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Pandemic influenza: Laboratory diagnosis

Preparation has been under way for some time in anticipation of the demands that will be placed on diagnostic virology laboratories during a pandemic.

ABSTRACT: Laboratory testing will be critically important during an influenza pandemic. Definitive and accurate diagnosis of the virus will be needed to facilitate the monitoring of the pandemic and allow for differentiation between epidemic and pandemic viruses, and to form a sound basis for surveillance, use of antiviral therapy, patient management, and infection control practices. To be effective, laboratories will need to communicate with clinicians about optimal specimen collection. Laboratories will also need to implement appropriate testing procedures, communicate results effectively, and maintain staffing and supplies for an anticipated tenfold increase in specimen volume over a period of at least 2 months.

Preparing for pandemic influenza poses a major challenge to diagnostic virology laboratories, who must select optimal specimens for diagnosis, determine a time frame for specimen collection and transportation of the specimens to the laboratory, process the specimens, select optimal tests to be performed, and report laboratory findings to both clinicians and local public health authorities. These requirements will have to be addressed as the global situation evolves from Phase 3 (sporadic human infections acquired from poultry) on the WHO pandemic preparedness scale to Phase 6 (widespread pandemic).

Although the next pandemic strain of influenza may be derived from the currently circulating H1 and H3 viruses, there is increasing evidence that the strain will most likely be a variant of the H5N1 avian influenza, now being spread by limited person-to-person transmission. Diagnostic virology laboratories are currently set up for same-day and next-day diagnosis of the H1 and H3 influenza viruses and other respiratory agents. Should the H1 or H3 virus become the pandemic strain, there will be a substantial increase in demand for testing beyond that needed in seasonal epidemics. Should a variant of the H5N1 virus become the pandemic strain, laboratories will have additional work to do differentiating between the pandemic strain and non-pandemic strains of influenza that may be co-circulating. Without appropriate planning, laboratory capacity could be overwhelmed by the increased amount of testing. On the assumption that the pandemic virus will be an H5 variant, diagnostic laboratories must prepare now for optimal specimen collection and transport, testing, communication of findings, and staffing.

Specimen collection and transport

Human influenza virus is present throughout the respiratory tract and although its highest concentration is in the bronchi, specimens from the nasopharynx (obtained by nasopharyngeal swabs or washes) are recommended for virus diagnosis (see **Figure**).[1] However, human infections with H5N1 currently occur predominantly in the lower respiratory tract, hence specimens of choice are those from the oropharynx and include sputum, although nasopharyngeal specimens are acceptable for more sensitive assays.[2] In more severe cases, endotracheal

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Nasopharyngeal swab insertion

Procedure

1. Put on gloves and other personal protective equipment as needed.
2. Collect specimen as described below.
3. Break off top of swab (it will snap off).
4. Place in transport medium.
5. Remove gloves and wash hands.
6. Ensure the specimen is labeled and ship to the BC Centre for Disease Control laboratory with completed requisition.

For nasopharyngeal swab (preferred)

- Using a slow, steady motion, insert a flexible swab several centimetres along the floor of the nose (straight back, not up the nose) over 6 cm or until the posterior nasopharynx has been reached (distance from nostrils to external opening of ear).
- Once at the appropriate depth or when resistance is met (the swab should pass into the pharynx relatively easily), rotate the swab several times and withdraw the swab.

For nasal swab

- Place one hand behind the patient's head to steady him or her, incline the head as appropriate and insert a cotton swab from a regular virus isolation tube into the nostril approximately 2 cm along the nasal septum (the centre of the nose).
- Rub the swab vigorously but gently along the lining of the septum several times to obtain cells. Vigorous swabbing is necessary to get cells onto the swab.

Figure. Specimen collection for virus diagnosis.

secretions and bronchoalveolar lavages are preferred. In addition, the H5N1 influenza has been recovered from stool specimens and plasma.[3] Although shedding may be prolonged, respiratory specimens should be collected within the first 3 days of illness.[4]

Should the H5N1 virus evolve to a strain that spreads readily, its concentration in the upper airway may increase, making specimens from the nasopharynx more acceptable. Transportation of specimens to the laboratory at the beginning of a pandemic is expected to follow the current pattern of practice, which includes the refrigeration of specimens after collection and during transport. As the pandemic evolves, enhanced transportation measures may be required. Health care workers will be expected to use appropriate personal protective equipment during collection procedures that generate aerosols and during high-risk processing steps in the laboratory.

Tests

Testing for influenza will be determined in response to the phase of the pandemic. For example, in our current state of pandemic preparedness, conventional immunospecific tests such as immunofluorescence microscopy and point-of-care tests are appropriate in the absence of specific risk factors. Isolation in cell culture continues to be performed by virology laboratories unless there are strong risk factors such as travel from an endemic country and direct contact with poultry or persons infected with the H5N1 virus. If risk factors are present, isolation in cell culture must be performed in a high containment biological safety level 3 (BSL-3/P3) laboratory. Nucleic-acid-based tests such as RT-PCR are considered ideal for the diagnosis of influenza, its differentiation into types A and B, and determination of the serotype. For patients with risk factors for having acquired the H5 virus, the diagnosis of influenza and determination of its H-type (H1, H3, H5, H7) can readily be accomplished in 1 day. In general, a preliminary discussion with a medical health officer is required for such cases.

In the event that H5 avian influenza virus begins to spread among our population, pandemic preparedness would increase to Phase 4 (family or small group outbreaks) through Phase 6 (widespread pandemic in the population). Under these circumstances, isolation of viruses in cell culture will be restricted to appropriately equipped BSL-3/P3 laboratories. Immunospecific testing by immunofluorescence microscopy will continue in virology laboratories, but at present these tests, as well as the point-of-care tests, can detect the virus as an influenza A but cannot definitively identify it at the subtype level as the H5 strain. Moreover, point-of-care tests have limited sensitivity and are not recommended for sporadic cases.[4] At the inception of a pandemic, it will be essential that

patients with any risk factors be tested for the presence of the H5 virus by RT-PCR. Identifying the H5 virus would allow for the implementation of measures such as patient isolation and administration of antiviral drugs to slow or even terminate the spread of the virus to the local population. In the event that the H5 pandemic strain becomes the predominant virus, then the use of immunospecific tests at local laboratories would be expected to prove adequate.

Based on projections of morbidity and mortality, it is anticipated that the demands on the laboratories for diagnostic testing would increase by up to tenfold over that experienced in seasonal epidemics.[5,6] Only RT-PCR assays with semiautomated extraction instrumentation would be able to cope with such a demand. Since RT-PCR is an established test in the province for the primary diagnosis of influenza, its use can readily be expanded to accommodate a tenfold increase in testing demands.

The highest demand on the laboratory is to be expected when the pandemic is expanding from infections among small clusters (Phase 4) to larger communities (Phase 5). At this stage, the unique clinical features of the illness may not as yet be sufficiently defined and clinicians would be particularly dependent on the laboratory to provide a definitive diagnosis, especially if the pandemic virus co-circulates with the epidemic variants in the winter months. When the pandemic reaches its peak and the clinical features of the illness have become better defined, diagnostic testing may be required for only severe or unusual cases (similar to testing for seasonal influenza at present). In this setting the laboratories will continue to perform testing for surveillance to provide the medical community with up-to-date information on the prevalence of the virus and the eventual decrease in H5 infections as the pandemic subsides.

At the peak of the pandemic, the reference laboratory will likely extend its mandate to monitoring for the appearance of viruses with resistance to antiviral drugs and providing serological confirmation of past infection and immunity to the virus. Testing for antiviral resistance will be critical for severe cases where the efficacy of the treatment may be in question. Serological testing will be important for health care workers and other essential personnel who may have recovered from infection or received a vaccine and are about to begin working with infected patients. In collaboration with the National Microbiology Laboratory, the reference laboratory will also continue to characterize pandemic isolates of virus at the genotype level to monitor for any genetic drift capable of affecting antiviral resistance or the efficacy of any vaccines that have been introduced.[7]

Communication

Communication of laboratory findings to clinicians, medical health officers, and epidemiologists will have to be augmented to address the increased volume of testing. A direct line of rapid communication will be required between the laboratory and medical health officers/epidemiologists. To monitor the geographic occurrence of cases, a web-accessible geo-mapping process will need to be introduced.

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Likewise, laboratories will have to establish an effective communication processes among themselves. This and other aspects of planning on laboratory preparedness have been undertaken at the national level by the Canadian Pandemic Influenza Laboratory Preparedness Network under the auspices of the Canadian Public Health Laboratory Network.

Staffing and supplies

To address demands posed by the anticipated increase in number of specimens and increased absenteeism due to

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illness of staff or their family members, laboratories will be expected to prioritize the testing being offered. Options will include the temporary reduction of testing for chronic infections such as hepatitis, syphilis, and HIV and the reassignment of technical staff to support influenza diagnosis. Likewise, it is anticipated that there will be an increase in test demands for the bacterial diagnosis on respiratory specimens, since bacterial infections following influenza may become a major concern.

Based on past experience, the pandemic can be expected to last for approximately 8 to 10 weeks.[8] It may then recur in successive waves, likely during the winter months. To meet the demands caused by an initial outbreak, laboratories are currently ensuring that adequate reagents and supplies are available and that the staff has been adequately trained to meet the testing demands. The issue of supplies is particularly sensitive since imported supplies and reagents can realistically be expected to become scarce as the pandemic progresses.

Summary

During an influenza pandemic, diagnostic virology laboratories will have a major role to play in patient management and surveillance, especially in the early phases of the pandemic. Preparation to meet this demand has been under way for some time. The infrastructure that will allow the laboratories to provide same-day diagnosis for a greatly increased number of specimens is being developed and is expected to be able to meet the needs of the province in the event of a pandemic. Once a pandemic occurs, the diagnostic process, from collecting specimens to reporting results, will continue to be refined as the pandemic strain of the virus and its clinical impact become better characterized, and ongoing coordination at the national level will keep the laboratories up to date on any new tests and algorithms that will require implementation.

Competing interests

None declared.

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