic.

Recommended Testing Procedures/Capacity

• Each PHL or designated laboratory (e.g. a hospital laboratory that has been designated to function as a PHL) should be able to diagnose influenza A by NAAT. These methods must be broadly reactive to be able to identify novel subtypes of influenza A.

• Each PHL or designated laboratory should be able to subtype positive influenza A specimens to distinguish seasonal influenza from novel subtypes.

• Each PHL that has CL3 capabilities should develop standard operational procedures for the culture and identification of novel subtypes of influenza A. The PHL or the designated laboratories that do not have this capability should develop a Memorandum of Understanding with another designated laboratory that has CL3 capability and could provide this service.

• Each PHL or designate should have appropriate PPE to allow for testing in a CL2 environment with enhanced precautions available for use.

• Provincial and other designated laboratories should have the capacity to meet the projected increased demands for testing for pandemic influenza.

• The NML has the mandate to provide additional, more specialized testing, including serological tests, AVR testing and antigenic characterization. However, depending on their resources and expertise, PHLs may collaborate with NML to develop these tests in order to provide additional surge capacity for specialized testing.
Specimen Collection

Each PHL or designated laboratory should be able to provide or advise on appropriate specimen collection.

- At present, the ideal specimen for a novel subtype is not known. Laboratories should be prepared to assess a broad range of specimen types, including NPS, throat swabs, mid-turbinate swabs, BAL or ET secretions and, potentially, plasma and stool from individuals with epidemiological features that put them at risk of a novel subtype of influenza A.
- As the pandemic progresses, information regarding the most appropriate specimen(s) will be provided.

Business Continuity Plan

- Each PHL or designated laboratory should develop a business continuity plan for influenza-related diagnostics and other essential non-influenza testing services, to include the following:
  1. Determination of the minimum requirements for human resources and materials in order to maintain uninterrupted, essential services during the critical phase of the pandemic. While the initial pandemic wave may last for at least 8 weeks (to vary by jurisdiction), laboratories must address limitations in such items as supplies, reagents and human resources.
  2. Development and publication of a prioritized list of laboratory services that will be reduced as needed during a pandemic.
  3. Development of stockpiles of reagents and supplies necessary to maintain these essential services and implementation of an inventory management system to maximize utilization of reagents and minimize loss due to reagent expiration. This should take into account the limited shelf life of essential perishable items.

Communication

MINIMUM REQUIREMENTS FOR TIMELY COMMUNICATION

- Each PHL or designated laboratory should develop or enhance communication links with its local PT public health departments, CPHLN and local laboratories. Information regarding novel subtypes will be updated from the NML through PILPN and then through CPHLN. While the reporting structure for pandemic response has not yet been developed, laboratories will play a key part and be integral to the response.
- Constant and timely updating of laboratory epidemiological information (data, specimen collection and routing, biosafety, etc.) during a pandemic needs to be developed further.
- PHLs and designated laboratories should take a lead role in developing and guiding specimen triage mechanisms (e.g. an essential clinical information form).