Guidance for Laboratory Testing for Detection and Characterization of Human Influenza Virus for the 2010 – 2011 Respiratory Virus Season

CPHLN RLSPC

CANADIAN PUBLIC HEALTH LABORATORY NETWORK
RÉSEAU DES LABORATOIRES DE LA SANTÉ PUBLIQUE DU CANADA

Executive Summary

This is the first influenza season after the H1N1 pandemic (pH1N1). It is assumed that pH1N1 will further adapt to humans and behave as a typical "seasonal" strain of influenza. However, because it is not yet clear if this adaptation has occurred, this season may see unusual activity in unexpected age groups similar to the 2009 pandemic. In addition, it is not clear whether the H3N2 strain or the pH1N1 strain or influenza B will predominate. As such, the discrimination between seasonal and pandemic influenza virus strains may continue to be important for surveillance, diagnosis, treatment and infection control.

As with other "regular" influenza seasons, circulating viruses must be monitored for changes in antigenic characterization and antiviral resistance. It is also important to note the need to track other common viral agents that co-circulate during the influenza season.

The Canadian Public Health Laboratory Network (CPHLN) has developed this document as a comprehensive *Best Practice Guidelines* for detection and characterization of human influenza virus during the 2010-2011 season. To ensure a consistent approach across Canada, this document highlights *Best Practices* for specimen collection, transportation, testing and biosafety from the perspective of Canadian public health laboratories.

The following summarizes *Best Practices* recommendations.

1. Population-based testing for influenza viruses should be carried out for surveillance (e.g. sentinel physician networks). Once influenza becomes widespread, diagnostic testing should focus on hospitalized patients with severe respiratory illness (SRI) or influenza-like illness (ILI), and patients for whom testing will assist decisions regarding care, infection control, or management of close contacts. Testing is also recommended for those who died of an acute illness, in which influenza is suspected, those with potential antiviral (zanamivir or oseltamivir) resistance and for adverse events (e.g. patients who are clinically ill and hospitalized; those who are deteriorating clinically).

2. Nasopharyngeal swab (NPS) is the specimen of choice for routine testing. Flocked swab should be used for collection, with either viral transport medium (VTM) or universal transport medium (UTM) for specimen submission. In SRI, endotracheal aspirate (ET) or bronchoalveolar lavage (BAL) should also be collected in addition to a NPS. Specimen type depends on assay validation and this varies from location to location. Autopsy specimens may include respiratory swab specimens and tissues. Further discussion is available in Appendix 1.

3. Nucleic acid-based testing (NAT) such as real-time reverse transcriptase polymerase chain reaction (rRT-PCR) is the method of choice for routine testing of influenza A and B. For monitoring antiviral resistance and antigenic variation, the virus should be isolated in cultures of Madin-Darby canine kidney (MDCK) or primary rhesus monkey kidney cells.

4. Rapid Influenza Detection Tests (RIDT), relying on antigen detection methods may be considered in remote areas or in resource-challenged environments. Due to limited sensitivity of RIDT, particularly for pH1N1 and influenza B strains, a negative result does not rule out influenza. Further, although some tests can differentiate influenza A from influenza B, currently available RIDT cannot differentiate between H1N1 and H3N2 strains. Therefore, the use of RIDT is not recommended for informing clinical decisions about diagnosis and management in individual patients.

5. Each province should ideally have at least one laboratory capable of genotypic testing for antiviral resistance and strain typing. Where this is not feasible, there should be arrangements to obtain this service.

6. Provincial public health laboratories (PHL) should submit a proportion (up to 10%) of community and hospital-based influenza isolates, to the National Microbiology Laboratory (NML) on an ongoing basis to monitor antiviral resistance and antigenic variations.

7. Co-circulation of other viral agents associated with ILI should be monitored during the influenza season as part of ongoing surveillance.

8. The decentralization of NAT testing for influenza virus to hospital laboratories and regional public health laboratories should be promoted to increase the diagnostic capacity required to meet increased demands.

Introduction

This *Best Practices* guidance should be used in conjunction with relevant provincial and territorial Pandemic Influenza guidelines. The Public Health Agency of Canada will be posting regular updates and related documents at www.phac-aspc.gc.ca.

This is the first influenza season after the H1N1 pandemic. It is assumed that pH1N1 will further adapt to humans and behave as a typical "seasonal" strain of influenza. However, because it is not yet clear if this adaptation has occurred, this season may see unusual activity in unexpected age groups similar to the 2009 pandemic. In addition it is not clear whether the H3N2 strain or the pH1N1 strain or influenza B will predominate. As such the discrimination between, seasonal and pandemic influenza virus strains may continue to be important for surveillance, diagnosis, treatment and infection control of persons presenting with ILI. The Pandemic Influenza Laboratory Preparedness Network (PILPN) of the CPHLN has developed this document as a comprehensive *Best Practices* guidance for detection and characterization of human influenza viruses for the 2010 – 2011 season.

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

1) Population based surveillance,
2) Diagnostic testing of individuals presenting with ILI.

Once influenza becomes wide spread in the community, treatment will be based on clinical presentation and testing of community samples should be reserved for community based surveillance programs. The remainder of testing focused on hospitalized patients and those with risk factors for severe disease where the results of the test may influence decisions regarding care and treatment, infection control, and management of close contacts. ***It is important that the above decisions should not be unduly delayed while waiting for test results*.**

Further, there continues to be a need to monitor influenza viruses from specific cases for antiviral resistance, as well as antigenic variation. There is also a need to track other common viral agents such as parainfluenza, human respiratory syncytial virus (RSV), adenovirus, rhinoviruses, human metapneumovirus, coronaviruses, etc, which co-circulate during the influenza season, from the standpoint of surveillance.

Surveillance

Population-based surveillance should include testing, with a rapid turnaround time, for influenza and other common respiratory viruses associated with ILI. Influenza A should be subtyped to distinguish pH1N1 from H3N2, with levels of subtyping dictated by the seasonal subtypes co-circulating with pH1N1 and their accompanying resistance patterns. Although it appears that the previous seasonal H1N1 strain has disappeared based on surveillance data from other areas of the world, laboratories should still have the capacity to identify this stain as the antiviral resistance pattern differs from pH1N1 and H3N2 strains. Community-based sampling should be increased as peak testing periods decline. A proportion of influenza isolates representing community-based cases as well as hospitalized patients should be sent to the NML to monitor for antiviral resistance and antigenic variation (See section on *Viral Characterization*). NML should continue to provide reference testing for phenotypic and genotypic antiviral

characterization and advise PILPN of any mutations associated with antiviral resistance other than the H275Y.

Diagnostic Testing

The following groups of patients should be considered for diagnostic testing once influenza becomes wide spread in the community:

1. Hospitalized patients with SRI and ILI.
2. Patients for whom diagnostic testing will assist decisions regarding care, infection control, or management of close contacts (e.g. residents/staff in long-term care facilities for outbreak investigations; those at risk of complications from influenza infection; those contacts at risk of serious outcome if infected by the index case and if not vaccinated).
3. Persons who died of an acute illness in which influenza is suspected.
4. Persons identified as part of the provincial sentinel surveillance system.

Testing is generally not indicated for clinical management of persons with uncomplicated ILI residing in communities where influenza is circulating.

Specimen Type and Collection

The ability to detect influenza virus depends on many factors including:

- Clinical illness
- Specimen collection with respect to onset of symptoms
- Age of the patient
- Type of specimen (preferred type outlined in table below)
- Collection swab
- Specimen transport
- Diagnostic test

Specimens should be collected within 5 days of onset of symptoms, preferably within 48 hours. Sampling beyond 5 days may be considered in patients with persisting or worsening symptoms regardless of age, in young children or the elderly, and in the immunocompromised[1]. Patients admitted to the hospital with suspected influenza should have specimens collected regardless of symptom duration.

Nature of Illness	Specimen of Choice	Alternative Specimens
Mild/Moderate ILI	Nasopharyngeal swab (NPS) Video demonstration of NPS collection can be accessed at http://www.youtube.com/watch?v=TFwSefezIHU	Deep nasal swab **WITH** a throat swab[a]
SRI	NPS AND endotracheal secretions or bronchoalveolar lavage (BAL)[b] if clinical condition of patient allows performance of BAL [b,c]	
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 performance of these specimens compared to NPS suggests that there is a reduction in sensitivity. Further review of data is provided in Appendix 1.
[b] National experience in ICU patients suggests that in some patients NPS may be negative whereas ET aspirates or BAL collected simultaneously are likely to be positive.
[c] There is no data currently available that compares the performance of ET aspiration to BAL to determine which is the preferred specimen. See Appendix 1 for further discussion.

Flocked swabs should be used for collection of nasopharyngeal, or nasal/throat combination specimens. Specimens collected with rayon on plastic or wires are suboptimal. Wooden swabs are inhibitory to nucleic acid-based testing, and therefore, not recommended.

Appropriate personal protective equipment (PPE) is required for collection (surgical mask, gloves and eye protection). The infection control/occupational health concern regarding the performance of a NPS potentially inducing a cough or sneeze was prominent early in the pandemic. At that time, there was no data that could be analyzed to address this concern. Although a N95 respirator mask is generally not required when collecting an NPS, the infection control/occupational health guidelines may differ between provinces. Laboratories should check with their local public health agencies and infection control practitioners for guidance.

Specimen Transport

Either VTM or UTM should be used for transporting respiratory specimens. Specimens should be transported to the laboratory as soon as possible, preferably within 72 hours on ice packs. If a longer delay is anticipated, specimens should be frozen at -70°C 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. If -70°C / dry ice is not available they should remain at 4°C and shipped as soon as possible. Specimens should be transported as diagnostic specimens per the usual practice for seasonal influenza specimens and no enhanced precautions are necessary.

Please ensure that the specimen tube and requisition are completed correctly and fully, with matching patient names and unique identifiers, with relevant clinical information.

Testing Methods

A number of methods are available for the detection of influenza, each of which has varying abilities. NAT protocols such as conventional or rRT-PCR with their high sensitivity, rapid turn-around time, and strain characterization features, together with high throughput and the ability for automation is the method of choice for seasonal and

pandemic influenza testing. The following table summarizes the testing options available for detection and characterization of pH1N1.

Test	Method	Turnaround Time[a]	Sensitivity for pH1N1[b]	Differentiation of pH1N1
NAT (RT-PCR [c])	RNA detection	24 – 96 h [6-8 h to perform test]	86 – 100%	Yes
Viral culture	Virus isolation	2 -10 days	-	Yes [d]
Direct and indirect immunofluorescence tests (DFA and IFA)	Antigen detection	2 – 4 h	47–93%	No
Point of Care tests (POC)	Antigen detection	0.5 h	10 – 69%	No

[a] The length of time needed from specimen collection until results are available. Note that testing patterns may vary between jurisdictions, and as such result reporting times may vary.
[b] Compared with rRT-PCR tests; rRT-PCR tests are compared to other molecular testing methods.
[c] Reverse transcriptase polymerase chain reaction. This includes the multiplex NAT.
[d] Requires further nucleic-acid based characterization.

1. Nucleic Acid Testing: NAT such as RT-PCR is the method of choice for detection and characterization of influenza due to its high sensitivity and specificity. Many commercially available kits and methods developed "in-house" are currently being used to diagnose and differentiate influenza types and subtypes. While they all have a high sensitivity, it can vary between assays[5]. Continued effort should be made to decentralize NAT testing and establish additional capacity in hospital laboratories. PHLs should take appropriate initiatives and help establish additional testing sites in the respective jurisdiction, ensuring mechanisms of central reporting of results are in place. Laboratories should optimize reporting strategies such that both positive and negative results are reported as soon as they are available.

2. Virus Isolation: Maintaining culture capacity is important to support the National and World Health Organization (WHO) surveillance programs as viral isolates are required for antigenic characterization in order to monitor for potential antigenic drift and antiviral resistance as the pandemic progresses. Influenza viruses including pH1N1 have been isolated using the Madin-Darby canine kidney (MDCK) and primary rhesus monkey cell lines, and commercially available co-culture preparations (RMIX: MDCK and mink lung; RMIX Too: MDCK and A549). For diagnostic testing, conventional tube cultures or shell vial cultures can be used. Centrifugation assisted shell vial methods offer the advantage of faster turnaround time compared to tube cultures. The cytopathic effect of the virus will depend on the cell line used, and confirmation of influenza A cultures will require DFA or NAT.

3. Direct and indirect immunofluorescence microscopy assays: Although the sensitivity of DFA for detection of influenza A virus in one study was 93% compared to RT-PCR[6] others have reported the sensitivity to be as low as 47%[3] indicating that these assays may not be sufficiently sensitive to rule out influenza A infection[7]. Also, additional testing is necessary for strain identification. If these methods are to be used, it has been suggested that an adequate sample must contain ≥ 60 columnar epithelium cells per test well[6].

4. Rapid Influenza Detection Tests: A number of RIDT are commercially available and are in routine use. Although the specificity of RIDT is reasonable, it is the poor sensitivity that limits the usefulness of RIDT in the management of individual patients. Data suggests the clinical sensitivity of these assays for detecting pH1N1 is widely variable, ranging from 10 - 100% [3,5,8-11] . Therefore, a negative RIDT result does not rule out influenza, especially pH1N1. Moreover, the potential exists for false positive test results, particularly at times of low disease prevalence. In general, RIDT should not be used to inform clinical decisions about diagnosis and treatment in individual patients especially when used to rule out influenza. However, RIDT based testing may be the only option and may have a role for determining the presence and relative prevalence of influenza in remote communities. It remains important to confirm RIDT positive cases with NAT.

If RIDT are used to assess influenza activity, the test limitations must be clearly understood and testing sites should train and educate healthcare professionals to ensure that specimen collection and testing is optimal[11] .The local PHL should provide assistance in choosing and validating RIDT assays. If these tests are to be used, concerned communities should ensure they have a stockpile of the test kits and the appropriate collection swabs.

5. Serology: Serology is not currently recognized as a front line testing method for influenza because of the inherently long turnaround time due to the need to obtain convalescent sera. Moreover, the current serological methods of hemagglutination inhibition and microneutralization are labour intensive. Serology may be considered for seroprevalence studies and for surveillance and in exceptional cases where appropriate sera are the only specimens available to make a retrospective diagnosis.

Viral Characterization

1. Antigenic characterization

Monitoring antigenic variation as the influenza season progresses is an important part of the surveillance program. Provinces should culture at least 10% of respiratory specimens in which influenza is detected to be submitted to NML for antigenic and genetic characterization, and phenotypic antiviral resistance testing. The selection criteria for antigenic characterization are as follows:

1. 10% of isolates identified during the early, mid and late season
2. Isolates associated with suspected animal to human transmission
3. Isolates associated with international travel
4. Isolates that cannot be subtyped
5. Isolates identified as non-human subtypes

2. Antiviral Resistance Monitoring

Although both the pH1N1 and the H3N2 strains of influenza A are resistant to amantidine, the resistance to neuraminidase inhibitors is currently relatively rare. As of 18 August 2010, of the tens of thousand specimens of pH1N1 tested, there were only 304 oseltamivir resistant viruses identified world wide. At the end of the 2008-2009 season, there were no oseltamivir resistant H3N2 viruses reported in Canada. Antiviral (AV) resistance testing will be done for surveillance purposes, but if the degree of

resistance increases it may play an important role in clinical management of patients. Testing isolates for antiviral resistance can be accomplished using phenotypic and genotypic platforms including sequence analysis of the NA gene or single nucleotide polymorphism (SNP) assays directed at regions of known resistance encoding mutation such as H275Y. NML should standardize SNP assays for antiviral-resistance mutations for all seasonal viruses to be distributed to public health laboratories when required.

The selection criteria for antiviral resistance testing are as follows:

	≤1% Positivity	>1% Positivity
Surveillance	• Temporal and geographic representation. PHLs to submit 10% of influenza isolates obtained from community based sampling such as Sentinel Physician Network to the NML[a]	• Temporal and geographic representation. PHLs to submit two random positive specimens per week obtained from community based sampling to the NML[a] • Outbreak of Influenza A in a new jurisdiction or institution.

[a] Surveillance criteria include routine submission of a subset of virus isolates to the NML.

Clinical application/criteria	• Failed therapy – ICU patient, 10 days post-treatment[a] • Positive test for influenza with ILI while receiving or after receiving prophylaxis • Positive test for Influenza A in a traveler returning from area where resistance is endemic • Persistent infection in immunocompromised host • Nosocomial transmission in clinical areas with immunocompromised hosts • Positive test from a case in contact with immunocomprimised case

[a] Additional phenotypic testing clinical criteria include ongoing deterioration in a patient with wild-type genotype.

Clinical failure in a patient being treated with antivirals: The definition for clinical failure in influenza infection has not been established. A study on 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 [13].

Laboratories performing rRT-PCR for influenza have the potential to assess viral loads in patient specimens obtained after antiviral therapy but this approach has, in most cases, not been adequately validated and is not routinely available. Data from a study of household contacts from the first pandemic wave suggest that, although only 13% of pH1N1 positive patients shed virus that could be isolated via culture at 8 days post infection, the virus could be detected by RT-PCR in 75% of patients (Gaston DeSerres, personal communication). A Vietnam study showed that only 12 percent of treated individuals were RT-PCR positive 5 days after treatment (no specimens were culture positive), and only 1 remained positive at 14 days post treatment [14]. These cases were considered clinically mild, suggesting that for those with an uncomplicated course, the majority will have cleared their virus 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 detecting the virus by RT-PCR in such patients is not well understood.

As routine repeat RT-PCR is **NOT** recommended, suspected failure of treatment should be based on the clinical response to the treatment (e.g. someone with worsening disease despite 10 days of antivirals and no other obvious cause such as bacterial superinfection). In such cases, follow-up specimens including endotracheal suctions and BALs should be collected for testing by RT-PCR and those showing substantial concentrations of virus should be forwarded for AV resistance testing.

Detection of Other Respiratory Viruses

Although experience from countries in the Southern Hemisphere suggest that pH1N1 continues to circulate, both H3N2 and influenza B have co-circulated in many areas (http://www.who.int/csr/disease/influenza/2010_10_08_GIP_surveillance/en/index.html), Canadian experience in the pandemic, and in previous influenza seasons, demonstrated that a number of other respiratory viruses such as parainfluenza and rhinovirus were co-circulating 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 the other viruses by all laboratories is not feasible. Therefore, a prioritized sampling method is advocated in instances where influenza is not detected, especially in patients with SRI, children under 5 years of age admitted with ILI, or ILI outbreaks in closed settings such as nursing homes.

Biosafety Considerations

International experience with pH1N1 indicates that it does not behave significantly different from seasonal influenza strains in the laboratory setting. Also, as the virus is widely circulating, it is no longer considered novel. Consensus among clinical virology laboratory experts is that standard CL-2 procedures used for respiratory virus detection are sufficient. It is important to note that no cases of accidental laboratory-associated infection with pH1N1 have been detected.

At present, the Pathogen Regulation Directorate (PRD) continues to recommend that clinical virology laboratories use CL-2 with additional precautions such as manipulation of specimens within a biosafety cabinet and enhanced personal protection during potential-aerosolizing procedures. It is also recommended that laboratory workers are vaccinated and appropriate work restrictions are in place for pregnant employees. It is anticipated that the PRD will further revise the biosafety advisory.

References

1. Harper SA, Bradley JS, Englund JA, File TM, Gravenstein S, Hayden FG, McGeer AJ, Neuzil KM, Pavia AT, Tapper ML, Uyeki TM, Zimmerman RK. Expert Panel of the Infectious Diseases Society of America.Seasonal influenza in adults and children--diagnosis, treatment, chemoprophylaxis, and institutional outbreak management: clinical practice guidelines of the Infectious Diseases Society of America. Clin Infect Dis. 2009; 48:1003-32.

2. http://www.cdc.gov/h1n1flu/guidance/diagnostic_tests.htm, September 29, 2009

3. Ginocchio, C.C., Zhang, F., Manji, R., Arora, S., Bornfreund, M., Falk, L., et al. Evaluation of multiple test methods for the detection of the novel 2009 influenza A (H1N1) during the New York City outbreak. J Clin Virol 2009; 45:191-5.

4. Mahony JB, Hatchette T, Ojkic D, Drews S, Gubbay J, Low D, Petric M, Tang P, Chong S, Luinstra K, Petrich A, Smieja M. Multiplex PCR tests sentinel the appearance of pandemic influenza viruses including H1N1 swine influenza. J Clin Virol 2009; 45:200-202.

5. Leblanc JJ, Li Y, Bastien N, Forward KR, Davidson RJ, Hatchette TF. Switching gears for a pandemic: validation of a duplex RT-PCR for simultaneous detection and confirmation of the novel influenza A (H1N1) variant. J Clin Microbiol. in press

6. Pollock NR, Duong S, Cheng A, Han LL, Smole S, Kirby JE. Ruling out novel H1N1 influenza virus infection with direct fluorescent antigen testing. Clin Infect Dis 2009; 49: 66-8.

7. Kapelusznik L, Patel R, Jao J, Patel G, Daefler S, Labombardi V, Calfee D. Severe pandemic (H1N1) 2009 influenza with false negative direct fluorescent antibody assay: Case series.J Clin Virol. 2009; Aug 28. [Epub ahead of print]

8. Balish, et al. Evaluation of rapid influenza diagnostic tests for detection of novel influenza A (H1N1) Virus - United States. CDC MMWR, 2009; 58: 826-9.

9. Hurt AC, Baas C, Deng YM, Roberts S, Kelso A, Barr IG. Performance of influenza rapid point-of-care tests in the detection of swine lineage A (H1N1) influenza viruses. Influenza and Other Respiratory Viruses, 2009; 3: 171-6.

10. Faix DJ, Sherman SS, Waterman SH. Rapid-test sensitivity for novel swine-origin influenza A (H1N1) virus in humans. N Eng J Medicine, 2009; 361:728-9.

11. Hatchette TF, Bastien N, Berry J, Booth TF, Chernesky M, Couillard M, Drews S, Ebsworth A, Fearon M, Fonseca K, Fox J, Gagnon JN, Guercio S, Horsman G, Jorowski C, Kuschak T, Li Y, Majury A, Petric M, Ratnam S, Smieja M, Van Caeseele P. Pandemic Influenza Laboratory Preparedness Network. The limitations of point of care testing for pandemic influenza: what clinicians and public health professionals need to know. Can J Public Health. 2009;100: 204-7.

12. WHO Weekly Update (Virological Surveillance Data)
http://www.who.int/csr/disease/swineflu/oseltamivirresistant20100820.pdf

13. de Jong MD, Tran TT, Truong HK, Vo MH, Smith GJ, Nguyen VC, Bach VC, Phan TQ, Do QH, Guan Y, Peiris JS, Tran TH, Farrar J. Oseltamivir resistance during treatment of influenza A (H5N1) infection. N Engl J Med. 2005; 353:2667-72.

14. ProMED-mail: Influenza pandemic (H1N1) 2009: Viet Nam, virus clearance – 20091011.3519.2009 11 Oct 2009. Available from: http://www.promedmail.org <last accessed November 23, 2009>

Appendix 1

During the Pandemic, PILPN was asked to review the literature regarding the ideal specimen for detection of influenza A.

It is clear from patients admitted to the ICU during the first wave that a nasopharyngeal swab (NPS) may result in negative test results, whereas lower respiratory track specimens such as ET aspirates or BAL collected at the same time tested positive. There is no data currently available that compares the performance of ET aspiration versus BAL to determine the preferred specimen type. What is important is that a deeper specimen needs to be collected in critically ill patients. One approach would be to test ET aspirate first and if negative in a patient with SRI, the patient should go on to have BAL as it can be used to investigate other causes of SRI that would be appropriate in this circumstance.

Thus, current recommendations for patients with severe disease is to collect both a NPS and deeper (endotracheal secretion or BAL) specimen. However, there is no data as yet available regarding the best sample for identification of pH1N1 in the community. As such, the following is a detailed assessment of the available literature what specimens is the best for identification of seasonal influenza A.

Nasopharyngeal aspirates (NPA) have been described as the ideal specimen. However data suggests that in children a nasal swab or nasopharyngeal swab is equivalent to NPA in influenza diagnostics. The data for adults is much more limited. There is some data to suggest that the sensitivity of throat swabs is lower than nasopharyngeal/nasal sampling but when molecular testing is used the difference was often not statistically significant. However, there are significant limitations to the currently available data that make drawing definitive conclusions regarding the true performance difficult including:

- Much of the data in the literature comparing throat and nasopharyngeal swabs that exists employs culture as the detection method. Culture is known to be less sensitive than NAT methods.
- Most of the data is comparing older swab formats. The newer swabs such as flocculated swabs increase the yield of cells and thus increase the sensitivity of influenza diagnostic testing.
- The vast majority of studies do not directly compare different specimens collected on the same patient at the same time but present aggregate data for a particular specimen collected from different patients during a specific influenza season.
- Influenza is often one of a subset of viruses examined and as such the numbers in some studies are very small.
- There is no data on whether the different methods of specimen collection induce coughing or sneezing which would be an important component of the risk assessment to determine the necessary personal protective equipment for different collection methods.

Posterior nasal swab (inserted 4-5 cm into the nostril and turned 3 times) using the flocked or standard NPS is an acceptable but slightly less sensitive method (85% sensitive) (Luinstra, 2009; Smieja, 2009).When the prevalence of illness is higher (i.e. when the novel virus is widespread in the community) it may be reasonable to justify the slightly lower sensitivity to allow for sub-optimal sampling methods. However in the early stages of the pandemic wave, when early identification of cases is the objective the more sensitive NP sampling methods need to be considered.

Nasal self-swabbing has been developed as a useful method for epidemiologic purposes, and may be useful in outbreaks. Sensitivity for a single swab is 85% compared with NPS, whereas serial swabbing will detect more cases than single NPS. Only one swab design (flocked Copan mid-turbinate swab) has been validated for this use (Smieja, 2008; Smieja, 2009).

Table 1

Reference	Population	Detection Method	Specimen Comparison	Results	Comments
(Heikkinen, Salmi & Ruuskanen, 2001)	101 children admitted to hospital with URTI (23 had IA)	Immunoflourescence	NS vs. NPA (cotton tip)	NS sensitivity 91% (73% - 98%)	Commented as easy and painless. No comment on cough or sneezing
(Schmid et al., 1998)	39 adults admitted to hospital with ILI (17 positive for IA)	Tissue culture	NPA vs. TS	TS had a 47% sensitivity compared to NPA	
(Covalciuc, Webb & Carlson, 1999)	Multiple age groups of Children and adults assessed in outpatients, ER, urgent care facilities	Tissue culture and OIA	Any combination of NPS, TS, Nasal aspirate or sputum. TS – rayon NPS- Dacron Number of specimens collected raged from 1-4 for each participant the mean was 2.2 per patient.	Nasal aspirate detected 79.6% of positives (culture) NPS detected 64.6% of positives (culture) TS detected 51.5% of positives (culture)	Difference between Th and NPS not significant (P=0.15) However, No indication how many direct NPS and throats were available for comparison. RT-PCR used to resolve discrepant results – identified 21 culture negative as positive (51 identified positive by culture)
(Ipp, Carson, Petric & Parkin, 2002)	Pediatric community based study 199 children	DFA and EIA	Paired NS vs. NPS (cotton tipped)	Sensitivity of NS compared to NPS was 86% and 87% using DFA or EIA as detection methods	NS shown to be significantly less painful
(Rawlinson et al., 2004)	Adult and children	Culture, IFA and PCR	Adults had paired TS and NS Children had NPA only	Comparing NPA to TS, the NPA is more sensitive (NPA detected influenza in 65/469 (13%) patients tested; TS detected 26/260 (10%) patients tested)	In the adult populations they took paired TS and NS for comparison in adults but do not present the data of this comparison. The bulk of the analysis is directed at comparing TS and NPA which were collected on different populations and thus not really a true comparison
(Herrmann, Larsson & Wirgart, 2001)	All ages (range 2 mo – 83 yrs)	Culture, DFA, RT-PCR and POC (FLU OIA)	Paired NPA vs. NPS (rayon tip) in a subset of patients (79/268)	Influenza A/B was detected in 52/105 (51%) of NPA vs. 40/79 (49.5%) of NPS	Not clear if the NPS and NPA were directly compared. NPA had more numbers than swabs
(Heikkinen, Salmi & Ruuskanen, 2002)	230 children median age 10 months	Tissue culture	Paired NPA and NS (cotton tip)	NS detected 11/12 (92%) positives detected by NPA	Only virus significantly reduced in NS was RSV

Reference	Population	Detection Method	Specimen Comparison	Results	Comments
(Frayha, Castriciano, Mahony & Chernesky, 1989)	125 hospitalized children	Tissue culture IFA	Paired NPA and NPS (cotton tipped wire)	Using culture NPS identified 50/59 cases (sensitivity of 84.7%) compared to NPA 55/59 cases (sensitivity of 93.2%)	True infection considered if virus was isolated by culture from wither specimen or positive in both specimens by IFA)
(Robinson et al., 2008)	137 children ≤17 yrs assessed in ER or in hospital	DFA NAT (real time)	Paired NPA (gold standard); TS and Saliva	5/7 Throats identified influenza by DFA, Other 2 throat swabs identified influenza by NAT	Although shows equivalence between TS and NPS only 7 Influenza infections identified
(Lambert, 2008)	295 children presenting to hospital with ARI	Real time NAT	Paired NS/TS combination and NPA	NPA identified 37/37 influenza A infections NS/TS combination identified 34/37 influenza A infections	Some of the TS/NS were collected by non HCWs (family members). Concordance of results of TS/NS between the two was the same. With an 8% likelihood of a false negative result in children presenting to hospital with ILI where a novel virus was in differential diagnosis, the authors felt that NS/TS was not suitable but during a pandemic, self (family) collection of TS/NS could be reasonable and decrease exposure of HCW.
(Hindiyeh, et al., 2001)	Not given	Culture/DFA compared to POC (FLUOIA)	Sputum, NS, TS	Sensitivity of NS for POC (compared to culture) = 46% Sensitivity of TS for POC (compared to culture) = 25%	Specimens were not paired for direct comparison and thus have different numbers in each group. Overall positivity rate for influenza based on specimen type: NS: 26/79 (33%) TS: 12/18 (67%)
(Pregliasco, 2004)	Children	RT-PCR / culture POC (Quickvue)	TS and NS	No data given on difference but in discussion suggests that reduced sensitivity may be related to specimen type (very vague)	Only paired NS and TS were collected in the first season the study was carried out
(Chan, Peiris, Lim, Nicholls & Chiu, 2008)	196 children hospitalized with ARI	Culture / DFA / real time RT_PCR	Paired NPA and NPS (flocked swab)	Using PCR both NPS and NPA detected all 41 positive influenza cases Sensitivity of NPS for DFA = 82.9% Sensitivity of NPA for DFA = 90.2%	Although m the viral load for influenza A in NPA was slightly higher than NPS specimens it was not statistically significant.

Reference	Population	Detection Method	Specimen Comparison	Results	Comments
(Abu-Diab et al., 2008)	455 children hospitalized with ARI	DFA	Paired pernasal (flocked swab) and NPA	Using DFA, pernasal swabs detected all 48 influenza case	Pernasal swabs described as ½ way to the nasopharynx (sounds like a deep nasal swab)
(Kaiser, Briones, & Hayden, 1999)	14 adults experimentally infected with A/Texas/36/91 (H1N1)	Culture and POC (Directogen)	Nasopharyngeal wash, NPS, TS, Throat gargle collected on all participants each day for 8 days (cotton tipped swabs)	Overall positivity: Nasopharyngeal - 64% of specimens positive by culture) NPS / throat gargle - 46% positive by culture TS – 24% positive by culture	Relative risk of getting positive culture with NPW compared to TS was 2.25 Mean viral load was highest in NPW > TG/NPS > TS Virus detected using culture up to day 6 for NPS but only day 3 for TS
(Smieja, 2009)	270 children and adults	PCR	Nasopharyngeal swab (flocked), self-collected nasal swab	NS have 85% sensitivity compared to NPS (40 of 47 influenza).	Serial self-collected NS detected slightly more cases (52/60) than single NPS (48/60)

References

Abu-Diab, A., Azxzeh, M., Ghneim, R., Zoughbi, M., Turkuman, S., Rishmawi, N., et al., (2008). Comparison between prenasal flocked swab and nasopharyngeal aspirates for detection of common respiratory viruses in samples form children. *Journal of Clinical Microbiolgy, 46,* 2414-2417.

Chan, K.H., Peiris, J.S.M., Lim, W., Nicholls, J.M., Chiu, S.S., (2008). Comparison of nasopharyngeal flocked swabs and aspirates for rapid diagnosis of respiratory viruses in children. *Journal of Clinical Virology, 42,* 65-69.

Covalciuc, K.A., Webb, K.H., Carlson, C.A., (1999). Comparison of four clinical specimentypes for detection of influenza A and B viruses by optical immunoassay (FLU OIA) and cell culture methods. *Journal of Clinical Microbiology, 37,* 397-3974.

Frayha, H., Castriciano, S., Mahony, J., Chernesky, M., (1989). Nasopharyngeal swabs and nasopharyngeal aspirates equally effective in the diagnosis of viral respiratory disease in hospitalized children. *Journal of Clinical Microbiology, 27,* 1387-9.

Heikkinen, T., Salmi, A.A., Ruuskanen, (2001). Comparative study of nasopharyngeal aspirate and nasal swab specimens for detection of influenza. *British Medical Journal, 322,* 138.

Heikkinen T, Marttila J, Salmi AA, Ruuskanen O., (2002) Nasal swab versus nasopharyngeal aspirate for isolation of respiratory viruses. *J Clin Microbiol*. 40(11):4337-9.

Herrmann, B., Larsson, C., Wirgart Zweygberg, B., (2001). Simultaneous detection and typing of influenza A and B by a nested reverse transcription-PCR: Comparison to virus isolation and antigen detection by immunofluorescence and optical immunoassay (FLU OIA). *Journal of Clinical Microbiology, 39,* 134-138.

Hindiyeh, M., Goulding, C., Morgan, H., Kenyon, B., Langer, J., Fox, L., et al., (2000). Evaluation of BioStar FLU OIA assay for rapid detection of influenza A and B viruses in respiratory specimens. *Journal of Clinical Virology, 17(2),* 119-26.

Ipp, M., Carson, S., Petric, M., & Parkin, P.C., (2002). Rapid painless diagnosis of viral respiratory infection. *Archive of Disease in Childhood, 86,* 372-373.
Kaiser, L., Briones, M.S., Hayden, F.G., (1999). Performance of virus isolation and Directogen Flu A to detect influenza A infection in experimental human infection. *Journal of Clinical Virology, 14,* 191-197.

Lambert SB, Whiley DM, O'Neill NT, Andrews EC, Canavan FM, Bletchly C, Siebert DJ, Sloots TP, Nissen MD., (2008). Comparing nose-throat swabs and nasopharyngeal aspirates collected from children with symptoms for respiratory virus identification using real-time polymerase chain reaction. *Pediatrics.* 122(3):e615-20.

Luinstra K, Castriciano S, Ackerman M, Petrich A, Chong S, Mahony JB, Smieja M. Improving Pre-Analytic Collection Systems: Inactivation and Preservation of Influenza A for Rapid Testing [poster]. *Clinical Virology Symposium*, Daytona FL April 19-22, 2009.

Pregliasco F, Puzelli S, Mensi C, Anselmi G, Marinello R, Tanzi ML, Affinito C, Zambon MC, Donatelli I., (2004). Collaborative Group Influchild. Influenza virological surveillance in children: the use of the QuickVue rapid diagnostic test. *J Med Virol.* Jun;73(2):269-73.

Rawlinson, W.D., Waliuzzaman, Z.M., Fennell, M., Appleman, J.R., Shimasaki, C.D., Carter, I.W., (2004). New point of care test is highly specific but less sensitive for influenza virus A and B in children and adults. *Medical Journal of Virology, 74,* 127-131.

Robinson, J.L., Lee, B.E., Kothapalli, S., Craig, W.R., & Fox, J.D., (2008). Use of throat swab or saliva specimens for detection of respiratory viruses in children. *Clinical Infectious Disease, 46,* e61-64.

Schmid, M.L., Kudesia, G., Wake, S., Read, R.C., (1998). Prospective comparative study of culture specimens and methods in diagnosing influenza in adults. *British Medical Journal, 316,* 275.

Smieja M, Petrich A, Mahony J, Chong S, Luinstra K, Buracond S. Defining a Reference Standard and Optimizing Sampling Strategy for Influenza Molecular Diagnostics [oral]. *AMMI-CACMID annual meeting*, Vancouver BC, February 27-March 2, 2008.

Smieja M, Singh P, Moss L, Pabbaraju K, Wong S, Fox J, Loeb M. Self- or Parent-Collected Nasal Mid-Turbinate Flocked Swabs versus Nasopharyngeal Swabs for Influenza Diagnosis in a Community-Based Study [poster]. *26th International Congress of Chemotherapy and Infection/AMMI* Canada, Toronto June 18-21, 2009.

The **Canadian Public Health Laboratory Network** is a nationally minded, proactive forum of public health laboratories.

PROUDLY LOCATED AT THE
NATIONAL MICROBIOLOGY LABORATORY

1015 ARLINGTON STREET
WINNIPEG MANITOBA R3E 3R2

INFO@CPHLN.CA
www.CPHLN.ca

CPHLN RLSPC

CANADIAN PUBLIC HEALTH LABORATORY NETWORK ✦ RÉSEAU DES LABORATOIRES DE SANTÉ PUBLIQUE DU CANADA