

Swine influenza H1N1

Is your laboratory prepared?

Sameera Aljohani, MD, FRCPC.

Influenza A virus is a member of the family *Orthomyxoviridae*. Influenza viruses are enveloped, with a segmented, single-stranded RNA genome. This family also contains influenza B and C viruses. Point mutations in the envelope protein hemagglutinin (H), referred to as antigenic drift, result in the emergence of new strains of influenza A and B viruses and the resultant annual outbreaks and epidemics. Sub-typing of influenza A virus is based on antigenic characteristics of 2 envelope proteins, H and neuraminidase (N). New influenza A virus subtypes emerge as the result of reassortment of H and N sequences from 2 different subtypes, referred to as antigenic shift. These new subtypes are responsible for influenza pandemics. There are currently 16 recognized H subtypes and 9 recognized N subtypes. Subtypes H1N1, H3N2, H2N2, and H1N2 have circulated, or are currently circulating widely, among humans. If, or when, the virus reassorts; a new genetically different and unique virus will form; and if transmitted from human to human easily, the new virus will likely produce an influenza pandemic with unpredictable mortality rates.

Swine influenza (swine flu) is a respiratory disease of pigs caused by type A influenza viruses, but human infections can, and do happen. Swine flu infection can be serious. In September 1988, a previously healthy 32-year-old pregnant woman in Wisconsin was hospitalized for pneumonia after being infected with swine flu and died 8 days later. A swine flu outbreak in Fort Dix, New Jersey, occurred in 1976 that caused more than 200 cases with serious illness in several people and one death.¹ Swine influenza A (H1N1) virus appears to spread the same way as seasonal flu. Flu viruses are

spread from person to person through coughing or sneezing of infected people. Sometimes people can get infected by touching contaminated surfaces or materials with flu viruses on it.¹

I. Special instructions. A laboratory should not perform culture on specimens if H1N1 influenza is suspected, unless performed under enhanced Biosafety Level 3 (BSL-3) laboratory conditions and with very close supervision.

Microbiology laboratories may perform rapid influenza antigen tests and direct fluorescent antibody staining on respiratory specimens from suspected cases, but only under BSL-2 conditions in a Class II biological safety cabinet. However, influenza A H1N1-specific reverse-transcriptase polymerase chain reaction (RT-PCR), is the preferred method because of its high sensitivity.²

II. Recommendations. *A. General.* Virology laboratories should not inoculate specimens suspected of containing influenza A H1N1 virus into cell culture. Only laboratories capable of performing culture under BSL-3 conditions with enhancements should perform culture to evaluate a suspected influenza H1N1 case.³ If these criteria are met, and culture is performed, consultation with the Ministry of Health laboratory is recommended. The use of rapid antigen tests for influenza is increasing in laboratories and point of care locations. These tests are among the least reliable for diagnosis of influenza, and should not be used alone to rule out H1N1 influenza in a suspected case, especially during the current pre-pandemic phase, and especially consider its performance for the current H1N1 strain is unknown.³

From the Department of Pathology and Laboratory Medicine, King Abdulaziz Medical City, Riyadh, Kingdom of Saudi Arabia.

Address correspondence and reprint request to: Dr. Sameera Aljohani, Department of Pathology and Laboratory Medicine, KAMC, Riyadh, Kingdom of Saudi Arabia. Tel. +966 (1) 2520088 Ext. 12817. Fax. +966 (1) 2520130. E-mail: Johanis@ngha.med.sa

B. Precautions. Culture diagnosis of suspected avian influenza A H1N1 requires enhanced BSL-3 laboratory conditions. Enhancements include the use of respirators, decontamination of all waste (solid and liquid), and showering of personnel before exiting. Molecular and rapid antigen testing can be performed on respiratory specimens under standard BSL-2 conditions in a Class II biological safety cabinet.³

C. Specimens. Respiratory specimens. The nasopharyngeal swab (NPS), as well as nasopharyngeal aspirate (NPA) and lower respiratory samples such as bronchoalveolar lavage and tracheal aspirates are the preferred specimens for detection of influenza A virus. But in Avian flu H5N1; throat swab was superior, all respiratory specimens should be acceptable. Nasal swabs and aspirates are acceptable, but may contain lower titers. We recommend 2 respiratory specimens per one patient, first the nasopharyngeal and the second is the throat swab, to be submitted in 2 different virus transport medium (VTM).

Specimen collection and handling. Detection of influenza A H1N1 is optimum if specimens are collected within the first 48 hours of illness. If possible, serial specimens should be collected over several days from the same patient to increase clinical sensitivity.³ As with seasonal flu, Dacron or rayon tipped swabs should be used for specimen collection, as other materials may inhibit RT-PCR. Swabs placed in viral transport medium are generally suitable for RT-PCR testing. The collection of lower respiratory specimens generates aerosols, and requires infection control precautions for influenza A H1N1, including the use of Personal Protective Equipments.³ Specimens should be stored at refrigerated temperatures 2-4°C. For virus isolation, specimens should be stored at refrigerated temperatures no longer than 2 days, or frozen at -70°C, and shipped on dry ice. Laboratories should follow current regulations for packaging and shipping hazardous materials. Transportations should be carried out under Ministry of Health directives.

E. Available laboratory tests. Rapid antigen tests. As rapid influenza antigen tests provide a result in 30 minutes or less, they significantly impact patient treatment and management. These tests are the Clinical Laboratory Improvement Amendments (CLIA) 88 regulated, and are widely used for diagnosis of influenza in central, point-of-care, and physician office laboratories. Several rapid antigen tests are commercially available, some of which are able to distinguish between influenza A and B types. Rapid antigen tests are less sensitive than culture or RT-PCR.³ While rapid antigen capture assays may detect influenza subtypes, including H1N1, currently available tests are NOT capable of distinguishing specific influenza A subtypes. Some evidence indicates that rapid

antigen tests are extremely insensitive for non-human influenza, and should not be used ALONE to rule out NON-HUMAN influenza in a suspect case, especially during the current pre-pandemic phase. Rapid antigen testing can be performed on respiratory specimens from suspected H1N1 influenza cases under standard BSL-2 conditions in a Class II biological safety cabinet where aerosols cannot be avoided.

Fluorescent antibody staining of antigens (DFA). The staining of influenza antigens with fluorescent antibody is an additional rapid test. This method can provide results in less than an hour. Fluorescent-labeled antibodies specific for influenza A and B viruses are available. Fluorescent antibody staining is generally considered to be more sensitive than rapid antigen tests. Specificity is high, but needs well-trained, experienced technologists.³ Fluorescent antibody staining can be performed on respiratory specimens from suspected influenza cases under BSL-2 conditions in a Class II biological safety cabinet.

Nucleic acid amplification. Nucleic acid amplification methods, such as RT-PCR and nucleic acid sequence-based amplification (NASBA), are becoming more commonly used for detection of influenza virus and other respiratory viruses. Test turn around time is around 5 hours. These are the most sensitive methods for detection of influenza viruses in general, including H1N1. Specificity depends on selection of the right primers and probes, optimization of amplification conditions, and interpretation of results. These tests need to have specific and strict protocols to avoid false positive results. The RT-PCR testing only for the H1N1 subtype is not recommended. Specimens from suspect cases should be tested for both influenza A and B, and currently circulating influenza A subtypes in addition to H1N1. Specimen processing should be performed within a biological safety cabinet in a BSL-2 laboratory.

Culture. Culture provides highly specific laboratory diagnosis of influenza, but this is considered to be too slow to impact patient treatment or isolation decisions. Culture is essential for detecting influenza infection missed by rapid testing, confirmation of non-culture results when disease prevalence is low, and to obtain isolates for characterization and surveillance. Do not perform culture on specimens if influenza A H1N1 is suspected outside a level III laboratory.³

Serology. Serologic test methods to detect influenza virus-specific antibodies are available. These methods include indirect fluorescent antibody (IFA), complement fixation (CF), hemagglutination inhibition (HI) and

neutralization. Serology has limited diagnostic value, as you need to collect both acute and convalescent sera to detect sero-conversion or a 4-fold rise in antibody titer.

Table 1 shows the guidelines for the requisition and submission of suspected/possible H1N1 specimens for routine laboratory investigations.²

Table 2 shows the safety guidelines for the handling and processing of suspected/possible (H1N1 influenza) specimens for routine laboratory investigations.⁴

F. Interpretation and reporting. Communication is the key to success. When a patient presents with suspected swine influenza, communication between the Microbiology laboratories, Molecular Laboratory, Hospital Infection Control, and the Ministry of Health is essential. Specimens from suspected H1N1 influenza cases should be referred to the local public health laboratory, as per Ministry of Health directives.

Table 1 - Guidelines for the requisition and submission of suspected/possible H1N1 specimens for routine laboratory investigations.²

- A plan in your laboratory has to be in place for a possible future influenza pandemic. The plan should include teams from infection prevention and control, virology laboratory, pathology, and laboratory medicine administration, hospital administration, emergency medicine, as well as from the nursing staff.
- Respiratory samples should be collected as well as other laboratory tests to be performed on newly admitted patients.
- Please ensure that specimens are properly closed and sealed:
 - Containers must have tight fitting lids or caps to prevent spillage.
 - Use Para film as additional preventative measure.
 - Place specimen in triple packaging system, and follow Transportation of Dangerous Goods (TDG) Guidelines as per international requirements (ITAT)
- Specimen transportation:
 1. Specimens of suspected H1N1 influenza or other potentially infectious materials should be placed in a well-constructed container with a secure lid, which prevents leakage during collection, handling, processing, storage, transport, or shipping. Use TRIPLE packing system as per TDG guidelines. Specimens that leave the hospital must be labeled with a BIOHAZARD label.²
 2. For safety reasons, specimens should NOT be sent via receiving or the hospital tube delivery system and should be communicated VERBALLY to the laboratory prior to sending the specimen.
 3. Requisition forms must be provided with the specimen and provide the full information.
 4. Please exclude all non-essential laboratory investigational testing.

If any of these requirements are not met, or in case of broken or specimen leakage, this will lead to the rejection of the specimen and no testing will be performed.

Table 2 - Safety guidelines for the handling and processing of suspected/possible (H1N1 influenza) specimens for routine laboratory investigations.⁴

The following activities must be performed in Biosafety Level (BCL)-2 facilities with appropriate BSL-2 work practices:⁴

- Always maintain Standard Laboratory Safety Precautions when handling specimens as outlined in the Microbiology Safety Manual.
- Perform all procedures in a biological safety cabinet.²
- Ensure availability of personal protective equipment and physical containment devices.
- Any procedure that may generate aerosols should be performed in a biological safety cabinet.
- Laboratory workers should wear protective equipment, including disposable gloves, solid-front or wrap-around gowns with cuffed sleeves, eye protection, and a surgical mask, or full-face shield, according to the risk of aerosols and exposure when performing specific manipulations. When working at a biological safety cabinet, a full-face shield is not necessary.¹
- Careful attention should be given to hand hygiene after removal of gloves and especially before touching the eyes or mucosal surfaces. Wash hands thoroughly with anti-microbial soap followed by the use of alcohol hand disinfectant.⁴
- Centrifugation of human specimens should be performed using sealed centrifuge rotors or sample cups. These rotors or cups should be unloaded in a biological safety cabinet.⁴
- Procedures performed outside of a biological safety cabinet should be performed in a manner that minimizes the risk of exposure to an inadvertent release of the etiologic agent.⁴
- Biological waste has to be collected in an autoclave bag and exposed to ultraviolet (UV) irradiation for a minimum of 30 minutes, sealed and placed in a second autoclave bag before transporting it to be autoclaved.
- Liquid waste must be collected in a suitable container containing 3% virkon, seal container, expose to UV irradiation for a minimum of 30 minutes, and place in an autoclave bag, seal, and place in a secondary autoclave bag before transporting it to be autoclaved.

No procedure should be undertaken in which there is any doubt about the ability to adequately contain the specimen and prevent the uncontrolled release of the virus.

Finally, is your laboratory ready? To ensure laboratory readiness and capacity to collect, diagnose, and ship patient's samples, it is not essential to have sophisticated techniques. The most important thing is to ensure appropriateness and effectiveness of simple laboratory safety standards. All laboratories, even community based or private laboratories must ensure availability of Personal Protective Equipment (PPE). The appropriate PPE for these types of rapid tests includes: laboratory coat, gloves, eye protection, and face mask (surgical, dental, medical procedure, isolation, or laser masks). There should also be appropriate packages for transportation of dangerous goods along with trained staff on how to pack and transports such specimens. Laboratory staff, especially those working in virology laboratories, should be fit tested for N95 masks as per National Institute for Occupational Safety and Health (NIOSH) regulations.¹ A first level laboratory should be able to collect and transport specimens to reference centers. A second level screening laboratory can perform simple tests such as rapid antigen detection and/or DFA, but they must have certified Biological Safety Cabinets, and can ship all positive cases to a reference laboratory. A third level laboratory should be able to perform RT-PCR, using approved primers and probes for H1N1. These tests should be performed and approved by a certified scientist in that field, and verification of the test result should be carried out before reporting the result. The local health authorities should be notified, and they should arrange shipping of samples to Centers

for Disease Control and Prevention or WHO as per local protocols.¹

Finally, a level 4 laboratory, should be able to test the samples and sequence the virus to detect its genomic background. This should be interpreted at an international level, to ensure that all viruses from across the globe have the same genetic characteristics and belong to the same strain responsible for outbreak or even pandemics. This must be controlled by the World Health Organization, and approved collaborative centers across the world.

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