Impact of Pandemic Influenza A (H1N1) on Laboratory Services

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Key Points

- Prior to the 2009 influenza pandemic, influenza was diagnosed in many laboratories by enzyme-linked immunosorbent assay (ELISA) or immunochromatographic tests (rapid influenza diagnostic tests; RIDT), immunofluorescence microscopy (direct fluorescent antibody tests; DFA), and/or shell vial or traditional virus culture. Some laboratories used nucleic acid amplification methods such as reverse-transcriptase-polymerase chain reaction (RT-PCR).
- With the emergence of pandemic influenza A/H1N1 (pH1N1), nucleic acid tests became the principle method for the detection of respiratory viruses. By April 28, 2009 the National Microbiology Laboratory had provided primers and a protocol capable of identifying this novel strain, and most public health laboratories (PHLs) across the country, as well as many academic hospital microbiology laboratories, quickly verified and implemented a RT-PCR method for the detection of pH1N1 based on amplification of the matrix (M) and hemagglutination (HA) genes.
- In addition to RT-PCR, multiplex assays were also used in some Canadian jurisdictions during the 2009 pandemic for detection of other circulating respiratory viral pathogens.
- In anticipation of more intense testing activities during the second pandemic wave, the Pandemic Influenza Laboratory Preparedness Network (PILPN) issued guidelines for laboratory testing for the detection of pH1N1. Based on these guidelines and testing capacity, the majority of provinces prioritized testing based on risk groups, with highest priority given to patients who were hospitalized or part of an outbreak investigation.
- To cope with the surge in influenza testing, personnel from other PHL departments were cross-trained to perform influenza tests. In addition, some PHLs suspended tests for other infectious agents, including norovirus PCR, viral culture on genital specimens, serology, respiratory viral and *Mycoplasma pneumoniae* culture, ova and parasite testing, bacterial typing, and HIV genotyping.
- The emergence of pH1N1 in the spring of 2009 changed the way many microbiology laboratories detect not only influenza virus but also other respiratory viruses causing respiratory infections.

Introduction

The emergence of pandemic influenza A virus (pH1N1) in the spring of 2009 changed the way many microbiology laboratories detect not only influenza virus but also other respiratory viruses causing respiratory infections. Despite initial concerns that it would lead to significant morbidity and mortality, the pandemic turned out to be moderate in severity in most regions. Public health laboratories across Canada played a significant role in responding and providing services not only to detect this new virus, but also in maintaining other services essential for patient/outbreak management. This review addresses the approaches to pH1N1 laboratory testing across Canada and a subset of countries with similar health care systems, and strengths and weaknesses of these approaches.

Indications for Testing

Utilization of diagnostic testing for influenza can be divided into two broad categories: individual patient and population management, and public health surveillance. Diagnostic testing aids in the

clinical management of patients, particularly those who would benefit from antiviral treatment, and plays a pivotal role in infection control. Across Canada this service varied among the provinces from being confined to one central laboratory to extensive point-of-care based testing at many sites. Alberta provincial laboratory performs influenza testing on specimens from the Northwest Territories (NWT) and from eastern Yukon. Testing for the Yukon territory is provided by British Columbia. Specimens originating from the Territories are tested using the same algorithm that these provinces use for their own samples. Follow-up testing such as subtyping, strain characterization and antiviral resistance testing was generally performed in reference settings such as provincial public health laboratories (PHLs) and a limited number of hospital-based laboratories. Subtyping of influenza viruses, which is important for ongoing surveillance activity, has also proven clinically helpful when subtypes with differing susceptibility patterns co-circulate, as occurred in the winter of 2008-2009 (1).

As part of the nationally coordinated influenza surveillance program, Canadian PHLs routinely submit a subset of influenza viruses to the National Microbiology Laboratory (NML), a World Health Organization (WHO) collaborating centre, for further characterization, including strain characterization by hemagglutination inhibition assays (HAI) and phenotypic antiviral resistance tests. These surveillance activities serve to monitor antigenic drift and shift, which can impact vaccine efficacy. Prior to the pandemic there was limited capacity for resistance testing at the provincial level, and the NML was the primary resource for antiviral resistance testing of specimens submitted for surveillance purposes, or in clinical cases where resistance was suspected.

Influenza Testing Prior to the Onset of the 2009 Pandemic

Prior to the pandemic, influenza was diagnosed in many laboratories by enzyme-linked immunosorbent assay (ELISA) or immunochromatographic tests (rapid influenza diagnostic tests; RIDT), immunofluorescence microscopy (direct fluorescent antibody tests; DFA), and/or shell vial or traditional virus culture. Some laboratories used nucleic acid amplification methods such as reverse-transcriptase polymerase chain reaction (RT-PCR). RIDT have a rapid turnaround time (TAT) and require little technical expertise, but are significantly less sensitive and specific than other methods (2). DFA offers higher sensitivity than RIDT, but requires technical expertise and specific equipment, and relies on health care workers collecting high quality samples. Classical isolation in cell culture has a potentially lengthy TAT of up to 14 days and requires transportation conditions that maintain virus viability. In addition, the presence of the influenza virus in cell culture needs to be confirmed by an alternate method (DFA or nucleic acid testing [NAT]). Neither RIDT, DFA, nor virus isolation can differentiate subtypes of influenza A virus. The most sensitive test for influenza detection and differentiation of subtypes of influenza A is RT-PCR (2). Real-time methodologies offer the advantage of decreased TAT (4-6 hours) compared to conventional PCR methods (8 hours). Because of the enhanced sensitivity and the ability of this methodology to subtype influenza, each PHL across Canada implemented nucleic acid amplification testing (NAAT) for influenza A detection as part of pandemic preparedness (3).

> The most sensitive test for influenza detection and differentiation of subtypes of influenza A is RT-PCR.

Serology, although historically utilized, is of limited use clinically, given it requires the submission of acute and convalescent samples. It is performed using hemagglutination inhibition assay (HAI) or microneutralization (MN) assays; the latter is labour intensive. Serology is generally reserved for seroprevalence surveillance studies and for vaccine research but may be used in the retrospective diagnosis of pandemic influenza when appropriate blood specimens are available.

In the pre-pandemic era, PHLs across Canada used an algorithmic approach which included a combination of various methods for the detection of respiratory viruses. As shown in Table 1, NAT played an important role in detection of influenza viruses, particularly in outbreak investigations. Some provinces including Alberta, British Columbia, Ontario, and Quebec used multiplex testing, which can detect influenza A and B as well as several other respiratory viruses including respiratory syncytial virus (RSV), adenovirus, rhinovirus/enterovirus, community coronaviruses, parainfluenza virus,

Table 1. Laboratory testing algorithm for pandemic influenza A (H1N1) in Canada

Province	Testing algorithm	Pre-pandemic	1st wave (Spring 2009)	2nd wave (Winter 2009)	Post-Pandemic (Spring 2010)
Alberta	Restriction:	None	None	In-patient, outbreaks, surveillance, or requests[A4]	None
	Method:	DFA[A1], multiplex[A2]	DFA[A1], multiplex[A2], influenza A/B RT-PCR[A3]	DFA[A5], influenza A/B RT-PCR[A6], multiplex[A7]	DFA[A5], influenza A/B RT-PCR[A6], multiplex[A7]
	Subtyping:	Sent to NML	Done at PHL	Done at PHL	Done at PHL
	Genotypic Resistance:	Surveillance specimens sent to NML	As requested, specimens sent to NML for confirmation	ICU and immunocompromised patient, community surveillance done at PHL, other clinically relevant requests	ICU and immunocompromised patient, community surveillance done at PHL, other clinically relevant requests
British Columbia	Restriction:	None	None	None	None
	Method:	Influenza A/B rRT-PCR[B8], multiplex NAAT[B9]	Influenza A/B rRT-PCR[B8], multiplex NAAT[B9]	Influenza A/B rRT-PCR, multiplex NAAT[B9]	Influenza A/B rRT-PCR, multiplex NAAT[B9]
	Subtyping:	Influenza A	Influenza A	Non-pH1N1 influenza A	Influenza A
	Genotypic Resistance:	Influenza A positive specimens	Representative specimens	Representative specimens	
Manitoba	Restriction:	None	None	Some screening was transferred to hospital labs. Done on all specimens meeting testing criteria[M10]. After Nov 20, 2009 (peak), only hospitalized (including ER/OU), immunosuppresed, at-risk persons and outbreak were tested[M10].	
	Method:	Viral culture, ELISA, influenza A rRT-PCR[M11]	Influenza A rRT-PCR[M12]	Influenza A rRT-PCR	Viral culture, ELISA, influenza A rRT-PCR[M11]
	Subtyping:	Outbreak specimens	All influenza A positive specimens	Influenza A positive specimens (influenza A RT-PCR detected at hospitals was subtyped at PHL)	All influenza A positives were tested for pH1N1. Outbreak specimens
	Genotypic Resistance:	Sent to NML	Sent to NML	Assay was developed but not implemented. Sent to NML	Sent to NML
New Brunswick	Restriction:	None	None	None[N13]	None
	Method:	Viral culture	Influenza A/B rRT-PCR	Influenza A rRT-PCR	Viral culture is used but molecular multiplex assay is currently being considered for routine testing.
	Subtyping:	Sent to NML	Positive influenza A	Positive influenza A. Untypable sent to NML	Positive influenza A
	Genotypic Resistance:	Sent to NML	Sent to NML	Sent to NML	Sent to NML
Newfoundland	Restriction:	None	Limited to those with travel history and contact of known cases. This was removed in the latter part of the first wave.	None[N13]	None
	Method:	DFA, culture	Influenza A/B rRT-PCR[N14] , DFA, culture	Influenza A rRT-PCR, DFA, culture	Plans to introduce multiplex
	Subtyping:	Representative numbers sent to NML	All positive influenza A for H1 & H3	All positive influenza A for pH1N1. Untypable sent to NML.	NA
	Genotypic Resistance:	Sent to NML	Sent to NML	Sent to NML	Sent to NML
Nova Scotia	Restriction:	None	None	Hospitalized patients, outbreaks, surveillance	None
	Method:	Influenza A/B & RSV RT-PCR & viral culture[N14]	pH1N1 rRT-PCR, influenza B/RSV, Viral culture[N15]	pH1N1 rRT-PCR, influenza B/RSV, Viral culture[N15]	Influenza A PCR, multiplex is being evaluated to replace viral culture method[N16].
	Subtyping:	All influenza A positives	Done	Done	Done
	Genotypic Resistance:	Sent to NML	Sent to NML	Assay was developed but not implemented. Sent to NML.	NA

Table 1. Continued

Province	Testing algorithm	Pre-pandemic	1st wave (Spring 2009)	2nd wave (Winter 2009)	Post-Pandemic (Spring 2010)
Ontario	Restriction:	None	None	None	None
	Method:	Virus culture, RIDT, influenza A/B PCR[O17], multiplex[O18]	Virus culture, RIDT, influenza A/B PCR[O17], multiplex[19]	Virus culture, RIDT, influenza A/B PCR[O17], multiplex[O19]	Virus culture, RIDT, influenza A/B PCR[O20], multiplex[O21]
	Subtyping:	Selected influenza positives	Hospitalized patients, outbreaks specimens, and 20% of all community patients	Hospitalized patients, outbreaks specimens, and 20% of all community patients	Hospitalized patients, outbreaks specimens, and all community patients until one dominant subtype is established in the province
	Genotypic Resistance:	Sent to NML	All outbreaks specimens, and select hospitalized, and community surveillance specimens	All outbreaks specimens, and select hospitalized, and community surveillance specimens	All outbreaks specimens, and select hospitalized, and community surveillance specimens
Quebec	Restriction:	None	Hospitalized patients only after May 15, 2009 and surveillance	Hospitalized patients and surveillance	None
	Method:	RIDT, viral culture, DFA, influenza A/B PCR, multiplex[Q22]	Influenza A PCR for screening and pH1N1 PCR done by 4 hospital labs and PHL	Influenza A PCR for screening and pH1N1 PCR done by 9 hospital labs and PHL	DFA, RIDT, culture, rRT-PCR, multiplex[Q23]
	Subtyping:	Done by PHL	Done by PHL and/ or NML for seasonal subtyping and confirmation	Done by PHL for seasonal subtyping and confirmation	Done by PHL for subtyping and confirmation
	Genotypic Resistance:	Sent to NML	Sent to NML	Done at PHL and sent to NML for confirmation	Done at PHL

[A1]DFA was performed on NP specimens received from < 5 yrs old, and outbreaks.

[A2]Multiplex testing was done on all DFA negative specimens.

[A3]DFA and multiplex negative specimens were tested using influenza A/B rRT-PCR

[A4]Restriction was implemented from October 28 to December 3, 2009.

[A5]DFA was performed on specimens received from < 1 yr old or on request.

[A6]Influenza A/B rRT-PCR done as a front line testing.

[A7]Specimens negative for DFA or influenza A/B rRT-PCR or surveillance specimens from community settings were tested using multiplex assay.

[B8]Specimens were tested using influenza A/B rRT-PCR. Selected specimens positive for influenza A were grown using cell culture method.

[B9]Multiplex testing was done on influenza A/B negative specimens received from hospitalized patients, children and from outbreaks.

[M10]Indication for testing included outbreaks, hospitalized and community surveillance. Restriction was removed once influenza A positivity rate fell below 10%.

[M11]Viral culture was used as a front-line testing method for all respiratory method. ELISA and subtype specific influenza A rRT-PCR was performed on outbreak specimens.

[M12]Influenza A rRT-PCR was used to screen specimens. Positive specimens were subtyped using pH1N1 RT-PCR.

[N13]No restriction was placed on testing. However, testing was prioritized based on patient settings with ICU patients given the highest priority, whereas lowest priority was placed on specimens from community settings.

[N14]During influenza season, RT-PCR was used to test specimens from outbreaks, hospitalized patients, and for community surveillance. Viral culture was done on specimens from community settings during influenza season and all specimens during off-season.

[N15]pH1N1 specific RT-PCR was used to screen all respiratory specimens. In addition, influenza B and RSV specific RT-PCR were used for specimens from hospitalized patients and outbreaks. If hospitalized, outbreak, and community surveillance specimens were negative for influenza A, influenza B, and RSV, virus culture was set-up.

[N16]It is anticipated that switch from viral culture to multiplex testing would require restricting multiplex testing to hospitalized patients, outbreaks, and community surveillance.

[O17]Influenza A/B RIDT was performed on all outbreak specimens. Outbreak specimens negative for RIDT method were tested using influenza A/B rRT-PCR. In addition, influenza A/B rRT-PCR was done on all hospitalized patients, high-risk groups in community settings, specimens from remote communities, and community surveillance specimens. Viral culture was performed on all specimens from all other community settings.

[O18]Multiplex testing was done on specimens from outbreak settings

[O19]Multiplex testing was done on hospitalized patients, outbreaks, community surveillance specimens, and specimens from remote communities

[O20]Influenza A/B rRT-PCR is done on all hospitalized patients, specimens from remote communities, and community surveillance specimens. Viral culture is performed on all specimens from all other community settings.

[O21]Multiplex testing is done on ICU patients, outbreak specimens, and on community surveillance specimens.

[Q22]In Quebec, front line testing for respiratory virus is done at the hospital laboratories. Each hospital establishes its own criteria for testing. Influenza A/B PCR testing and subtyping for outbreak specimens is carried out by the provincial laboratory.

[Q23]In post-pandemic period, hospital laboratories are responsible for establishing testing criteria and methodology. At the PHL, outbreaks and specimens from surveillance programs are tested using rRT-PCR assay.

and human metapneumovirus. In addition to NAT, specimens particularly from community settings were also tested by isolation in cell culture.

Response to the 2009 Influenza A Pandemic

Many countries, including Canada, had developed pandemic preparedness plans during the past decade. In general, these plans stated that, during a pandemic, particularly in the early phase, laboratory-based testing was essential for detection of the virus and determining prevalence and transmission characteristics of influenza virus, which may impact control measures and management (3).

With the emergence of pH1N1, NAT tests became the principle method for the detection of respiratory viruses in many laboratories. The performance characteristics of rapid methods such as RIDT and DFA were initially unknown for the newly emerged influenza virus and thus further complicated the diagnostic picture. Early in the pandemic several reports, including one from the Centers for the Disease Control and Prevention (CDC), evaluated performance characteristics of RIDTs for the detection of pH1N1 (Table 2). This study reported a sensitivity ranging from 40 % to 69% (2). Other studies using one of the same RIDTs (BinaxNOW Influenza A&B) reported sensitivities as low as 10% (4-6). Similarly, the sensitivity of ClearView ® Exact Influenza A&B was low (19%) compared to the CDC rRT-PCR method (Table 2) (7). Even though RIDTs offer a faster TAT, and can be performed outside the laboratory setting, the positive and negative predictive value is significantly impacted by prevalence rendering them of little use for detection of pH1N1 influenza. Therefore, negative RIDT results required confirmation with NAT.

Similarly, the performance characteristics of various DFA assays for detection of pH1N1 have been evaluated (8-9). As shown in Table 2, the sensitivity of DFA was comparable or superior to RIDT, ranging from 46.7% to 93% (8, 10). The United States Food and Drug Administration (FDA) Emergency Use Authorization (EUA) was given for the use of at least one commercial DFA assay that was specific for pH1N1, but the authorization was terminated along with all other pandemic-specific EUAs on June 23, 2010 (9).

As mentioned earlier, NAT is the most accurate testing method as it can specifically and reliably detect and distinguish influenza A virus subtypes. At the onset of the pandemic in Mexico, CDC, Public Health Agency of Canada (PHAC) and WHO identified the virus by genomic sequence analysis (11).

By April 28, 2009 the NML had provided primers and a protocol capable of identifying this novel strain, and most PHLs across the country, as well as many academic hospital microbiology laboratories,

Table 2. Performance characteristics of diagnostic test methods for detection of pandemic influenza A (H1N1)

Reference	Platform Evaluated	Sensitivity %	Specificity %
Rapid Influenza Diagnostic Test (RIDT)			
Balish *et al*	Binax NOW Influenza A & B BD Directigen EZ Flu A + B QuickVue A + B	40 49 69	-- -- --
Vasoo *et al*	BinaxNOW Influenza A & B BD Directigen EZ Flu A + B QuickVue A + B	38.3 46.7 53.3	100 100 100
Hawkes *et al*	BinaxNOW Influenza A & B	62	99
de la Tabla	ClearView® Exact Influenza A + B	19	100
Ginocchio *et al*	Binax NOW Influenza A & B 3M Rapid Detection Flu A + B	9.6 40	
LeBlanc *et al*	BinaxNOW Influenza A & B	13	100
DFA Test			
Sandora *et al*	Simuloflour Flu A/B DFA	57.3	>99
Gniocchio *et al*	D3 Respiratory Virus Reagents	46.7	94.5
Hawkes *et al*	Influenza A/B Chemicon	83	96

quickly verified and implemented a RT-PCR method for the detection of pH1N1, based on RT-PCR of the matrix (M) and hemagglutination (HA) genes (12-13). By April 30, 2009 CDC released the rRT-PCR protocol for detection of all influenza A viruses and pH1N1 subtyping which was adapted by many laboratories in Canada (14). By May 7, 2009, many countries around the world had access to the CDC protocol and were able to perform rRT-PCR to detect pH1N1.

In addition to rRT-PCR, multiplex assays became an important tool in the detection of respiratory viral pathogens. These platforms virtually eliminated the need for virus culture on routine specimens in many laboratories. Not only are these assays generally more sensitive than culture (4), from an operational perspective, they were preferred given that manipulation of virus culture required enhanced precautions, including the use of an N95 respirator as the pH1N1 was initially considered a Containment Level 3 (CL 3) pathogen by the Health Canada Pathogen Regulatory Directorate. NAT-based detection did not require these enhanced precautions as NAT does not require live virus.

Laboratory Response Testing in Different Countries/Jurisdictions

United Kingdom

In the United Kingdom, the Health Protection Agency (HPA) initiated a 'containment' phase of the pandemic response after the first two confirmed cases of pH1N1 were identified on April 27, 2009. Initially, testing and confirmation was performed at the national reference laboratory of the HPA. By June 4, 2009, this approach was decentralized to the network of regional HPA laboratories due to the overwhelming demand for testing (15, 16). The HPA also launched the "First Few Hundred Project" (FF100), a surveillance project initiated to collect detailed demographic, exposure, clinical treatment and outcome, and virologic data for laboratory confirmed cases of pH1N1 and their close contacts during this phase. To determine the extent of community transmission, persons who contacted their public health nurse were sent self-sampling kits for nasal swab collection and pH1N1 testing. The data showed that between May 28 and June 30, 1,385 swabs were collected by this approach; pH1N1 was confirmed in 91 (7%) of the submitted swabs (15, 16). It is important to note that specimens collected and sent by inexperienced individuals may not yield the ideal quality of specimen, and thus may affect sensitivity of the tests.

In the first few weeks of the pandemic, regional microbiology network (RMN) laboratories were able to manage the increased demand for testing; however, as the number of cases increased, it became apparent that laboratories had reached testing capacity. In response, the RMN developed plans to transport specimens to other network laboratories, and increased capacity by performing several runs per day, 24 hours a day, seven days a week. To facilitate this sudden increase in testing, equipment was rapidly procured, resulting in an overall testing capacity of 5,500 tests/day across England (16). In addition, administrative support was enhanced, making it possible for both positive and negative results to be telephoned to submitters. In the last week of June 2009, more than 10,000 tests were carried out by RMN laboratories. By the end of June, widespread transmission in communities resulted in the implementation of a "treatment only" phase during which suspected cases were no longer routinely tested for influenza virus, and patients with respiratory symptoms consistent with influenza were treated with antivirals (16).

Australia (New South Wales)

The first case of pH1N1 in Australia was reported in Queensland on May 9, 2009. The Australian Department of Health and Ageing declared DELAY phase in the first week of May. In this phase, cases were actively sought in travellers returning to Australia and in the general community, with the aim of preventing spread of the virus. Although the pandemic response was under the jurisdiction of individual states or territories, many of these jurisdictions aligned their recommendations, including laboratory testing, with national guidelines. On May 22, 2009, the CONTAIN phase was initiated in which all suspected cases were encouraged to be tested for pH1N1 (17, 18). This was implemented as a mechanism to control virus spread in communities. Subsequently, on June 23, the PROTECT phase was implemented with laboratory testing that focused on those with moderate to severe disease or those from high-risk populations. In addition, continued testing was recommended for patients admitted to hospital, and a representative group of samples from the community were tested for surveillance purposes. In New South Wales (NSW), collection of respiratory specimens and testing

for pH1N1 required authorization by public health or designated clinicians (19), as well as the completion of a dedicated web-based form by the submitter. The results were entered and reported in a single web-based system, NetEpi. In NSW, molecular testing for pH1N1 was only carried out at two public reference laboratories in Sydney. As the volume of testing increased, six other testing laboratories serially joined the response using commercial assays. During the PROTECT phase, RIDTs were performed in private laboratories. Adamson *et al*. reported that during the early stages of the pandemic, results were delivered in a timely manner but this could not be maintained during late CONTAIN and PROTECT phases, as laboratories became overwhelmed by community requests for testing that were not required for public health or clinical management (19). Although actively discouraged, the large and sustained volume of community test requests continued into the PROTECT phase and, given NSW adopted a policy whereby no specimens were rejected, these excessive volumes had a severe and detrimental effect on service delivery, in particular TAT. Based on the experience it was suggested that in the future, targeted testing should be considered in a laboratory response to an infectious disease emergency (19).

United States (North Shore Long Island Jewish Health System, New York City)

In the United States, CDC supplied reagents and protocols for NAT detection of pH1N1 to state public health microbiology laboratories. Many state laboratories supported clinical laboratories by performing influenza A RT-PCR and/or subtyping. In the state of New York, the state microbiology laboratory was initially approved to perform molecular testing for detection of pH1N1. A study done by Crawford *et al.* reported their experience during the early part of the pandemic (20). The microbiology laboratory of North Shore Long Island Jewish Health System (NSLIJSH), which serves 15 hospitals and regional physician practices in Metropolitan New York, received specimens from 20 students with influenza-like illness who presented to one of the hospital emergency departments on April 24, 2009. Some of these specimens tested positive for influenza A virus and were subsequently sent to CDC for subtyping. CDC reported that 28 out of 35 samples were positive for pH1N1. By April 29, 2009, the number of specimens submitted daily to the laboratory increased to greater than 7.5 times the pre-pandemic average with a total of 308 specimens. The laboratory responded by extending work hours, expanding laboratory space, implementing a laboratory information system for the new tests, and establishing a communication system designed to handle increased telephone call volumes. During this time, a multiplex molecular assay was implemented to test respiratory specimens. In addition, the rapid shell-vial culture protocol was modified to screen cultures at 24 rather than 48 hours to improve TAT on culture-positive specimens. By the end of June, over 34,000 tests were performed including RIDT, DFA, culture and multiplex molecular testing. This testing volume was similar to the average number of tests performed during an entire seasonal influenza season. Since many of these tests required expertise, management of personnel became the biggest challenge. Staff from other departments were recruited and cross-trained to deal with increased influenza testing. As a result of increased workload and extended work hours, a mandatory off-duty rotation was implemented to ensure all employees were adequately rested and the laboratory could sustain long-term testing capabilities. The laboratory's preparedness to respond to emergency situations such as bioterrorism had allowed them to adapt to this challenge efficiently. In addition, it was felt that rapid implementation of NAT methodology was

the key to their successful response to pH1N1 (20).

Canada

Similar to Australia, the pandemic influenza response was under the direction of provincial health jurisdictions in Canada. The first Canadian cases of pH1N1 were reported in Nova Scotia and British Columbia on April 26, 2009. As a result, PHLs across Canada implemented NAT to detect this new virus. This was possible since every PHL across Canada had established testing capabilities as a part of pandemic preparedness plans and were using NAT on outbreak specimens prior to the pandemic. In addition, the concern for biosafety precautions and the rapid expansion of rRT-PCR detection of the pH1N1 meant that many labs moved to testing with molecular methods rather than performing viral culture. Alberta, British Columbia, and Ontario PHLs expanded the use of multiplex testing in their testing algorithms as other respiratory viruses were also circulating during the pandemic (Table 1). Marchand-Austin *et al.* analyzed 83 respiratory outbreaks reported from long-term care facilities (LTCF) in Ontario between April 20 and June 12, 2009 that were tested by a commercial multiplex assay (21). Among outbreaks tested, 37%, 27%, and 20% were caused by enterovirus/rhinovirus, parainfluenza 3, and human metapneumovirus, respectively, whereas only one (1%) of the outbreaks was caused by pH1N1 (21). These data showed that in addition to pH1N1, other respiratory viruses were circulating during the pandemic and actually caused the majority of LTCF outbreaks. This information was critical to the management and control of outbreaks and prevented unnecessary use of antivirals for prophylaxis and therapy during the outbreaks.

During the first wave in the spring, all respiratory specimens were tested using NAT (Table 1) and those that tested negatively by NAT were subsequently cultured. In addition, Manitoba and Ontario used RIDT to screen outbreak specimens for detection of influenza, with follow-up NAT. In Quebec, RIDT was primarily used in the pre-screening process for infection control purposes rather than for diagnosis of influenza infection. The high specificity of the RIDT allowed public health units to manage an outbreak as presumptive pH1N1 when an RIDT test was reported positive for influenza A.

The importance of rapid subtyping was recognized, and most PHLs across Canada implemented subtyping assays. Genotypic resistance testing was carried out only in British Columbia and Ontario, particularly during the first wave as the majority of PHLs continued to rely on NML for resistance testing (Table 1). As per the previously established nationwide surveillance program, NML continued to receive influenza A positive specimens for further characterization including confirmation, subtyping, strain typing and resistance testing. Overall, the first wave in Canada lasted for approximately

The Canadian Public Health Laboratory Network (CPHLN) and the Pandemic Influenza Laboratory Preparedness Network (PILPN)

Established in 2001, the Canadian Public Health Laboratory Network (CPHLN) is a national association of public health laboratory professionals which provides a forum for public health leaders to share knowledge and expertise in an atmosphere of trust. CPHLN's mission is to provide leadership and consultation in all aspects of the public health system through the continued development of a proactive network of public health laboratories to protect and improve the health of Canadian. CPHLN Issue/Task Groups are the conduit through which issues important to public health laboratories are identified, investigated and addressed or operationally implemented on a national level. Issue/Task groups will also be used as subject matter expert groups to deliberate and analyze special issues on behalf of CPHLN and to implement specific initiatives.

As a CPHLN task group, the Pandemic Influenza Laboratory Preparedness Network (PILPN) works to enhance public health laboratory preparedness for pandemic influenza and other potential public health threats by establishing networks and collaborations among federal, provincial, hospital, regional, and local public health laboratories, clinicians and federal epidemiologists. PILPN is responsible for making recommendations on expected response during a pandemic event to public health laboratories. Laboratories play a critical role in the response to pandemic influenza, thus planning guidelines related to public health response are coordinated by PILPN culminating to Annex C: Pandemic Influenza Laboratory Guidelines of the *Canadian Pandemic Influenza Plan for the Health Sector.*

8-16 weeks; the pandemic peaked at slightly different times in the different provinces.

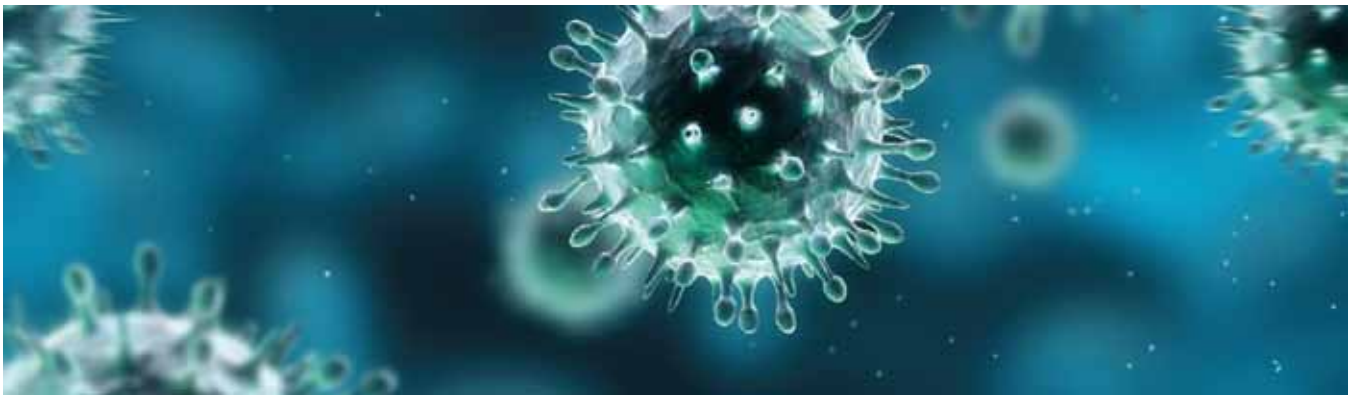
During the first wave, all respiratory specimens were tested for influenza A virus. However, as the pandemic progressed, with widespread transmission in communities, laboratories implemented strategies to selectively test specimens from patients who were deemed most likely to benefit from a definitive laboratory diagnosis. These individuals included hospitalized patients, select high-risk persons (e.g. patients with risk factors such as pregnancy, immunocompromised status or who are young children etc.) or populations (e.g. Aboriginal communities) and outbreak cases (i.e. persons involved in a pH1N1 outbreak in long-term care facilities, where prophylaxis and infection control practices are important in the control and prevention of further transmission of the virus). This led PHLs to make appropriate arrangements in anticipation of the second wave in the fall of 2009, which was expected to be of higher intensity than the first wave. In the fall of 2009, the Pandemic Influenza Laboratory Preparedness Network (PILPN) issued guidelines for laboratory testing for the detection of pH1N1 (22). It stated that molecular testing was the preferred method for detection of pH1N1, and that testing should be done for community surveillance and on patients with severe influenza-like illness, high risk groups, patients who died of acute illness in which influenza was suspected, and for outbreak investigations. Testing was not recommended for uncomplicated infections in patients living in communities where pH1N1 transmission was widespread. Based on these guidelines and testing capacity, the majority of provinces prioritized testing based on risk groups, with highest priority given to patients who were hospitalized or part of an outbreak investigation (Table 1). In conjunction with the PILPN Guidelines, Alberta, Ontario and Nova Scotia additionally rejected specimens from uncomplicated community patients with no risk factors during their peak period in the second wave. There were no major changes with respect to subtyping and resistance testing between the two waves, except that Alberta and Quebec introduced genotypic resistance testing on intensive care (ICU) and immunocompromised patients, and for community surveillance to improve their TAT. Other PHLs continued to send their specimens to NML for resistance testing. There were some laboratories that had verified resistance testing (whereby the resistance assays have been tested and certified in a quality control process) but never introduced it into routine services. This may be due to the fact that oseltamivir-resistant pH1N1 cases were rare, with only a few hundred cases reported worldwide (23).

On average, provinces reported receiving 10 times more specimen submissions for influenza testing during the second pandemic wave than previous influenza seasons.

During the spring of 2010, the Canadian Public Health Laboratory Network (CPHLN) surveyed the impact of the pandemic on PHLs across Canada. The assessment was developed to determine challenges faced by each PHL in the areas of laboratory capacity, capability, TAT, stockpiling, and surveillance (24). Overall, PHLs were well prepared to respond to the pandemic as a result of their pandemic preparedness plans. Based on the survey, 73% of PHLs stated that they had sufficient staff to meet pandemic needs. This was achieved by cross-training personnel from other departments to perform influenza testing. In addition, some PHLs suspended tests for other infectious agents, including norovirus PCR, viral culture on genital specimens, serology, respiratory viral and *Mycoplasma pneumoniae* culture, ova and parasite testing, bacterial typing, and HIV genotyping, in order to cope with the increased number of influenza tests done.

On average, provinces reported receiving 10 times more specimen submissions for influenza testing during the second pandemic wave than previous influenza seasons. In order to maintain 24 hour TATs, PHLs introduced extended working hours by switching to 6 or 7 day work weeks in addition to deploying more staff to influenza testing. In addition, many PHLs, including British Columbia, Ontario and Quebec decentralized their testing with many more laboratories, especially those affiliated with academic hospitals, performing molecular testing for pH1N1, thus increasing overall testing capacity.

No major issues were reported with respect to reagents and equipment, given the majority of PHLs were able to purchase and stockpile



reagents as soon as pH1N1 was identified in late April 2009. However, many provinces reported local shortages of viral transport medium and nasopharyngeal swabs.

To address some of these issues, PHLs recommended focusing resources on solving staffing issues, equipment, data management, and space. These recommendations were identified by PHLs across Canada, though they were not necessarily prioritized in the same order by each PHL.

In addition to testing for pH1N1, PHLs across Canada played an essential role in the surveillance of pH1N1 and other respiratory viruses. Laboratories continued to send specimens to NML as part of the nationwide surveillance program. In addition, laboratory results were shared with their provincial epidemiology partners on a real-time basis. Immediately prior to the pandemic, the Canadian PHLs installed a number of Laboratory Liason Technical Officers in most PHLs to speed and improve communication of results and trends to stakeholders. This timely endeavour, in addition to streamlining the necessary communication required at the time, has been met with an overwhelmingly positive response by recipient PHLs. The anticipatory pandemic response by the laboratories demonstrated not only that they excelled in rapid testing of specimens for clinical management, but also in providing valuable data for the surveillance and characterization of the epidemiology of the new virus.

Summary and Conclusions

The pH1N1 pandemic response in Canada measured the ability of microbiology laboratories, including PHLs, to respond to the emerging threat. Although the pandemic was not as severe as originally anticipated, pandemic preparedness plans definitely contributed to the successful diagnostic management of the pandemic by PHLs. By adapting the national and provincial pandemic preparedness plans to the situation, in conjunction with guidance from national groups such as CPHLN, laboratories across Canada were able to respond efficiently and effectively. During the pandemic, PHLs actively monitored their testing algorithms to assess the effectiveness of testing, laboratory capacity and capability, and these algorithms were modified as required. The pandemic also had a lasting effect on respiratory viral testing. Nucleic acid testing, including multiplex testing for the detection of respiratory viruses, became the method of choice universally, and many laboratories are looking into ways to replace respiratory virus culture with multiplex testing. In addition, the importance of subtyping and antiviral resistance testing with respect to the management of patients and influenza surveillance was reaffirmed. Finally, the pandemic demonstrated the importance of having a strong surveillance system to detect not only new influenza strains but also other pathogens. Established surveillance systems allow public health professionals to identify, characterize and implement control measures to minimize the effect of any new pathogens that may be emerging.

Many of the enhancements to laboratory infrastructure, surveillance, and inter-laboratory communication networks in Canada and globally as a result of the pandemic will provide significant additional benefits. In particular, they will improve laboratory response to future emerging infectious diseases and bioterrorism threats, regardless of the pathogen. It can also be anticipated that the speed and quality of both the specific and aggregate information coming out of virology labs will have greatly improved as a result of this pandemic response. It thus becomes imperative that the improvements made during the pandemic not only be preserved but further enhanced, putting laboratory networks in a position to respond effectively when faced with the next emerging disease.

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