14. Laboratory Services

> Laboratories everywhere had turned to influenza. … In Germany, in Italy, even in revolution-torn Russia, desperate investigators searched for an answer. But by the fall of 1918 these laboratories could function only on a far-reduced scale. Research had been cut back and focused on war. … Laboratories in both Europe and the United States were affected, but Europeans suffered far more, with their work limited by shortages not only of people but of everything from coal to heat to money for petri dishes.
>
> The Great Influenza, J.M. Barry

In the event of an influenza pandemic, Ontario laboratories will play a significant role in the detection, surveillance and characterization of the influenza virus. Laboratory confirmation of pandemic influenza in a person, or within the population, in Ontario, or Canada, will trigger many of the planned pandemic responses, including the initiation of various public health measures and the use of anti-viral agents.

Laboratory testing takes place within three distinct, yet highly interrelated, health service settings: hospital laboratories, public health laboratories and community laboratories.

The National Microbiology Laboratory (NML), in Winnipeg, will play a key role in confirming the presence of the pandemic strain in Ontario and in ensuring timely exchange of laboratory surveillance and scientific information among all levels of government. This laboratory houses Canada's only Containment Level 4 laboratory.

The information provided in the Laboratory chapter of the 2006 OHPIP has been written in the form of "guidelines" only, in order to give the user some flexibility, acknowledging that laboratories must rely on professional expertise and experience once a pandemic arises. As of the writing of this text, the exact nature of the influenza agent responsible for the next pandemic is unknown. The guidelines have been developed based on currently available scientific knowledge, and experience with inter-pandemic influenza strains, and on information collected and derived from previous pandemics and currently circulating strains with pandemic potential.

14.1 Objectives

- To assist laboratories preparing for a pandemic through the provision of guidelines and planning tools.
- To identify the laboratory services required during a pandemic and those that can be curtailed.

14.2 General Guidelines for Pandemic Planning

Influenza is a zoonotic disease, and currently the largest natural public health threat to humans. All mammalian influenza viruses, including those that infect humans, are maintained in birds, the natural reservoir for influenza A viruses. Other animals, typically pigs, can also play a role in the emergence of new and potentially pandemic influenza A strain, by providing a vessel for viral reassortment.

Recently, human infection with avian influenza A/H5N1 viruses has heightened awareness and the potential for the emergence of a pandemic influenza strain. In response to this threat, Ontario laboratories are working towards increasing laboratory capacity for the diagnosis of influenza and the early detection of emerging pandemic strains. Although much attention and investigation has focused on influenza A/H5N1, these guidelines are also applicable to other

future pandemic threats, such as those possibly posed by H9 or the highly pathogenic H7, or any other influenza type which may arise with pandemic potential.

These guidelines address pandemic influenza virus threats, in general, and not human infection with avian influenza A/H5N1, or other avian influenza types. (See Chapter 14A: Laboratory Tools - Laboratory Information—Avian Influenza—Interim Guidelines). For additional information on avian influenza infection in humans, please see the Laboratory Annex, Canadian Pandemic Influenza Plan at http://www.phac-aspc.gc.ca/cpip-pclcpi/.

Table 14.1: Overview of Pandemic Responsibilities by Laboratory Type

WHO Pandemic/ Phase	All Laboratories	Provincial Public Health Laboratories	National Laboratory
Interpandemic Period: **Phase 1** No new influenza virus subtypes have been detected in humans. **Phase 2** A circulating animal influenza virus subtype poses a substantial risk of human disease.	Develop a laboratory preparedness plan to support the response to an influenza pandemic, which addresses laboratory services, operational requirements and human health resources. Assess and address laboratory surge capacity, including human resources, infrastructure, testing, supplies, equipment and other. Educate and train personnel for pandemic influenza response, including updates on scientific information as it becomes available, bio-safety guidelines and management of respiratory specimens during an influenza pandemic. Develop suspended testing guidelines and testing algorithms, which include those services considered *essential* during a pandemic, in order to address anticipated human resource and laboratory supply shortages in the event of a pandemic, as well as a surge in testing requests for specimens submitted because of respiratory illness. Maintain essential routine laboratory diagnostic services and surveillance for influenza. Initiate training and cross-training of laboratory personnel in order to ensure a rapid, expert laboratory response, even in the face of decreased numbers of personnel. Develop and practice emergency response protocols. Survey all laboratory employees for annual influenza vaccine uptake and encourage vaccination of all personnel. Assess the need for anti-viral stockpiling for chemoprophylaxis on site.	As per All Laboratories, plus the following: Laboratories designated as influenza testing sites should implement molecular diagnostic methods for influenza virus, and training, if not already in place. Ensure laboratory participation in proficiency testing programs for molecular based and other testing. Participate in laboratory-based surveillance testing programs, and ensure adequate and representative specimens are forwarded to the National Microbiology Laboratory (NML) as per their direction. Engage and maintain research in support of efficient and rapid diagnosis and study of influenza viruses. Disseminate precise and accurate communications to clients, stakeholders, employees and other laboratories. Adopt testing and reporting and surveillance protocols from the NML and PHAC for influenza diagnosis, etc. as available.	Monitor preparedness and laboratory capacity for seasonal influenza. Continue to provide and/or develop anti-viral susceptibility testing methods. Transfer sub-typing and susceptibility testing expertise/methodology to designated Public Health Laboratories as appropriate. Develop, evaluate and provide reagents such as RNA controls and monoclonal antibodies to Public Health Laboratories as appropriate. Provide on-going direction regarding laboratory based surveillance programs. Disseminate precise and accurate communications to Provincial and Territorial (P/T) laboratory partners.

WHO Pandemic/ Phase	All Laboratories	Provincial Public Health Laboratories	National Laboratory
Pandemic Alert Period: **Phase 3** Human infection(s) with a new subtype, but no human-to-human spread or spread to a close contact only. **Phase 4** Small cluster(s) with limited human-to-human transmission but spread is highly localized, suggesting that the virus is not well adapted to humans. **Phase 5** Larger cluster(s) but human-to-human spread still localized, suggesting that the virus is becoming increasingly better adapted to humans, but may not yet be fully transmissible.	As per interpandemic period, if not already implemented, plus the following: Review operational and other plans to manage increased number of requests for influenza testing. Ensure clearly labeled specimens from patients with suspected novel influenza are sent to the National Microbiology Laboratory or P/T Public Health Laboratories. Survey all laboratory personnel for annual vaccine uptake. Consider institution of active surveillance for influenza-like illnesses (ILI) among laboratory personnel. Ensure guidelines for specimen type, collection, transportation and testing, as provided by National and Provincial Pandemic guidelines, are implemented. Review priorities for testing and initiate plan to suspend testing for non-essential laboratory services. Enact pandemic plans as needed.	As per interpandemic period, plus the following: Under National direction, enhance laboratory-based surveillance of influenza virus subtypes. Ensure clearly labeled and appropriate specimens from patients with suspected novel influenza are sent to the National Microbiology Laboratory (NML) or other P/T Public Health Laboratories. Ensure increased capacity for molecular diagnosis of influenza. Rapidly communicate information regarding identification of novel influenza sub-types to NML and submit to NML for confirmation. Inform Medical Director immediately if novel influenza sub-type is identified. Culture of novel subtypes of influenza viruses should be carried out in CL-3 facility. Ensure laboratory protocols are communicated as they evolve to all provincial and other influenza testing laboratories. Monitor anti-viral resistance.	As per interpandemic period plus the following: Provide technical support to the Public Health Laboratories and Ministries of Health and Agriculture, as requested, by providing confirmatory testing and analyzing novel influenza virus subtypes-including avian isolates and human isolates with pandemic potential, including sub-typing, RNA sequencing, and drug sensitivity testing. Work with Provincial Public Health Laboratories to ensure that the diagnostic reagents required for the identification of "pandemic alert" strains are available and are used safely and effectively. Provide guidance on containment and safe handling of respiratory specimens obtained from potential cases of pandemic influenza. Work with provincial laboratory partners to ensure appropriate surveillance guidelines are in place and enacted. Regularly and routinely engage in pandemic drills.
Pandemic Period: Phase 6 Increased and sustained transmission in general population.	All of the above.	Enact operational and other plans to manage increased number of requests for influenza testing. Submit select specimens from possible pandemic influenza patients to Provincial Public Health Laboratories designated as influenza testing sites or to National Microbiology Laboratory, as appropriate. Distribute updated guidelines on all aspects of specimen management and diagnostic testing to healthcare providers and hospital and community laboratories, as appropriate. Work with National partners to monitor the pandemic virus and conduct special studies as required, or to address other aspects of the response. Ensure communication with employees, clients and stakeholders is clear, precise and	Work with provincial and global partners to characterize new pandemic viruses including sub-typing, RNA sequencing, and anti-viral sensitivity, and to monitor changes over time. Work with provincial public health laboratories to ensure the availability and the safe and effective use of diagnostic tests and reagents. Conduct reference/confirmatory testing for positive samples, and perform viral culture. Culture will be particularly important at the beginning of the pandemic wave (to confirm entry of the pandemic strain in Canada and to obtain baseline information on viral characteristics) and again, as the wave subsides, in order to detect the emergence or re-emergence of other respiratory viruses in the population. Take lead for research and

WHO Pandemic/ Phase	All Laboratories	Provincial Public Health Laboratories	National Laboratory
		up to date.	investigation of pandemic influenza virus, including development of improved diagnostic methods, sequencing, anti-viral sensitivity testing, immune response, etc. Update surveillance guidelines as appropriate. Provide accurate scientific information and updates to guidelines as available.
Postpandemic Period: Return to Phase 1	Evaluate pandemic response. Revise pandemic response and protocols, as appropriate. Conduct special post pandemic viral studies, as appropriate.		

For a list of pandemic planning activities by laboratory sector in Ontario (i.e., hospital, community and public health) see Chapter 14A: Laboratory Tools.

14.3 Guidelines for Hospital Laboratories

It is difficult to predict the impact of an influenza pandemic on hospital laboratory services. Given that there are significant differences in the breadth and depth of laboratory services provided in an academic or teaching hospital and those provided in a community hospital, it is not practical to identify lists of tests that will or will not be performed during a pandemic.

The primary role of hospital laboratories is to support the acute care provided in their facilities. The types of tests that may be reduced or curtailed will depend on the care needs of each facility's patients.

Hospital laboratories should maintain all services required to safely and optimally manage all hospitalized patients within their facility. In the event of a pandemic, it is likely that only the most acutely ill patients will be admitted and cared for in the hospital setting and elective procedures will be postponed. While acutely ill patients may require significant laboratory testing, the decrease in elective procedures and admissions should decrease the need for laboratory testing.

In preparation for a possible pandemic, hospital laboratories should review and develop appropriate plans. See Chapter 14A: Laboratory Tools - The Laboratories Pandemic Planning Self Assessment Tool. Planning should address, but not be limited to:

- human resources
- specimen collection, processing and testing
- equipment and supplies
- biosafety and biocontainment
- contingency and collaborative plans
- communication and education of employees and clients
- transportation requirements.

14.4 Guidelines for Community Laboratories

Ontario's community laboratories have identified a suggested list of tests that would be required to support the provision of basic health

care to the population as a whole and to those affected by influenza (see Table 14.2). The list of suggested tests varies depending on the severity of the pandemic. During a pandemic with a low attack rate, most routine tests would continue to be conducted. During a pandemic with a moderate or severe attack rate, some routine testing could be reduced or temporarily suspended to increase capacity for other testing.

Table 14.2: Community Laboratory Activity by Severity of the Pandemic

Low (15%) Attack Rates	**Moderate (25%) Attack Rates**	**Extremely Severe Attack Rates**
Continue most testing; suspend or reduce routine screening tests to free up laboratory capacity or address resource shortages	Maintain capacity to provide essential tests and, depending on capacity and resource availability	Maintain capacity to provide tests required to support basic medical care for population
Continue most testing The following routine and screening tests could be **reduced or temporarily suspended** if necessary**:** • HDL cholesterol • TSH • Cervicovaginal specimen – Pap testing • Vitamin B12 • Ferritin • Cholesterol Total • Triglycerides • Folate • Target Drugs of Abuse • Estradiol • Drugs of Abuse • Drug Screen	Provide all required tests listed for severe attack rate **PLUS**: • anti-HAV IgM • HBsAg • Stool Culture • Cervical Culture • Throat Culture • Glycosylated Haemoglobin • Sputum Culture	Provide the following tests required for basic medical care: • CBC • INR • Sodium • Potassium • Calcium • Chloride • ALT (SGPT) • Glucose • Urinalysis (dipstick only) • Creatinine • Blood Culture • Wounds • Bilirubin (for neonatal assessment only) • HBsAg (for needlestick follow-up only) • Anti-HCV (for needlestick follow-up only) • TSH to diagnose hyperthyroidism (if required) • Tests required to monitor patients on therapeutic drugs (e.g., anti-epileptics) • Selected histology specimens (e.g., suspected melanomas)

Note: Table 14.2 defines the severity of the pandemic by attack rates (i.e., 15%, 25%, 35%), but this is not the only factor that will determine severity. A low (15%) attack rate of a highly virulent influenza strain that causes more secondary complications and high mortality rates would create the same kind of "severe" demands on laboratory services as a less virulent strain with a higher attack rate. The laboratory system's response will be driven by the demands for testing created by the pandemic. The intent is to maintain the services required to respond to the population's medical needs.

14.5 Guidelines for Public Health Laboratories

The province's public health laboratories conduct virology and other microbiology related testing to:

- support surveillance for, and detection of, emerging and reportable infectious diseases
- aid in the care and treatment of patients
- track disease emergence and spread within Ontario.

These laboratories also serve as reference testing centres in support of hospital and community laboratories. During a pandemic, laboratory resources may have to be redirected in order to

meet the demand for influenza related diagnostics and care. The capacity of all laboratories to maintain current levels of service during a pandemic will depend in part on the availability of laboratory and human health resources at the time. In anticipation of limited resources, the following suspended testing guidelines have been developed for the Ontario Public Health Laboratories.

Table 14.3: Recommended Public Health Laboratory Activities by Severity of the Pandemic

Note: This table is a guide only. Generally, those tests not listed will not be offered. Additional testing will be performed as requested by the Medical Officer of Health or as deemed necessary by infectious disease specialists and microbiologists.

TEST	Low (15%) Attack Rate Most testing continues to be offered except screening tests in low risk populations	Moderate (25%) Attack Rate A number of tests continue to be offered, as human and other resources allow	Severe (35%) Attack Rate Testing performed primarily for diagnostic purposes
Influenza Testing (Molecular/Other)	Yes	Yes	Yes
Mycobacterium tuberculosis	Yes	Yes	Yes
CSF, Joint, Pleural, other sterile fluids, etc. culture.	Yes	Yes	Yes
HIV (including viral load)	Yes	Serology only	Serology only
Direct Testing RSV and Rotavirus	Yes	Respiratory only	Respiratory only
ELISA for C. difficile Toxin	Yes	Yes	No
Virus Culture	Yes	Yes	Yes
NAAT for C. trachomatis and N. gonorrhea	Yes	Yes	Yes
Hepatitis (Diagnostic Only)	Yes	Yes	Yes
Malaria, Babesia, Critical Specimens (e.g. CSF)	Yes	Yes	Yes
B. pertussis	Yes	Yes	Outbreaks only
Throat Culture	Yes	Yes	No
West Nile Virus/Arboviruses	Yes	Yes	No
Diagnostic Serology	Yes	Yes	Yes
Prenatal Screening	Yes	Yes	High risk populations only
Syphilis	Yes	Yes	Yes
Specimens related to the investigation of Bioterrorism	Yes	Yes	Yes
Sputum Culture and Smear	Yes	No	No
Stool Culture	Yes	Yes	Outbreaks only
Mycology Culture for invasive and normally sterile sites	Yes	Yes	Yes

Antibiotic Sensitivity	Yes	Critical specimens ONLY	Critical specimens ONLY
Chlamydia Culture	Yes	Sexual assaults only	Sexual assaults only
Water Testing, on order of Medical Officer of Health	Yes	Yes	Yes

Note: Table 14.3 defines the severity of the pandemic by attack rates (i.e., 15%, 25%, 35%), but this is not the only factor that will determine severity. A low (15%) attack rate of a highly virulent influenza strain that causes more secondary complications and high mortality rates would create the same kind of "severe" demands on laboratory services as a less virulent strain with a higher attack rate. It is to be presumed that those tests not listed, will not be offered, unless approved by the Laboratory Director, or the Medical Microbiologist(s).

14.6 Influenza Testing in the Event of a Pandemic

Given the large number of people who will be affected by a pandemic and the potential severity of illness, there will be a significant increase in the volume of testing requested to diagnose influenza related illness. At the same time, there will be a decrease in the available laboratory workforce, due, for example, to personal illness or illness within families.

As entire laboratories may be rendered non-operational when the influenza pandemic hits their region or city, a minimum of four public health laboratories in Ontario should have the capacity for complete and rapid influenza diagnosis and typing using molecular methods. Selection of these four or more sites should be based upon several considerations and not upon geographic location alone. For example: 1. Does the site have additional available local expertise, (e.g. University, Academic Health Science Centre, College)? 2. Is the site relatively accessible by ground and air, regardless of season (e.g. sites located very far north, may be difficult to reach, particularly in winter months)? 3. Does the site have adequate numbers of personnel to train and cross-train (e.g. given the anticipated absenteeism rates)? 4. Do potential human resources exist locally that could fulfill the need for additional personnel, (e.g. Medical Laboratory Technology students, University students, Medical or Nursing students). 5. Does the laboratory serve a relatively large proportion of the population that is not otherwise served, or could be served, by federal institutions or other local (e.g. hospital virology laboratories) institutions? 6. Is the site physically remote (e.g. greater than 200 km) from other designated testing sites? Sites in close physical proximity may suffer the same human resources, transportation, supplies, etc., issues, should pandemic influenza strike their region, given that they share the same geographic area.

Further, it is advisable to support a site dedicated to the molecular characterization of virus, including sequencing, anti-viral sensitivity testing, and research and development. Although culture for diagnosis of influenza will not be routinely offered during a pandemic, this particular facility will require a certified CL-3 laboratory in order to propagate virus for study. Given that manipulations involving growth of the novel agent, this should not be performed in the same laboratory that is simultaneously culturing material that may contain interpandemic, seasonal human influenza. Culture should be limited to virus in specimens already identified, for example by other Public Health Laboratories, as being of a novel or pandemic strain. Culture in these instances would be for the purpose of further characterization (e.g. antiviral sensitivity testing) and study of the virus. Moreover, the National

Pandemic Influenza Laboratory Preparedness Network (PILPN) has identified the need for additional laboratories across Canada, with pandemic influenza expertise, to support National Laboratory partners during a potentially overwhelming pandemic period.

All other public health laboratories, as well as hospital laboratories, should have, at minimum, the capacity to screen specimens by molecular methods for the pandemic strain (e.g. molecular detection of the pandemic strain hemagglutinin). These laboratories should receive training in RT-PCR protocols for the molecular detection of H1, H3, H5, H7 and possibly H9 sub-types. The use of standardized protocols, as developed and distributed by NML, is preferred, and laboratories should participate in molecular proficiency testing programs and incorporate RT-PCR or real time RT-PCR into standard influenza laboratory activities. All specimens which test positive for pandemic or novel influenza strains would then be forwarded to the previously identified full service public health laboratory sites for further testing and molecular sub-typing. In addition, specimens, particularly early in the pandemic period, will be forwarded to the National Microbiology Laboratory, or designate, for confirmation, as well as for the purpose of additional viral studies.

Description of Laboratory Tests for Influenza Virus

Laboratories in the province of Ontario, within the public health and hospital sectors, routinely offer a variety of tests for the detection of interpandemic influenza strains including: rapid antigen testing, nucleic acid based amplification, virus isolation, immunofluorescence testing and serology. To support the rapid diagnosis of influenza A virus infection, laboratory test results should be available within 24 hours.

Nucleic Acid Amplification Testing

The Ontario Public Health Laboratories will offer reverse transcriptase polymerase chain reaction (RT-PCR) as the test of choice for the screening and sub-typing of influenza viruses. Several hospitals are also able to offer RT-PCR for influenza diagnosis. In the event of a pandemic, RT-PCR will be the mainstay of laboratory based influenza diagnosis. Updated protocols will be made available through the National Microbiology Laboratory.

Immunofluorescent Assays

Direct (DFA) or indirect (IFA) immunofluorescence antibody assays using standard reagents may provide a potentially rapid laboratory diagnosis of influenza in regions where RT-PCR is not available.

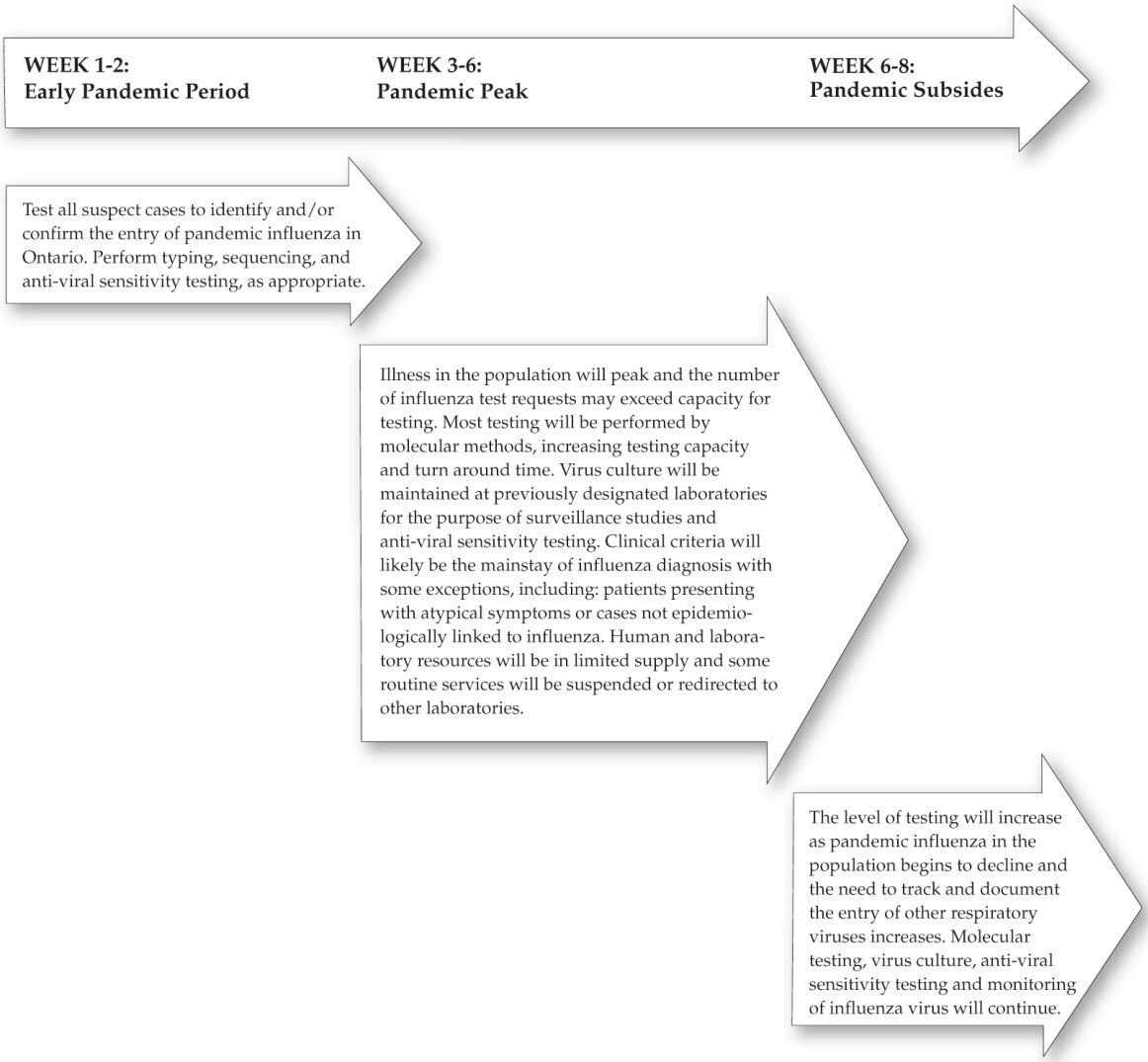
Virus Isolation

Growing virus in cell culture is the current gold standard for influenza diagnostics. With the exception of initial inoculation of tube cultures with primary specimens, viral culture including manipulation and characterization of viral agent recovered from cultures should be conducted in a Containment Level (CL)-3 facility using CL-3 operational practices. Material recovered from all cell cultures may be removed from the CL-3 facility for further analysis once viable virus has been inactivated. During a pandemic, virus isolation together with antigenic and genetic analysis will be used to characterize and monitor the pandemic influenza virus.

Culture using Madin Darby Canine Kidney (MDCK) or Primary Monkey Kidney (PMK) cell lines using standard protocols will likely detect potential new pandemic strains.

Conventional tube culture may take 4 to 7 days. Rapid shell vial methods may reduce this to 1 to 3 days. The ability of currently used or commercially available monoclonal antibodies to detect a pandemic strain will need to be determined. The most reliable means to identify isolates may be achieved by RT-PCR.

Figure 14.1: Influenza Testing During the First Wave of the Pandemic



Typing and Sub-typing

The Ontario Public Health Laboratories will use rapid typing and sub-typing by molecular methods to detect new pandemic strains. All specimens identified as being of the pandemic strain will be forwarded to the National Microbiology Laboratory, or designate, for confirmation.

Rapid Testing

If used and interpreted appropriately, rapid EIA (enzyme immunofluorescent assay) based, commercially available diagnostic tests for influenza can aid in: the diagnosis and management of patients

who present with signs and symptoms of influenza-like illness; and the management of potential outbreaks.

Some rapid tests identify both influenza A and influenza B, and can distinguish between them; others will identify both influenza A and influenza B but do not distinguish between the two. Sensitivity and specificity may vary for rapid tests depending on specimen type. Specimens should be collected as close to the onset of symptoms as possible, and not later than 4 to 5 days after symptom onset. Children often shed virus longer and have higher viral titres, which leads to higher test sensitivity in this population.

Median sensitivities of rapid diagnostic tests are generally 70 to 75% when compared to viral culture, and specificities are in the range of 90 to 95%. False-positives are more likely to occur when prevalence is low (i.e., at the beginning and end of the influenza season). False-negatives are more likely to occur when disease prevalence is high (i.e., typically during the peak of influenza season).

Commercial rapid antigen tests are not currently recommended for the diagnosis of influenza due to a pandemic influenza strain.

Serology

The clinical utility of serological tests in the event of a pandemic will be limited. Tests that specifically detect antibodies to the pandemic strain will be required. Traditional haemagglutinin inhibition (HAI) provides type specific diagnosis by a single high titre or a rise in antibody between acute and convalescent specimens.

Laboratory Case Definition

A laboratory proven case will be any case that is identified as positive for the pandemic strain of influenza by more than one method and/or in which reactivity is confirmed by a second laboratory. Initial and early cases identified as pandemic influenza should be forwarded to the National Microbiology Laboratory, or designate, for confirmation.

14.7 Specimen Collection and Handling Guidelines

General

The ability to detect virus is directly related to the quality of the specimen collected and its rapid transportation to the laboratory and appropriate storage prior to testing. Specimens containing large numbers of virus-infected cells are preferred. Acute and convalescent serum samples may be recommended in some circumstances.

The following information should be recorded on the specimen requisition:

- patient name
- date of birth
- date of collection
- specimen type
- name and contact information of submitter
- symptoms (including date of onset)
- travel history
- contact with other persons with laboratory-confirmed influenza
- health card number
- outbreak number (if applicable).

Ensure that the specimen container is appropriately labeled with the patient identifier and that this identifier matches the one on the requisition.

Those persons collecting specimens should see Chapter 7: Infection Prevention

and Control and Occupational Health and Safety for information on infection control precautions.

Collection Times

Based on information available for seasonal, interpandemic strains of influenza virus, specimens should be collected as close to the onset of symptoms as possible, preferably within the first 3 days, as viral shedding will be highest at this time and may drop to undetectable levels by 5 days. Children and immune-compromised patients typically shed virus for longer periods, and it may be that specimens can be effectively collected up to 7 to 10 days following the onset of symptoms in these populations. If serology is indicated, an acute phase serum should be collected soon after symptom onset, and not later than 7 days after onset. A convalescent serum specimen can be collected 10 to 14 days (or as late as 21 days) after collection of the acute serum specimen.

Specimen Type

The choice of specimen may vary with the type of test being performed -- particularly with direct antigen testing. As a general guideline, the usual recommended specimen type for detecting interpandemic strains of influenza is the nasopharyngeal swab (NPS). Other acceptable specimens include: nasaopharyngeal aspirate, nasal swab, throat swab, sputum, bronchoscopically-obtained specimens (e.g. Bronchoalveolar Lavage, BAL), pleural fluid, and lung biopsy and lung tissues. Swabs and transport media intended for bacteriological testing are *not* suitable for influenza testing.

Although the pandemic influenza strain is not yet known, avian influenza A/H5N1 virus is considered a potential pandemic threat. NPS is currently the recommended specimen type for human influenza testing; however, recent data suggests that the recovery of the avian influenza A/H5N1 virus infecting humans may be improved with the collection of throat swabs and lower respiratory tract specimens. At this time, the optimal specimen type and the correct timing of specimen collection are unknown for either pandemic or avian influenza infections in humans. As additional information becomes available, and these viruses continue to evolve, more definitive recommendations can be made. The Canadian Pandemic Influenza Plan currently recommends collecting different types of respiratory specimens, including NPS, NP aspirate nasal washings, throat swabs and sputa on multiple different days for the detection of avian influenza virus in humans. There have also been cases of avian influenza A/H5N1 detected from the stool and blood of infected patients so the collection of stool (especially in those who have significant gastrointestinal symptoms) and blood should be considered. For patients with central nervous system (CNS) symptoms, a cerebrospinal fluid (CSF) specimen may be warranted.

Preferred specimens

Nasopharyngeal Swab

Gently tilt the patient's head back to about 70^0 from vertical. Bend the wire swab, while in the sterile package, to give it a slight arc. Insert the thin, flexible swab into the nostril and back into the nasopharynx (i.e., the point approximately midway between the tip of the nose and the earlobe), and leave in place for a few seconds. Then slowly withdraw using a gentle, rotating motion. Use a separate swab for the second nostril. Place the swab(s) in the transport medium and break (or cut) the shaft so it can be

contained within the appropriate transport tube with the cap securely closed.

Nasopharyngeal Aspirate
Nasopharyngeal secretions are aspirated through a catheter connected to a mucus trap and fitted with a vacuum source. Attach suction catheter to the mucus trap and suction apparatus. Measure the distance from the patient's nostril to the nasopharynx (half the distance from the nostril to the base of the ear). Hold the tubing at that location, and when inserting the tubing into nasopharynx, do not advance the tube beyond that point. Start suction and pass the tube along the base of one nostril into the nasopharynx. Apply suction to obtain secretions from the nasopharynx. Sample both nostrils with the same suction catheter, without moving fingers from the measured position on the tubing. Rinse the suction catheter by aspirating the viral transport medium (VTM) through the catheter.

Other specimens

Throat Swab
Swab both tonsils and the posterior pharynx. Place the swab in the appropriate transport medium and break (or cut) the shaft so it can be contained within the appropriate transport tube with the cap securely closed.

Nasal Wash
Instruct the patient to sit comfortably with head slightly tilted back. The patient should be instructed to say "K" during the procedure to keep the pharynx "closed". Apply 1 to 1.5 ml of washing fluid (sterile physiological saline) into the nostril. Instruct patient to lean head forward and collect fluid into specimen container. Repeat with alternate nostrils until 10 to 15 ml of specimen is collected. Dilute wash 1:2 in the appropriate transport medium.

Nasal Swab
Insert a dry swab into the nostril, parallel to the palate, as far as the anterior end of the nasal turbinate, and leave in place for a few seconds. Then slowly withdraw using a gentle, rotating motion. Specimens can be obtained from each nostril using a single swab. Place the swab tip in the appropriate transport medium and break (or cut) the swab shaft so it can be contained within the appropriate transport tube with the cap securely closed.

Serum
Collect blood as per the usual protocol for serum samples (3 to 5 ml of whole blood in a serum tube).

Specimen Handling
Specimens should be collected and transported in the appropriate viral transport medium and shipped to the laboratory immediately following collection (i.e., on ice, if possible). Avoid transportation of specimens using a pneumatic tube. Specimens for the direct detection of viral antigens can be refrigerated prior to processing. If specimens for virus isolation must be stored before shipping, they should be refrigerated immediately. If specimens cannot be processed within 48 to 72 hours, they should be frozen at -70°C.

Packaging, shipping and transport of specimens must comply with the requirements of the Transportation of Dangerous Goods Regulations, Transport Canada, (http://www.tc.gc.ca/tdg/menu.htm) and the Dangerous Goods Regulations, International Air Transport Association (http://www.iataonline.com).

14.8 Biocontainment and Biosafety Guidelines for Laboratories Processing Pandemic Influenza

General Guidelines

Risk assessment governs the level of biosafety and containment required when handling an infectious organism. Performing an adequate risk assessment is difficult in the case of preparing for a pandemic as there are several unknowns. In cases where there is incomplete information, it is prudent to take a conservative approach to specimen manipulation. Thus, the level of biosafety required for the handling and processing of specimens will be assessed and updated as required by the Office of Laboratory Security, Centre for Emergency Preparedness and Response (CEPR), in consultation with the CPHLN (Canadian Public Health Laboratory Network), NML (National Microbiology Laboratory), CDC (Center for Disease Control), and WHO (World Health Organization).

Factors which need be considered include:

- pathogenicity of the agent and the infectious dose
- consideration of the outcome of exposure
- natural route of infection
- other routes of exposure possibly resulting from laboratory manipulations (aerosol, ingestion)
- stability of the agent in the environment
- concentration of the agent
- the presence of a suitable host
- laboratory activity planned (e.g. RT-PCR versus culture)
- availability of effective prophylaxis or therapeutic intervention.

Laboratory directors are responsible for ensuring that safe and comprehensive policies are in place. It is incumbent upon all laboratory employees to follow and abide by these policies as it is in the best interest of themselves, their colleagues and their communities.

In spite of stringent and safe laboratory practices, the laboratorian must always be alert to the possibility of a laboratory accident. Efforts to minimize accidents and transmission of infection in humans are of the highest priority during an influenza pandemic.

Infection Control Practices in the Laboratory

Available engineering and administrative controls must be assessed and appropriate measures must be in place before handling potentially infectious specimens.

To minimize contact, droplet and air-borne or aerosol transmission of influenza virus when handling patient specimens, laboratorians should adhere to standard or enhanced precautions (when appropriate).

Standard Precautions

- hand hygiene
- PPE when handling blood, body substances, excretions or secretions
- avoid use of sharps
- environmental hygiene
- appropriate waste management.

Personal Protective Equipment

- masks
- disposable gloves
- protective eyewear
- long sleeved, cuffed gown.

PPE should be worn by all laboratory workers handling specimens from a patient being investigated for pandemic influenza; and they should be removed prior to leaving the designated laboratory area.

Droplet and air-borne precautions should be employed for procedures such as growing virus in culture, manipulating cultures or tissues, or manipulating patient specimens (e.g. lung tissue) which may result in the generation of aerosols containing virus. Depending on the available engineering controls, precautions may include the use of high efficiency masks and negative pressure rooms if available.

Decontamination and Disinfection

Influenza virus is inactivated by alcohol and by chlorine. Environmental surfaces should be cleaned with a neutral detergent followed by a disinfectant solution.

All contaminated liquid and solid wastes must be decontaminated prior to disposal, preferably by autoclaving.

Direct Hazards

Influenza virus can survive for at least a short time (hours) on surfaces or in the laboratory environment. This provides a potential direct means of infection for laboratory workers.

Indirect Hazards

An indirect hazard may exist through secondary reassortment with a human or animal influenza virus as influenza viruses are known to exchange genes by the process of reassortment. For secondary reassortants to be generated, several events need to occur including: infection of the laboratory worker with wild type virus not of the pandemic strain, a concurrent laboratory acquired infection with the pandemic or novel strain, and a reassortment event between the two strains.

To reduce the chance of infection with wild-type virus, laboratory workers should be vaccinated with current seasonal influenza vaccine.

Laboratory employees with a possible work exposure may be asked to refrain from returning to work until the end of the incubation period, providing they remain symptom free.

Depending on epidemiological and agricultural circumstances, laboratory personnel potentially exposed to a pandemic or novel strain of virus should also avoid visiting some mammalian (e.g., equine and porcine) and avian facilities until a minimum of 14 days after the possible occupational exposure. It is of note that H5 and H7 strains are reportable to the Office International des Epizooties/World Organization for Animal Health (OIE, **http://www.oie.int/eng/oie/en_oie.htm**). Such an event would have enormous impact on animal and human health and on the economy and the agricultural industry.

Containment Levels (CL)

The Public Health Agency of Canada currently uses a classification system based on containment level, which indicates the containment required for handling an organism safely in a laboratory setting. The containment level required for work with a particular agent is based on the manipulations generally associated with laboratory-scale research and clinical procedures. Information in this document is based on information currently available. Updated information is available from the Office of Laboratory Security, Public Health Agency of Canada at (phone) 613-957-1779, or (fax) 613-941-

0596 or **http://www.phac-aspc.gc.ca/ols-bsl/index.html**.

Containment Level 1 (CL-1)

This level applies to the basic laboratory handling of agents requiring containment level 1. CL-1 requires no special design features beyond those suitable for a well-designed and functional laboratory. Biological safety cabinets are not required. Work may be done on an open bench top, and containment is achieved through the use of practices normally employed in a basic microbiology laboratory.

Containment Level 2 (CL-2)

This level applies to the laboratory handling of agents requiring containment level 2. The primary exposure hazards associated with organisms requiring CL-2 are through the ingestion, inoculation, and mucous membrane route. Agents requiring CL-2 facilities are not generally transmitted by the airborne route, but care must be taken to avoid the generation of aerosols (aerosols can settle on bench tops and become an ingestion hazard by contamination of the hands) or splashes. Primary containment devices such as biological safety cabinets and centrifuges with sealed rotors or safety cups are to be used, as well as personal protective equipment (gloves, laboratory coats, protective eyewear). Environmental contamination must also be minimized by the use of hand washing sinks and decontamination facilities (autoclaves).

Containment Level 3 (CL-3)

This level applies to diagnostic, research and clinical laboratories, production facilities, or teaching laboratories handling agents requiring containment level 3. These agents may be transmitted by the airborne route, often have a low infectious dose and can cause serious or life-threatening disease. CL-3 emphasizes additional primary and secondary barriers to minimize the release of infectious organisms into the immediate laboratory and the environment. Additional features to prevent transmission of CL-3 organisms are appropriate respiratory protection, HEPA filtration of exhausted laboratory air, as well as strictly controlled laboratory access.

Containment Level 4 (CL-4)

This is the maximum containment available and is suitable for facilities manipulating agents requiring containment level 4. These agents have the potential for aerosol transmission, often have a low infectious dose, and produce very serious and often fatal disease; there is generally no treatment or vaccine available. This level of containment represents an isolated unit functionally and, when necessary, structurally independent of other areas. CL-4 emphasizes maximum containment of the infectious agent through complete sealing of the facility perimeter with confirmation by pressure decay testing; isolating the researcher from the pathogen by containing the individual in a positive pressure suit (most common) or containing the pathogen in a Class III biological safety cabinet line (rare); and decontaminating air and other effluents produced in the facility.

Operational Practices

For the receipt and processing of human clinical specimens and tissues from suspicious human novel or pandemic influenza cases, a CL-2 laboratory is required to safely:

- perform routine diagnostic testing of serum or blood samples
- manipulate inactivated virus particles or portions of the viral genome

- package specimens for transportation to the appropriate diagnostic laboratory for testing
- perform rapid antigen testing
- carry out RT-PCR.

For the receipt and processing of human clinical specimens and tissues from suspicious human novel or pandemic influenza cases, a CL-2 laboratory and the use of additional operational practices are required to safely:

- aliquot or dilute specimens
- perform diagnostic testing that does not require propagation of virus
- perform nucleic acid extraction on untreated specimens
- prepare smears using heat or chemical fixation.

Additional operational practices include:

- wearing protective clothing (e.g., protective solid front gowns, gloves, and N-95 respiratory protection) in accordance with the risk of exposure when handling specimens
- manipulations that may produce aerosols should be carried out in a certified biological safety cabinet
- centrifugation of respiratory and tissue specimens should be carried out in sealed centrifuge cups or rotors, both of which are unloaded in a biological safety cabinet.

For human clinical specimens from confirmed novel or pandemic influenza cases, a CL-3 laboratory, including the use of respiratory protective equipment, is required to safely:

- perform work or diagnostic tests that involve propagation of viral agents *in vitro* or *in vivo*
- recovering viral agents from cell cultures
- manipulating or concentrating virus.

CL-3 facilities must be certified by Health Canada officials prior to conducting this work.

Canada's single CL-4 facility is located in Winnipeg, Manitoba.

Specimen handling and testing of specimens potentially containing a pandemic strain of influenza A should be performed in areas physically isolated from those in which routine inter-pandemic human strains or avian or animal strains are being cultured.

Table 14.4: Containment Levels Required for Different Testing Methods

Laboratory Method	Containment Level
Virus Isolation/Manipulation	CL-3
Immunofluorescence on direct specimens	CL-2
RT-PCR/NAT	CL-2
Rapid Testing	CL-2
Serology	CL-2

14.9 Guidelines for Laboratory Based Surveillance

Early detection of a novel, unusual or pandemic strain of influenza in humans in Ontario will guide the pandemic response. Implementation of disease control strategies, including the use of vaccines and antivirals, will be triggered by changes in the epidemiology and virology of the pandemic virus. Ontario's ability to identify the entry of a pandemic virus into the province or detect changes in the virus depends on laboratory based surveillance information. Laboratory surveillance activities may change as a reflection of newly available information.

Objective

The fundamental objective of laboratory based surveillance is to quickly detect, monitor (for antigenic drift and shift in circulating influenza strains), and characterize novel or pandemic influenza viruses anywhere in Canada. Once the virus is identified and characterized, vaccine strains can be compared to circulating strains, or new vaccines developed in response to circulating strains. Surveillance for antiviral susceptibility or resistance would be on-going.

Special Considerations

During the interpandemic period or as soon as possible, intra-provincial protocols should be developed that detail studies (including seroprevalance studies) required both during and following the pandemic period.

Anti-microbial susceptibility testing and prevalence studies of agents responsible for secondary infections to influenza must be included in the planning.

Table 14.5: Laboratory Surveillance by Pandemic Period and Phase

Objective by Pandemic Period	Pandemic Phase	Surveillance Activities
Interpandemic Period: *To establish baseline influenza activity and to facilitate early detection of new emergent strains.*	**Phase 1** Phase 1: No new influenza virus subtypes have been detected in humans. An influenza virus subtype may be present in animals, but the risk of human infection is considered low. and Phase 2: A circulating animal influenza virus subtype poses a substantial risk of human disease.	Virus isolates obtained under the following conditions should be sent to the National Microbiology Laboratory (NML) as part of routine surveillance. All laboratories performing virus isolation are to submit both pre-season and early isolates. In addition to these, laboratories are asked to send up to 10% of all season influenza isolates; including 5% during the early season, 5% during the latter part of the season and any unusual isolates, isolates from unusual clusters, or isolates for which the patient has an epidemiological link to an area of concern, to the NML for viral sub-typing. The latter will be treated as priority specimens by the NML. Isolates of persons whose influenza illness is related to travel to an area of concern. Isolates collected during peak activity, usually January, which are representative of the season. Late season isolates after major outbreak activity ends. Isolates of a type or subtype present as a minor component (10% or less) of the year's epidemic. Influenza A isolates that cannot be sub-typed. Isolates from persons receiving antiviral agents or from their contacts should they become ill. Isolates obtained during in-depth investigations of influenza outbreaks occurring in otherwise healthy, immunized populations. Isolates from cases of suspected animal-to-human transmission of influenza virus. **Susceptibility Testing:** On early and late isolates as appropriate. **Sequencing:** On early and late isolates as appropriate.
Pandemic Alert Period: ***To ensure detection and characterization of novel or unusual virus immediately upon introduction into the province of Ontario, including strain identification, drift and/or shift, and anti-viral sensitivity.***	**Phase 3** Human infection(s) with a new subtype, but no human-to-human spread or rare instances of spread to a close contact only	As in the Interpandemic Period, with heightened surveillance as directed by the NML and the Pandemic Influenza Committee (PIC). Testing, typing, sequencing and susceptibility testing of select isolates from newly identified "clusters" of influenza-like illness (ILI), ILI outbreaks, travel-related disease, or cases/clusters with atypical clinical presentation and an exposure history or history of travel to an area of novel strain activity. Influenza viruses can be rapidly detected and typed using molecular methods. Ontario Public Health Laboratories and some Ontario hospital laboratories can provide this technology with increased capacity over standard methods. The NML is available as an out of province resource and, in addition to sub-typing, will perform susceptibility testing and sequencing. All isolates identified as being of a novel strain, or any other unusual or non-typable isolates should be forwarded to the NML for further study and for confirmation. Enhanced surveillance includes: • "ring" surveillance of contacts of those known to be infected with the novel or pandemic influenza strain. • increased testing, including anti-viral sensitivity testing, especially in those not responding to treatment, or those receiving prophylaxis that develop symptoms of influenza-like illness. • sub-typing, sequencing and anti-viral sensitivity testing of a select (1%) proportion of the specimens submitted from patients hospitalized with Influenza A. • sub-typing, sequencing and anti-viral sensitivity testing of those persons presenting with severe respiratory illness (SRI) and/or unexpected outcomes of severe ILI, and a significant travel history.

Objective by Pandemic Period	Pandemic Phase	Surveillance Activities
		Primary care providers should be reminded to query travel and test for influenza. in severe ILI patients, particularly in those who have recently traveled or had close contact (i.e. within 1 meter) with travelers to any novel/unusual/pandemic influenza affected area, or resided in or visited an area where mass unexplained die offs of domestic fowl, ducks or wild birds have occurred. Local public health authorities should be notified for all patients with severe ILI with a positive travel history or close contact with an ill traveler to an area of concern.
	Phase 4 Small cluster(s) with limited human-to-human transmission but spread is highly localized, suggesting that the virus is not well adapted to humans	Public Health authorities in Canada are advised to continue increased vigilance for the surveillance, recognition, reporting and prompt investigation of influenza. Thus, continue as per Phase 3.
	Phase 5 Larger cluster(s) but human-to-human spread still localized, suggesting that the virus is becoming increasingly better adapted to humans, but may not yet be fully transmissible	As per Phase 4. Heightened surveillance to be continued and consistent with direction provided by PIC and NML.
Pandemic Period: ***To assist with the identification of the affected population(s) thereby facilitating identification of high risk groups in order to inform public health actions. To monitor and characterize the virus, including strain identification, drift and/or shift, and anti-viral sensitivity.***	**Phase 6** Increased and sustained transmission in general population Several outbreaks in at least one country and spread to other countries)	Sustainable surveillance activities are maintained. Both human and laboratory resources may be scarce by this period depending upon how widespread the pandemic virus is in Ontario. Molecular testing will be the mainstay of both diagnosis and surveillance. Unusual, novel, or non-typable specimens will be forwarded to the NML for further investigation. Towards the end of this phase, and as prevalence of pandemic strain starts to subside, heightened surveillance for other respiratory viruses must be initiated.
Postpandemic Period return to Phase 1		Return to pre-pandemic activities. Review and analyze pandemic period and activities. Revise pandemic plans as needed.

14.10 Guidelines for Laboratory Personnel

Vaccination Status

Laboratory employees should be vaccinated against the currently circulating strain of influenza. This vaccine is not expected to provide protection against the novel or pandemic strain, but it will reduce the opportunity for concurrent infections and the possibility of reassortment of the novel strain with a strain already well adapted to humans and capable of human to human transmission. It may also help to discriminate between ILI symptoms as the consequence of infections with the seasonal strain, or as the consequence of infection with a laboratory acquired strain, following occupational exposure. See Chapter 14A: Laboratory Tools for a form to assist with collecting information on routine vaccine uptake by laboratory employees.

Prophylaxis

Personnel employed in the practice of virus culture and the manipulation of cultured virus should do so using CL-3 containment. Some laboratories may elect to stock antivirals in the event of accidental exposure or to offer prophylaxis to employees engaged in high risk activities in accordance with provincial policy (when established). Laboratory employees potentially exposed or at risk should undergo appropriate medical evaluation.

Education

Laboratory leaders are responsible for ensuring that employees are well informed and continually updated on issues related to pandemic influenza. Employees must be properly trained to handle, process, test and ship specimens potentially containing pandemic strains of influenza. For those employees who will have direct patient contact, please see "Preventing Febrile Respiratory Illness" which contains recommended infection control practices.

Archived Serum

Laboratories may choose to collect and store serum on all employees in advance of a pandemic for retrospective analysis.

Medical Surveillance

Medical surveillance of employees at risk of occupational exposure should help ensure appropriate and timely medical intervention and decrease the opportunity for transmission.

Employees working in laboratories with potentially pandemic, novel, or avian strains should report fever or respiratory symptoms to their supervisors. Employees should be evaluated for possible exposures and the course of illness closely monitored. Employees exposed but not yet sick, should remain away from work, until appropriate laboratory investigations have been completed, and return to work only pending results and physical well being.

Emergency Preparedness at Home

Laboratory personnel are reminded of the importance of planning for family and home. See the following guide for this planning:
http://www.health.gov.on.ca/english/public/program/emu/emerg_prep/emerg_prep_mn.html

14.11 Next Steps

The laboratory sector will continue to:

- refine its pandemic plan
- develop stockpiles of equipment and supplies
- develop competency-based HHR/ deployment plans for use during a pandemic.