INTRODUCTION
A well-organized network of laboratories, capable of rapidly and correctly identifying and subtyping influenza viruses is critical to recognizing and managing an influenza pandemic. Recognition of novel strains of influenza virus will be dependent upon early detection and sampling of initial clinical cases associated with the pandemic. Since the symptoms of influenza are nonspecific and are similar to those caused by a number of respiratory pathogens, laboratory testing is crucial to identify the causative agent as a form of the influenza virus. It is essential that the Missouri State Public Health Laboratory (MSPHL) be prepared for the emergence of novel influenza strains and influenza pandemic.

OBJECTIVES
- Provide laboratory resources for rapid detection of novel human or animal influenza viruses.
- Monitor changes in the circulating viruses during the pandemic.

BACKGROUND
The MSPHL is a collaborating laboratory in the World Health Organization’s Global Influenza Surveillance Network. Year-round respiratory specimens from designated sentinel laboratories are sent to the MSPHL where they are tested by viral culture and polymerase chain reaction (PCR). The resulting positive influenza A specimens are then subtyped by PCR and viral culture. A representative number of influenza A and B isolates from viral culture are then forwarded to Centers for Disease Control and Prevention (CDC) for further antigenic characterization. In addition, a representative number of samples subtyped as 2009 Influenza A H1N1 pdm are sent to CDC for antiviral resistance testing. All specimens that cannot be subtyped and a subset of samples from vaccine failures are forwarded to CDC for further testing. Daily reports of laboratory-confirmed cases of Influenza A and B viruses, are sent by HL7 messaging to CDC via the Public Health Laboratory Interoperability Project (PHLIP).

The MSPHL maintains a fully trained technical virology staff. In the summer of 2007, MSPHL moved into a new state-of-the-art facility that contains an extensive biosafety level 3 (BSL-3) laboratory. Additional scientists have been trained in the CDC PCR testing methods to provide back-up and support during a pandemic or public health emergency. The MSPHL participates in year-round laboratory-based surveillance via the National Respiratory and Enteric Virus Surveillance System (NREVSS). The MSPHL continues the Sentinel Surveillance Program with providers participating in the CDC Influenza Sentinel Provider Network. Providers send up to nine specimens per season October through May and/or if the providers see any unusual influenza activity.

Trainings and exercises are part of the preparedness activities that MSPHL participates in throughout the year. The MSPHL exercises the laboratory influenza-testing plan by maintaining scientist’s competencies in polymerase chain reaction (PCR) testing and maintains Clinical Laboratory Improvement Amendments (CLIA) certification.
The Missouri Department of Health and Senior Services’ (DHSS) MSPHL, Bureau of Communicable Disease Control and Prevention and Bureau of Immunization Assessment and Assurance in cooperation with local public health agencies (LPHAs) perform year round, outbreak and seasonal influenza surveillance. In support of this influenza surveillance, MSPHL and program staff conduct training sessions at DHSS area or district health offices. Such training provides hands on opportunities for health care professionals to ask questions and gain knowledge on issues related to seasonal, avian and pandemic influenza, data collection and interpretation, laboratory testing issues, and vaccinations. These trainings serve as an opportunity to review packaging and shipping protocols, review protocols for safe specimen collection and testing procedures utilized by the MSPHL, reporting mechanisms and responsibilities.

MSPHL in cooperation with the Council of State and Territorial Epidemiologists (CSTE) and other DHSS staff conducted six training exercises throughout the state of Missouri. Specialists in epidemiology, emerging diseases, laboratory and veterinary public health attended these training exercises. Participants learned to recognize and manage a human case of Highly Pathogenic Avian Influenza (HPAI), comprehend laboratory diagnosis and specimen collection, and review the investigation process of a possible human-to-human HPAI transmission. Finally, those attending the trainings participated in an exercise and case study to allow participants to walk through an investigation and response to HPAI detection among poultry.

Other aspects of the Laboratory Pandemic Plan are exercised while participating in DHSS Disaster Situation Room (DSR) drills that are held annually.

**PLANNING ASSUMPTIONS**

- As a member of the Association of Public Health Laboratories, the MSPHL will utilize laboratory tests and methods recommended by the CDC in cooperation with the Association of Public Health Laboratories (APHL). The MSPHL will utilize all testing algorithms as disseminated by the APHL and CDC.
- MSPHL will continue to conduct year-round testing for influenza viruses in order to characterize circulating influenza strains and to monitor for novel influenza subtypes.
- MSPHL will provide advanced testing, utilizing laboratory tests and reagents supplied by the CDC and World Health Organization. These testing procedures are not available to most clinical laboratories.
- During influenza pandemic, the MSPHL will work with CDC to provide guidelines for specimen management and diagnostic testing as the pandemic evolves.
- MSPHL will maintain testing supplies and the capacity to meet the public health surveillance needs of the state. MSPHL will not accept specimens solely for diagnostic purposes. MSPHL will process only specimens of public health significance.
- MSPHL scientists are cross-trained in an effort to assist with testing of greatest need. There is an acknowledgement that certain testing may be delayed or redistributed to other laboratories in order to meet more pressing or other critical testing demands. The laboratory has worked with program staff to develop plans for specimen referral and triage.
• MSPHL will utilize the Missouri Laboratory Response Network (MOLRN) to contact member laboratories throughout the state with up-to-date testing recommendations and information.

• The Midwest Regional Center of Excellence for Biodefense and Emerging Infectious Diseases Research will be a resource for laboratory surge capacity.

• The pandemic intervals will determine testing strategies and testing algorithms. Highest test load is expected to occur during the early stages when the novel virus demonstrates efficient human-to-human transmission. During the peak of pandemic, laboratory testing is expected to decrease as more patients will be treated without laboratory confirmation. During the pandemic peak, testing will be provided for the purpose of surveillance of the pandemic strain and for antiviral resistance. Once the cases begin to decline, the MSPHL will continue testing for surveillance of the pandemic strain as well as other circulating influenza viruses.

LABORATORY RESPONSE NETWORK (LRN)

The LRN became operational in August 1999 with the objective to ensure an effective laboratory response to bioterrorism. The LRN is an integrated network of local clinical laboratories (sentinel labs), state and local public health laboratories (reference labs) and federal laboratories (CDC, The United States Army Medical Research Institute for Infectious Diseases, Food and Drug Administration). MOLRN is a network of Missouri laboratories that are fully equipped and trained to respond quickly to acts of chemical or biological terrorism, emerging infectious diseases and other public health threats and emergencies. MOLRN includes MSPHL, which serves as Missouri’s LRN reference laboratory, plus sentinel laboratories within the state. See http://www.bt.cdc.gov/lrn/biological.asp.

SENTINEL LABORATORIES

According to the 2010 MOLRN survey of sentinel laboratories within Missouri, 83% perform rapid diagnostic testing for influenza viruses on respiratory specimens. Of these, 11% have the capability to perform high-complexity viral testing, including the use of viral isolation techniques. In addition, 8% of Missouri’s sentinel laboratories are capable of performing PCR or immuno-fluorescence (IF) testing for rapid detection and sub-typing. These laboratories could be utilized for surge capacity should the situation warrant the need. Hospital laboratories should not attempt to isolate influenza viruses from patients with suspected novel influenza virus infections.
MISSOURI STATE PUBLIC HEALTH LABORATORY TESTING CAPABILITIES
The MSPHL receives samples for testing for respiratory viruses from multiple sources:

● Sentinel influenza surveillance sites.
● Respiratory outbreak investigations.
● Suspected cases of novel influenza virus, including avian flu.
● As a reference laboratory, MSPHL receives influenza isolates from commercial and hospital laboratories.

The MSPHL performs several different tests for influenza diagnosis:

● RT-PCR: The MSPHL performs the CDC FDA-approved influenza assays for detection of influenza A and B viruses. This test detects all influenza A viruses, including A/H5N1.
● Specimens positive for influenza A are subtyped for human seasonal H1 and H3 viruses.
● Tissue Culture: Some specimens are inoculated into tissue culture tubes for confirmatory testing by culture as needed. Results are available within 2 to 14 days. The subtyping results are available within 10 to 21 days.
● Antiviral Resistance: Select portion of influenza A positive samples is tested for the markers of antiviral resistance. The MSPHL has acquired instrumentation and protocols to perform antiviral resistance testing.

If the sample is from a patient who meets the criteria for a suspected case of highly pathogenic avian influenza virus, only RT-PCR testing is performed. The sample is not inoculated into tissue culture, where the virus would be amplified. If these test results suggest the presence of a novel influenza virus, the sample is sent to the CDC for confirmatory testing.

It should be understood that the laboratory procedures used for testing may change depending on the characteristics of the pandemic strain.

● MSPHL maintains year-round capability to perform real time polymerase chain reaction (RT-PCR) testing for influenza A and B viruses using CDC FDA-approved RT-PCR assays. These assays will detect influenza A and B virus and include assays to subtype currently-circulating human influenza A viruses, including highly-pathogenic influenza A/H5N1.
● MSPHL maintains testing capacity for outbreak investigations, reference testing as well as testing as for other novel influenza viruses.
● MSPHL continues to participate in a specimen exchange program with the Kansas State Public Health Laboratory and the Wadsworth Center in New York, the College of American Pathologists (CAP) proficiency-testing program and the CAP bioterrorism proficiency testing program and will maintain its status as a certified laboratory within the Select Agent Program.
● As part of the LRN, MSPHL has the capability of transferring samples to the nearest appropriate partner laboratory if the laboratory cannot perform the required tests or becomes overwhelmed.
● The MSPHL has exercised and drilled the use of the Emergency Management Assistance Compact (EMAC). EMAC is another resource which could be employed should the need arise for additional testing beyond the capacity of the MSPHL. Relationships have been cultivated with the Midwest Research Institute (Kansas City, Missouri) and the Midwest
Regional Center of Excellence for Biodefense and Emerging Infectious Diseases Research (St. Louis, Missouri) as potential outlets for surge capacity testing.

**PANDEMIC INFLUENZA: LABORATORY ROLES AND RESPONSIBILITIES**

**Sentinel and Other Private Laboratories:**

**Pandemic Planning**
- Inventory current levels of diagnostic supplies, including personal protective equipment. Assess anticipated needs for equipment and supplies, and determine trigger point for ordering additional resources. Consider a back-up source for supplies.
- Identify key laboratory personnel whose roles are critical to maintaining laboratory operations.
- Train employees in management of respiratory specimens.
- Institute surveillance for flu-like illnesses among laboratory personnel.
- Cross-train employees to perform rapid diagnostic tests and report results.
- Qualified personnel should be identified to staff laboratory for 24/7 capabilities.
- Ensure employees are trained in the proper packaging and shipping of suspected novel influenza strains to MSPHL.

**Pandemic Response**
- Scale up to manage increased requests for influenza testing.
- Continue to expedite specimens from possible pandemic influenza patients to MSPHL.
- Maintain surveillance for flu-like illnesses among laboratory personnel.

**Missouri State Public Health Laboratory**

**Pandemic Planning**
- Follow CDC guidance related to possible emerging novel viruses, including implementation of new testing algorithms, changes in laboratory procedures, availability of testing reagents, etc. as pandemic evolves. Testing protocols will be determined by CDC algorithms and may be modified with each stage of the pandemic. The laboratory receives RT-PCR test kits and consumables through the CDC Influenza Reagent Resource (IRR). These kits and consumables are supplied as a set to ensure the availability or necessary items during peak demand. Due to the highly variable nature of the influenza virus, these kits are managed on a national level and cannot be stockpiled by the laboratory.
- Inventory current levels of supplies, assess anticipated needs for equipment and supplies and determine trigger point for ordering additional resources. Include specimen mailing kits in assessment. Arrange for back-up manufacturer source for supplies and equipment.
- Enhance lab-based influenza surveillance by increasing designated sentinel sites.
• Utilize the MOLRN and Health Alert Network to send out Health Alerts to educate sentinel laboratories, LPHAs, physicians and other network partners on how to notify DHSS if novel influenza infection is suspected.

• Institute surveillance for flu-like illnesses among laboratory personnel.

• Educate sentinel laboratories within Missouri which have BSL 3 facilities on the highly pathogenic nature of certain emerging novel influenza viruses. Respiratory virus cultures should not be performed in most clinical laboratories and such cultures should not be ordered for patients suspected of having highly pathogenic A/H5 (Asian Linage) virus infection. See http://www.cdc.gov/flu/h2n2bsl3.htm.

• Continue ongoing training of sentinel laboratories and LPHAs in proper specimen collection, handling and packaging and shipping procedures. See http://health.mo.gov/lab/virology/pdf/sphl_avianflu_instructions.pdf.

• Communicate expeditiously to the DHSS Division of Community Public Health (DCPH) any confirmation of a novel virus within the state.

• Continue to supply specimen collection kits and maintain courier service to all counties to facilitate receipt of novel influenza strain at the MSPHL.

Pandemic Response

• Educate sentinel laboratories on the evolving novel influenza strain testing procedures as well as supply updated information received from CDC on an ongoing basis to MOLRN laboratories, LPHAs and other associated partners using Health Alerts, MOLRN broadcasts, updated website information and by other communication means as necessary. See http://health.mo.gov/lab/virology/respiratoryvirstesting.php.

• Utilize technicians cross-trained during regular flu season to perform RT-PCR procedures and to report results as requests for influenza testing increases.

• Redirect laboratory staff to areas of greatest need, i.e. assist in specimen collection kit assembly, extraction, reporting, and telephone call triage.

• Utilize temporary staff as needed to meet increased staffing needs.

• Follow CDC guidance related to the novel virus, including institution of new testing algorithms, changes in procedures, availability of testing reagents, etc. as pandemic evolves.

• Supply updated information received from CDC on an ongoing basis to MOLRN laboratories, LPHAs and other associated partners using Health Alerts, MOLRN broadcasts, updated website information and by other communication means as necessary. See http://health.mo.gov/lab/virology/respiratoryvirstesting.php.

• Communicate expeditiously to the DCPH, initial confirmation of the novel virus within the state and trends and movement of the virus throughout the state as the pandemic evolves.

• Continue to supply specimen collection kits and maintain courier service to all counties to facilitate receipt of novel influenza strain at the MSPHL.

LABORATORY INFORMATION MANAGEMENT SYSTEM (LIMS)

Beginning in 2009, the MSPHL began the implementation of a Laboratory Information Management System (LIMS). Initially, LIMS provides for the electronic transfer of patient demographics, specimen information and results within the DHSS as well as to its federal partners. The long term capabilities and benefits of this system will be to interface with sentinel and other clinical laboratories, hospitals, health clinics and healthcare institutions to facilitate the exchange of electronic laboratory information.
REFERENCES:
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http://health.mo.gov/lab/

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http://health.mo.gov/emergencies/ert/alertsadvisories/index.php

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http://health.mo.gov/emergencies/panflu/panfluplan.php

New York City Department of Health and Mental Hygiene Pandemic Influenza Preparedness and Response Plan; pp 95-101

CDC Pandemic Influenza
http://www.flu.gov/

WHO Influenza Network
http://www.who.int/influenza/gisrs_laboratory/en/

CDC Laboratory Network for Biological Terrorism (LRN)
http://www.bt.cdc.gov/lrn/