COMMENTARY

A Decentralized Molecular Diagnostic Testing Plan for Pandemic Influenza in the Ontario Public Health Laboratory System

Steven J. Drews, PhD, FCCM D(ABMM)[1-3]
Anna Majury, DVM, PhD[4,5]
Frances Jamieson, MD, FRCPC[1,3]
Garth Riley, BSc[1]
Tony Mazzulli, MD, FRCPC[1-3]
Donald E. Low, MD, FRCPC[1-3]

ABSTRACT

The Ontario Public Health Laboratories system (OPHL) is in the midst of a six-year plan to implement molecular tools for pandemic influenza diagnostics in one central and three regional public health laboratories. This plan has been formulated as a consequence of: 1) experiences gained through severe acute respiratory syndrome (SARS), and comments of the members of the Expert Panel on SARS and Infectious Disease Control (i.e., the Walker report); 2) a review of pandemic preparedness literature; 3) historical and epidemiologic discussions about previous pandemics; and 4) suggestions made by various pandemic working committees. The OPHL plan includes: 1) an aggressive restructuring of the overall molecular microbiology testing capacity of the OPHL; 2) the ability to shift influenza testing of samples between designated OPHL laboratories; and 3) the development of screening tools for pandemic influenza diagnostic tests. The authors believe that investing in increased molecular testing capacity for regional laboratories outside the greater Toronto area will be beneficial to the OPHL system whether or not an influenza pandemic occurs. Well-trained technologists and microbiologists, and the introduction of new technologies, will facilitate the development of a wide variety of molecular tests for other infectious diseases at public health laboratories geographically distant from Toronto, thus enhancing overall laboratory testing capacity in the province of Ontario.

Key words: Pandemic planning; influenza; molecular testing; RT-PCR; diagnosis

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

1. Ministry of Health and Long-Term Care, Public Health Laboratories Branch, Etobicoke, ON
2. Department of Microbiology, Mount Sinai Hospital, Toronto, ON
3. Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto
4. Ministry of Health and Long-Term Care, Kingston Public Health Laboratory, Kingston, ON
5. Department of Microbiology and Immunology, Queen's University, Kingston

Correspondence and reprint requests: Dr. Steven J. Drews, Ministry of Health and Long-Term Care, Public Health Laboratories Branch, 81 Resources Road, Etobicoke, ON M9P 3T1, Tel: 416-235-5703, E-mail: steven.drews@ontario.ca

Many Canadian public health laboratories are navigating a comprehensive series of preparations to enable these institutions to effectively detect and diagnose pandemic influenza. The laboratory component of these preparations in Ontario has been guided by the experience of severe acute respiratory syndrome (SARS),[1] comments of the members of the Expert Panel on SARS and Infectious Disease Control (i.e., the Walker report),[2] reviews of the published literature on pandemic planning, historic and epidemiologic data from previous pandemics,[1,3-7] and interactions with pandemic preparedness working groups (http://www.phac-aspc.gc.ca/cpip-pclcpi/ann-c_e.html).[8]

THE PLAN

Within the next five years, the OPHL will introduce automated molecular diagnostic tools for the diagnosis and characterization of pandemic influenza virus. Appropriate personnel will be trained to use these new tools in four designated OPHL testing facilities chosen based on multiple factors, including: geographic location, population size, and proximity to academic and clinical centres of excellence. Pandemic influenza screening tools will be created to triage specimen testing and to capture the maximum amount of clinical and epidemiologic data. The OPHL will increase pandemic surge capacity through the creation of redundancies in molecular testing capabilities across the province. The resulting system will function not only for the diagnosis of influenza, but also for other pathogens in settings such as sporadic cases, outbreaks, epidemics and pandemics. The OPHL is at the start of the second year of a six-year pandemic influenza plan, as described in Table I.

The following sections provide the rationale for the OPHL plan.

Why an automated molecular platform was chosen for pandemic influenza diagnostics

Molecular testing was chosen for pandemic influenza diagnostic testing for several reasons. Currently available rapid antigen detection kits have not been validated for avian or pandemic influenza and are not recommended by the World Health Organization for avian or pandemic

influenza diagnostics (http://www.who.int/csr/disease/avian_influenza/guidelines/labtestsMarch07web.pdf). Culture of all suspect pandemic isolates requires Containment Level (CL)-3 facilities which is not feasible in Ontario (few available approved facilities), whereas molecular methods can be performed in CL-2 facilities (http://www.searo.who.int/en/Section10/Section1027/Section1091_4305.htm). Molecular tools also allow for the characterization of difficult to culture strains (ProMed-Mail Archive # 20050509.1277) and the detection of drug-resistant viruses as they emerge.[9-13]

As of March 2007, OPHL verified a new automated real-time reverse transcriptase polymerase chain reaction (RT-PCR) for diagnosis of influenza A and B. This methodology allows for testing of large numbers of samples with a relatively short turnaround time when compared to culture-based diagnostic methods,[14,15] and these assays can be used with rapidity to help direct antiviral therapy in patients infected with influenza (McGeer, A et al., 47th Interscience Conference on Antimicrobial Agents and Chemotherapy, 2007, L-732).

Emphasis on the use of molecular testing was largely due to the experiences of Central Public Health Laboratory (CPHL) personnel during SARS. The number of respiratory swab samples received by CPHL in Toronto for viral culture and identification for the period between February and August of the years 2002, 2003 (SARS)[1] and 2004 inclusive were available for analysis (Table II). These data, and the results of an informal retrospective survey of employee opinion with respect to the concomitant increase in workload during that critical period, indicate that OPHL must develop a testing model and diagnostic capacity to deal with anticipated five- to tenfold surges in testing in the event of a pandemic. Other factors increasing the workload during a pandemic may include multiple sample submissions per patient,[16] and emergence of a pandemic influenza strain in a series of waves or at the same time as seasonal influenza.[17-20]

TABLE I

Yearly Objectives of Molecular Diagnostic Testing Plan for Pandemic Influenza

Year	Objectives
1 (near completion)	• four molecular diagnostics laboratories for the OPHL systems will have been established and technologists will be identified and trained • an automated extraction protocol and a real-time reverse transcriptase PCR (RT-PCR) assay will be introduced • a pandemic influenza pre-analytical screening tool will be created by the OPHL • one OPHL site will maintain molecular subtyping capability for influenza
2	• modifications to protocols will be made to increase surge capacity of real time RT-PCR protocols • automated RT-PCR assays for the determination of antiviral resistance will be introduced into four OPHL laboratories • commercial respiratory virus panels for the purpose of outbreak investigations will be introduced into one OPHL site for study purposes • logistic issues surrounding pandemic preparedness will be studied, and yearly pandemic preparedness exercises will be initiated • communications links will be strengthened with clients
3	• nucleic acid sequencing for antiviral resistance mutations and virulence determinants will be introduced into one OPHL site • logistics standard operating procedures (SOPs) will begin to be implemented • pandemic exercises will be expanded • functionality of commercial respiratory virus panels will be studied
4	• molecular subtyping capabilities will be transferred to the remaining three OPHL testing sites • pandemic exercises will be expanded • functionality of commercial respiratory panels will be studied • nucleic acid sequencing for antiviral resistance mutations and virulence determinants will be introduced into one OPHL site • logistics SOPs will be tested • yearly pandemic exercises will be expanded • functionality of commercial respiratory virus panels will be studied
5,6	• respiratory virus panels for outbreak investigations will have been introduced into all four OPHL testing sites • pandemic logistics SOPS will be functional • all four laboratories will have the capacity to identify and characterize influenza strains • pandemic exercises will be routine • communications links with clients will be established and resilient

Why pandemic influenza testing was decentralized to four regions

A decentralized testing plan will be effective because a pandemic strain may not affect all regions within a single jurisdiction at the same time.[5,21,22] To allow for increased testing capacity and capacity shifting between regions, four molecular diagnostics testing facilities have been created in the OPHL system from distinct regions in Ontario (i.e., Northwestern, Southwestern, Central and Eastern). A key goal is to decrease the transportation time from physicians in the regions to molecular testing facilities across the province. The number of sites was limited to four to prevent over-extension of resources in training of staff and validation of assays, and to limit the amount of work required to ensure competency for the Ontario Laboratory Accreditation (OLA) process. Other criteria for choosing the location of testing sites included accessibility to laboratory staff, proximity to ground and air transportation routes, ability of each individual laboratory to absorb molecular testing, and proximity to a centre of excellence (i.e., a University, College or other institution) for collaboration purposes and continued education of staff.

Quality assurance (QA) in a decentralized system is an achievable goal. Common documentation regarding protocols and policies is maintained by a centralized (Toronto) document information management system. Proficiency of laboratory staff is ensured by continuing education, site visits (audits), a centralized training program, examinations and enrolment into internal and external proficiency testing programs. Since each site is independently licensed, QA documentation must be performed at each site to meet licensing and OLA requirements. Decentralization does incur some additional costs. Redundancy requires additional human resources in multiple locations to verify and validate assays and maintain competency and to ensure clear communications with personnel at geographically distant locations. Travel and shipping costs are also incurred to ensure competency.

Why a pre-analytical pandemic screening tool was created and steps were taken to strengthen communications with clients

Data on leaking respiratory specimens received by CPHL for the years 2002 (Pre-SARS) and 2003 (SARS) indicate that the number of inappropriate samples (e.g., leak-

TABLE II

Mean Increases in Respiratory Samples Processed by CPHL Before, During and After SARS

Month	# Respiratory Samples			Mean Pre- and Post-SARS	Fold Increase in SARS vs. Mean
	2002 (Pre-SARS)	2003 (SARS)	2004 (Post-SARS)		
February	1696	1167	1082	1389	NI
March	1385	1564	1024	1204	1.5
April	930	2906	539	734	4.0
May	435	755	283	435	1.7
June	246	1620	276	276	5.8
July	190	505	159	190	2.7
August	286	432	172	286	1.5

NI - No increase; CPHL - Central Public Health Laboratory Toronto

TABLE III

Leaking Respiratory Specimens Received by CPHL as an Indicator of Inappropriate Pre-analytical Processes: Pre-SARS vs. SARS

Month	% Respiratory Samples Leaking		Fold Increase in SARS vs. Pre-SARS
	2002 (Pre-SARS)	2003 (SARS)	
February	3.1	5.0	1.6
March	3.5	7.2	2.1
April	5.5	12.4	2.3
May	3.2	6.3	2.0
June	2.8	16.9	6.0
July	4.2	13.2	3.1
August	7.3	6.5	NI

NI - No increase; CPHL - Central Public Health Laboratory Toronto

ing) sent to CPHL for respiratory virus identification during SARS was two to six times higher than during the pre-SARS period (Table III). Discussions with OPHL employees working during SARS uncovered the perception that inappropriate pre-analytical processes resulted in significant laboratory resources being wasted and may have hampered downstream analytical and post-analytical processes. To prevent this problem from occurring again, the OPHL has compiled guidelines for clients entitled "Pandemic/avian influenza in suspected cases- specimen collection and transportation guidelines", available as a "Labstract" from the following url: (http://www.health.gov.on.ca/english/providers/pub/labs/labstracts/panflu_avian_LAB-SD-015-000.pdf).[23] This communication informs clinicians, public health personnel and other stakeholders of the pre-analytical steps required for a sample to be tested for avian or pandemic influenza (by molecular diagnostics) during the current pandemic alert period. Specimens will only be accepted for pandemic influenza testing if this guideline is followed and all fields of the OPHL laboratory requisition plus additional requested data are supplied and complete.

The creation of specimen collection and transportation guidelines alone is not sufficient to strengthen communications between the laboratory and its clients. Instead, these tools act as a means of generating discussion with clients, which provide the laboratory with input on pre-analytical processes and allow the laboratory to educate the clients on the characteristics of molecular testing.

What if the influenza pandemic is a non-event?

Molecular technology is highly adaptable, can be effectively scaled-up, and easily transferred, implying that: 1) influenza panels can be utilized as diagnostic tools during the periods of seasonal influenza; 2) trained personnel can undertake molecular testing for other pathogens; and 3) other molecular diagnostic tools can be developed for use in the setting of disease outbreaks, or for a novel non-influenza pandemic or epidemic. The various resources invested now under the umbrella of pandemic planning can be justifiable in highly trained staff and an infrastructure is available for the benefit of patients regardless of whether or not an influenza pandemic arises in the future.[24] Decentralization of molecular testing allows for flexibility within a well-connected laboratory network, and ensures that molecular diagnostic testing continues, even during times of natural or human disasters.[25,26]

CONCLUSIONS

The expansion of molecular testing capacity to four sites has involved the investment of resources at several levels including: increased human resources funding, increased workspace allotted for molecular testing at all sites, and the restructuring of physical laboratory space at others, purchase of new equipment common to all sites and the development of standardized molecular testing protocols. The ability of the authors to undertake this plan would not have been possible without the support of government, as well as the administrative, financial, technical, scientific and clinical personnel who invested their fiscal resources and time into the development and implementation of the plan.

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RÉSUMÉ

Le système des laboratoires de santé publique de l'Ontario (LSPO) est à mi-parcours d'un plan de six ans visant à mettre en œuvre des outils moléculaires pour le diagnostic de la grippe pandémique dans un laboratoire central et trois laboratoires régionaux de dépistage sanitaire. Le plan en question a été formulé d'après : 1) les leçons de la crise du syndrome respiratoire aigu sévère (SRAS) et les commentaires des membres du Comité d'experts sur le SRAS et la lutte contre les maladies infectieuses (rapport Walker); 2) l'examen de la documentation sur la préparation à une pandémie; 3) les analyses historiques et épidémiologiques des pandémies antérieures; et 4) les suggestions de divers comités de travail sur les pandémies. Le plan des LSPO englobe : 1) une restructuration approfondie de l'ensemble des outils de dépistage basés sur la microbiologie moléculaire dans les laboratoires; 2) la possibilité de transférer d'un LSPO désigné à un autre l'analyse des échantillons grippaux; et 3) l'élaboration d'outils de sérodiagnostic de la grippe pandémique. Selon les auteurs, le fait d'investir davantage dans la capacité de dépistage moléculaire des laboratoires régionaux à l'extérieur du Grand Toronto serait bénéfique pour le système des LSPO, peu importe si une pandémie de grippe survient ou non. Des technologues et des microbiologistes bien formés, ainsi que l'implantation de nouvelles technologies, faciliteront l'élaboration d'un vaste éventail de tests moléculaires pour d'autres maladies infectieuses dans les laboratoires de dépistage sanitaire éloignés de Toronto, ce qui devrait améliorer globalement la capacité de dépistage en laboratoire en Ontario.

Mots clés : planification entourant une pandémie; grippe; tests moléculaires; RT-PCR; diagnostic