The Limitations of Point of Care Testing for Pandemic Influenza: What Clinicians and Public Health Professionals Need to Know

Todd F. Hatchette, MD¹ and Members of the Pandemic Influenza Laboratory Preparedness Network (PILPN)*

ABSTRACT

As the world prepares for the next influenza pandemic, governments have made significant funding commitments to vaccine development and antiviral stockpiling. While these are essential components to pandemic response, rapid and accurate diagnostic testing remains an often neglected cornerstone of pandemic influenza preparedness. Clinicians and Public Health Practitioners need to understand the benefits and drawbacks of different influenza tests in both seasonal and pandemic settings. Culture has been the traditional gold standard for influenza diagnosis but requires from 1-10 days to generate a positive result, compared to nucleic acid detection methods such as real time reverse transcriptase polymerase chain reaction (RT-PCR). Although the currently available rapid antigen detection kits can generate results in less than 30 minutes, their sensitivity is suboptimal and they are not recommended for the detection of novel influenza viruses. Until point-of-care (POC) tests are improved, PILPN recommends that the best option for pandemic influenza preparation is the enhancement of nucleic acid-based testing capabilities across Canada.

Key words: Pandemic influenza; seasonal influenza; point of care testing; nucleic acid amplification testing

La traduction du résumé se trouve à la fin de l'article.

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As the world prepares for the next influenza pandemic, governments have made significant funding commitments to vaccine development and antiviral stockpiling. While these are essential components to pandemic response, rapid and accurate diagnostic testing remains an often neglected cornerstone of pandemic influenza preparedness. As outlined in Annex C of the Canadian Pandemic Influenza Plan,¹ accurate laboratory testing is essential to:

- identify the earliest Canadian cases of a novel influenza strain;
- support public health surveillance;
- facilitate clinical management in limited circumstances;
- monitor circulating influenza viruses for anti-viral resistance.

Laboratory and epidemiologic data will also identify triggers that will escalate pandemic phases and dictate appropriate response measures. Recently, the advantages and limitations of currently available influenza testing methods have been reviewed.² Understanding the limitations of these tests is essential when interpreting results. This manuscript briefly reviews tissue culture, rapid antigen detection, and nucleic acid-based testing. It also discusses the limitations of these methodologies and the options for pandemic testing and planning from the perspective of the front-line clinician and public health official.

Tissue culture

The traditional gold standard for the diagnosis of influenza has been virus isolation using tissue culture. Its turnaround time can be from 1 to 10 days, however, early in the pandemic, this technology will be restricted to a very small number of containment level (CL-3) facilities with approved protocols and trained staff able to safely carry out testing procedures.

Nucleic acid testing (NAT)

Nucleic acid testing utilizes sequence-based amplification methods to detect viral RNA. The method most commonly used is reverse transcriptase polymerase chain reaction (RT-PCR) which can be used to detect or subtype influenza A viruses in 4-8 hours. Data show that the increased yield of viral identification by NAT over culture ranges from 8-800% (Table 1).³⁻⁷ This increased sensitivity and improved turnaround time has made NAT the new diagnostic "gold standard". However, NAT requires specialized technical knowledge and equipment and extensive quality assurance, which often restricts their use to larger virology laboratories. Current guidelines suggest that during seasonal influenza, antiviral therapy should be initiated as soon as possible after symptom onset and within 48 hours.^{8,9} The ability to provide rapid and accurate diagnosis while the patient is under observation would be ideal, however even the most rapid methods have a turnaround time of several hours.

Despite these disadvantages, there are many features that make NAT the most effective platform for preparing for the next influenza pandemic:

1. In addition to superior sensitivity and specificity, NAT technologies do not require growth of the virus; thus they are safe to perform in all virology laboratories.

Author Affiliations

1. Division of Microbiology, QE II Health Science Centre, Halifax, NS

* Members of PILPN: Nathalie Bastien, Jody Berry, Tim F. Booth, Max Chernesky, Michel Couillard, Steven Drews, Anthony Ebsworth, Margaret Fearon, Kevin Fonseca, Julie Fox, Jean-Nicolas Gagnon, Steven Guercio, Greg Horsman, Cathy Jorowski, Theodore Kuschak, Yan Li, Anna Majury, Martin Petric, Sam Ratnam, Marek Smieja, Paul Van Caesele.

Correspondence: Dr. Todd F. Hatchette, Division of Microbiology, QE II Health Science Centre, 5788 University Avenue, Room 315, Halifax, NS B3H 1V8, Tel: 902-473-6885, Fax: 902-473-7971, E-mail: todd.hatchette@cdha.nshealth.ca

Table 1. Summary of Literature Demonstrating the Increase in Yield Using RT-PCR Compared to Cell Culture for the Detection of Influenza Virus in Clinical Specimens

Reference	Molecular Method	Conventional Method	Influenza-positive Specimens Detected by Culture	Influenza-positive Specimens Detected by NAT	Increased Yield
Gharagabhi et al., 2008 ³	Real-time RT-PCR [Artus influenza RT-PCR Kit (Qiagen, Hamburg)]	DFA + cell culture (RhMK MDCK)	155/169 (92%)	167/169 (99%)	8%
Van de Pol et al., 2007 ⁴	Real-time RT-PCR (in house)	DFA in children + cell culture (LLC-MK2, R-HELA, HEp-2)	8/18 (44%) in children 2/18 (11%) in adults (using PCR as the reference method)	18/18 18/18	125% 800%
Zitterkoff et al., 2006 ⁵	Real-time RT-PCR (in house)	R-MIX	*Day 0 – 43/50 (86%) Day 2 – 22/50 (44%)	Day 0 – 50/50 (100%) Day 2 – 34/50 (68%)	16% 55%
Espy et al., 2006 ⁶	Real time RT-PCR (in house)	RMIX	49/557	92/557	88%
Ellis et al., 1997 ⁷	Conventional RT-PCR	DFA + cell culture (MDCK, RhMK)	200/619	246/619	23%

* If the initial samples were positive, subsequent samples were collected at 48 hours.

Abbreviations: DFA (direct fluorescence antibody test); RT-PCR (reverse transcriptase polymerase chain reaction); RhMK (Rhesus monkey kidney cells); MDCK (Madin-Darby Canine Kidney Cells); LLC-MK2 (rhesus monkey kidney cell line); R-HELA (cervical adenocarcinoma); HEp-2 (cervical adenocarcinoma); R-MIX (co-culture of mink lung and MDCK cell line).

Table 2. Performance Characteristics of Commercially Available Point of Care Test Approved in Canada

Name of Kit	Manufacturer	Percentage of Canadian Laboratories Using Kit (n=90)	Pub Med (English language/Human)*	Sensitivity (%)	Specificity (%)	References
DIRECTIGEN FLU A + B	Becton Dickinson and Company	56	15	22-96	93-100	15-17
BINAX NOW INFLUENZA A & B or NOW FLU A NOW FLU B TEST KIT	Binax Inc.	18	14	53-80	93 -100	18-20
XPECT FLU A/B	Remel	6	1	48	99	26
DIRECTIGEN FLU A TEST KIT	Becton Dickinson and Company	4	17	62-100	80-100	12-14
QUICKVUE INFLUENZA A+B TEST	Quidel Corporation	3	12	22-95	76-100	21,22,24
FLU OIA TEST KIT	Thermo Biostar Inc.	0	9	54-93	73-97	21-23
BD DIRECTIGEN EZ FLU A+B	Becton Dickinson and Company	0	2	39-69	94-99	10-11
IMMUNOCARD STAT FLU A & B	Meridian Bioscience Inc.	0	2	67-80	98-99	19,25
ACTIM INFLUENZA A & B TEST	Medix Biochemica OY AB	0	0	N/A	N/A	N/A
QUICK S INFLU A/B TEST	Innovatek Medical Inc.	0	0	N/A	N/A	N/A
CLEARVIEW FLU A/B TEST	Wampole Laboratories Inc.	0	0	N/A	N/A	N/A
INFLU A RESPI STRIP	Coris Bioconcept	0	0	N/A	N/A	N/A
INFLU A&B TEST KITS	Coris Bioconcept	0	0	N/A	N/A	N/A

* Indicates the number of articles published examining the performance characteristics of the POC of interest on human specimens in English as listed on PubMed using the search words "influenza" and the name of the specific POC of interest.

Note: Data from June 2006.

Source: Medical Devices Bureau, Health Canada.

2. Although the initial capital equipment expenditure is high:

- The platform can be used to test for numerous pathogens and can be quickly adapted to novel pathogens;
- Many RT-PCR assays can be manufactured "in house", thus reducing cost and assuring availability in a time of international crisis;
- The majority of these reagents can be stored for years, facilitating stockpiling.

3. The increasing use of NAT in routine clinical circumstances will help reduce wastage, as stockpiled supplies can be put into a rotating inventory system so they can be used for other diagnostic work prior to expiration.

Point of care (POC) testing

The currently available near-patient or POC tests use rapid antigen detection technologies that are relatively simple and can generate results in less than 30 minutes; however the accuracy of these tests is suboptimal when compared to RT-PCR.

A recent survey conducted by the Pandemic Influenza Laboratory Preparedness Network (PILPN), found that 72/90 (80%) of Canadian public health and hospital laboratories responding use POC tests in their diagnostic algorithms. Only 6 of 14 POC tests which are currently licensed in Canada were used (Table 2). The vast majority of surveyed labs use them as the primary method of diagnosis.

A PubMed database search for studies published in English and using human specimens revealed five of the approved kits in Canada do not have any published performance data or peer-reviewed comparative studies (Table 2). As outlined in Table 2, performance characteristics vary greatly for these assays.¹⁰⁻²⁶ Performance depends on the type of specimen tested, the timing of collection, age of the patient, and the skill with which the specimens are collected and the tests performed.^{14,16,23,27,28} Generally, studies reporting better performance are done by trained personnel using optimal specimens such as nasopharyngeal aspirates or swabs. In routine clinical practice, however, POC tests are often performed by per-

Table 3. Predictive Values Depend on the Prevalence of Influenza in the Community

	Off Season	Height of Influenza Season
Prevalence of Influenza	1,000/100,000 (1%)	25,000/100,000 (25%)
Individuals affected	1000	25,000
Individuals not affected	99,000	75,000
Kit sensitivity	80%	80%
Kit specificity	97%	97%
True positives	800	20,000
False positives	2970	2250
True negatives	96,030	72,750
False negatives	200	5000
Positive predictive value (PPV)	21.2%	89.9%
Negative predictive value (NPV)	99.8%	93.6%

sonnel who lack familiarity with test procedures and interpretation skills, using suboptimal specimens such as throat swabs.

For any diagnostic test, understanding performance characteristics in the context of disease prevalence is essential. Table 3 outlines how positive and negative predictive values are influenced by the prevalence of influenza in the community. For diagnosis of seasonal human influenza, the relatively high specificity of most POC tests allows the clinician to be fairly confident in the accuracy of a positive result from a patient presenting with influenza-like illness (ILI) during the influenza season. However, during periods of low prevalence (i.e., summer months), positive results need to be confirmed with more specific methods such as RT-PCR or culture.^{27,28} The primary limitation of currently available POC tests is poor sensitivity, which translates into an inability to rule out the diagnosis of influenza. This is important in the context of pandemic influenza planning when optimal sensitivity is required to detect the arrival of the pandemic strain in Canada.

The World Health Organization (WHO) does not recommend the use of rapid POC tests for the diagnosis of novel influenza viruses.²⁷ The U.S. Food and Drug Administration has also issued a cautionary notice for rapid tests because their performance has not been established for influenza A subtypes, other than A/H3N2 and A/H1N1.²⁸ Although some assays can identify a wide range of influenza A subtypes including avian influenza viruses,²⁹ they cannot differentiate between a novel influenza virus or seasonal human influenza. In addition, current POC tests have an analytical sensitivity approximately 1000-fold less sensitive than culture for both avian and human virus strains and only detect H5N1 viruses in high concentrations.^{30,31} This may partially explain their poor performance in diagnosing human H5N1 infections. Since 1997, only 8 (17%) of 48 patients with RT-PCR or culture-confirmed H5N1 infection could be diagnosed by POC tests.³²

In addition to the poor sensitivity, there are other features of POC tests which make them suboptimal for pandemic planning:

- Single use;
- Limited shelf-life (one to two years), leading to significant wastage and excessive cost if stockpiled for pandemic preparedness purposes;
- They must be procured as kits that are manufactured abroad, making them potentially unavailable during a time of international crisis.

The development of a POC test that has the performance characteristics of RT-PCR, that is easy to use and interpret, and has a turnaround time of less than 30 minutes would be the optimal influenza diagnostic test. This test would also have to be reason-

ably priced, have a prolonged shelf life, and ideally, be able to subtype influenza, or at the very least distinguish novel or potential pandemic isolates from seasonal human influenza. The Centers for Disease Control and Prevention is committing significant resources to develop new and improved POC technologies. Additional attention must be paid to development of new technologies and to optimizing current approaches. Moreover, such tests will need to be rapidly evaluated under field conditions in a pandemic setting, likely against a reference standard including molecular testing, to justify their use for clinical or public health decision-making.

CONCLUSION

Front-line clinicians need to be aware that the positive and negative predictive values of POC tests depend on the prevalence of influenza. Although there may be some utility in using POC tests during seasonal influenza, their sensitivity is poor and they are not recommended for the diagnosis of novel influenza strains. For public health officials and policy-makers, understanding the limitations of these POC tests and the comparative advantages of molecular platforms is essential when trying to decide how to most wisely invest the limited resources available for diagnostic testing that supports provincial and territorial pandemic preparedness plans. To develop and optimize rapid and clinically relevant POC tests for potential pandemic influenza strains, a significant research investment towards laboratory diagnostics is required. By investing in molecular technologies in preparation for the next pandemic, Canada will not only improve its diagnostic and surveillance capabilities for seasonal influenza, but will create the infrastructure to assess new diagnostic tests under field conditions and rise to the challenge of novel pathogens. Until improvements in POC tests are developed and realized, PILPN believes that the best option for pandemic influenza preparation is the enhancement of nucleic acid-based testing capabilities across Canada (Figure 1).

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RÉSUMÉ

Tandis que le monde se prépare à la prochaine pandémie de grippe, les gouvernements se sont engagés à financer substantiellement la mise au point de vaccins et la constitution de réserves d'antiviraux. Même s'il s'agit là de volets essentiels à une intervention en cas de pandémie, le diagnostic rapide et exact demeure une pierre angulaire, souvent négligée, de la préparation à la pandémie de grippe. Les cliniciens et les praticiens en santé publique doivent comprendre les avantages et les inconvénients des différents tests de dépistage tant en saison de grippe qu'en situation de pandémie. La mise en culture est depuis longtemps le test de référence servant au diagnostic de la grippe, mais il faut de 1 à 10 jours pour obtenir un résultat positif, comparativement aux méthodes de détection des acides nucléiques telles que l'amplification par la polymérase après transcription inverse (RT-PCR) en temps réel. Bien que les trousses courantes de détection des antigènes puissent donner un résultat en moins de 30 minutes, leur sensibilité est sous-optimale, et elles ne sont pas conseillées pour déceler les nouveaux virus de la grippe. Tant que les tests au point de service ne se seront pas améliorés au Canada, le RPLPI recommande un renforcement dans tout le pays, de la capacité de dépistage du virus à l'aide d'un test d'amplification des acides nucléiques comme étant le meilleur moyen de se préparer à une pandémie de grippe.

Mots clés : pandémie de grippe; grippe saisonnière; dépistage au point de service; test d'amplification des acides nucléiques