2009 H1N1 and Seasonal Influenza
Guidance for Laboratory Testing and Reporting of 2009 H1N1 and Seasonal Influenza in the 2009-2010 Flu Season
January 28, 2010

Introduction
This document provides guidelines for laboratory testing, specimen collection, sample submission, and referral of cases of 2009 H1N1 and seasonal influenza. Information from previous CDPH documents and updates (noted above) has been consolidated to provide one document that contains the most recent testing guidelines.

Objective
The predominant circulating influenza virus is currently 2009 H1N1; however, sporadic infections with seasonal influenza A/H3 have also been detected. CDPH advises all Respiratory Laboratory Network (RLN) laboratories to test for influenza B in addition to influenza A, subtypes H1 and H3, and 2009 H1N1.

Highlights
RLN laboratories should:

- Report all influenza A, B, subtype and confirmation results to VRDL on a weekly basis.
- Continue testing of all hospitalized, ICU, and fatal cases, including severely ill pediatric cases.
- Refer the following RNA extracts to VRDL for further testing and confirmation: 1) fatal cases, 2) severely ill pediatric cases, 3) cases with a CT > 29, and 4) five cases with CT < 30 per month for antiviral resistance testing.
- Refer autopsy tissue specimens of fatal cases to VRDL for further testing and histopathologic analysis at CDC.
In addition, bacterial isolates associated with 2009 H1N1 influenza cases should be submitted to CDPH Microbial Diseases Laboratory (MDL) for characterization of secondary bacterial infections.

Testing Prioritization and Reporting

- **Highest Priority for PCR Testing:**
  - Fatal cases with influenza-like illness (ILI*).
  - Hospitalized patients with ILI.
  - Outpatients with ILI who are being evaluated by an influenza sentinel surveillance provider.
  - Pregnant women with ILI.

- **Testing as resources permit, at the discretion of the LHJ**
  - Health care workers with ILI (those who directly interact with patients or the patient care environment).
  - At the direction of LHJs as part of an investigation of selected ILI clusters when testing results may impact public health interventions.
  
  *ILI is defined as fever >37.8°C (>100°F) and a cough and/or sore throat.

- **Reporting**
  RLN laboratories are also requested to continue reporting influenza PCR results each week to the Viral and Rickettsial Disease Laboratory (VRDL). Our new format requests that, if possible, reports of laboratory data be broken down into categories as follows:

<table>
<thead>
<tr>
<th></th>
<th>2009 H1N1</th>
<th>Seasonal A/H3</th>
<th>Seasonal A/H1</th>
<th>Seasonal B</th>
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<tbody>
<tr>
<td>Outpatient</td>
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<td>Hospitalized</td>
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<td>Outbreak</td>
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Testing and specimen guidelines for suspect cases of 2009 H1N1

In some cases, upper respiratory tract specimens (i.e. nasopharyngeal swabs) from severely ill and fatal 2009 H1N1 cases have tested PCR-negative for influenza when lower respiratory tract or autopsy specimens from the same patient tested positive.

With influenza, viral antigens and nucleic acids may be focal and sparsely distributed in patients. Extensive sampling of both the upper and lower airways ensures the best chance of detecting the virus by immunohistochemical stains, PCR assays, and culture.
For fatal cases, local health jurisdictions should work with their pathologists, medical examiners and coroners to collect both fresh-frozen and fixed tissue at time of autopsy.

CDPH specimen collection guidelines are posted on the VRDL website at: http://www.cdph.ca.gov/programs/vrdl/Pages/DiagnosticTestingforSwineInfluenzaA(H1).aspx

- **Respiratory specimen collection**
  
  o At a minimum, collect a nasopharyngeal swab (nasopharyngeal wash or nasopharyngeal aspirate are also acceptable). Oropharyngeal (throat) swabs are acceptable, but may not have as high a yield. If oropharyngeal specimens are collected, they should be accompanied by a specimen from the nasopharynx. Place the swabs in a standard container with 2-3 ml of viral transport media (VTM).
  
  o If the patient is hospitalized with pneumonia, specimens from the lower respiratory tract (e.g., tracheal aspirate, bronchoalveolar lavage) should also be obtained.
  
  o Use dacron-tipped swabs only. Cotton or calcium alginate swabs are not acceptable for PCR testing.

- **Tissue specimen collection**
  
  The preferred specimens are fresh-frozen and wet fixed tissue specimens representing extensive samples from the following pulmonary sites in addition to specimens from other organs showing pathology:
  
  o Central (hilar) lung with segmental bronchi, right and left primary bronchi, and trachea (proximal and distal);
  
  o Representative pulmonary parenchyma from the right and left lung;
  
  o For patients with suspected myocarditis, encephalitis, rhabdomyolysis or gastrointestinal symptoms, specimens should include myocardium (right and left ventricle), CNS (cerebral cortex, basal ganglia, pons, medulla, and cerebellum), skeletal muscle and gastrointestinal tract, respectively;
  
  o Specimens should be included from any other organ showing significant gross or microscopic pathology.

- **Tissue specimen submission**
  
  o Wet tissue: multiple 1 x 2 cm pieces of tissue from sites listed above in 10% neutral buffered formalin.
  
  o Fresh-frozen tissue: (sent *separately* on dry ice): single piece of tissue as listed above.

Paraffin-embedded tissue blocks: blocks can be submitted in addition to wet and fresh-frozen samples and are the preferred type of specimen to submit in cases where tissues have been in formalin for a significant time. Prolonged fixation (>2
weeks) may interfere with some immunohistochemical and molecular diagnostic assays.

Specimens from fatal cases should be sent to VRDL using the specimen submittal form available at: http://www.cdph.ca.gov/programs/vrdl/Documents/SwineInfluenzaSurveillanceSpecimenSubmittalForm520.pdf

Please call 510-307-8585 before sending specimens so that testing can be expedited.

• **Referral of RNA extracts to VRDL for influenza testing**
  
  For the 2009-10 influenza season, VRDL requests that all public health laboratories in the RLN submit:
  
  o RNA extract or original specimens on all fatal cases of 2009 H1N1 or seasonal influenza
  
  o RNA extract or original specimen on all pediatric cases hospitalized in an ICU with 2009 H1N1 or seasonal influenza (severe pediatric influenza surveillance)
  
  o Five RNA extracts per month on influenza-positive specimens with CT<30 for surveillance for antiviral resistance.

• **Bacterial Isolates from H1N1 Patients with Secondary Bacterial Infections**

  CDPH is interested in characterizing secondary bacterial infections due to group A streptococcus, *Staphylococcus aureus* (MRSA and MSSA), and *Streptococcus pneumoniae* in 2009 H1N1 patients. CDPH requests that available isolates of these bacterial pathogens from 2009 H1N1 patients be submitted to the Special Pathogens Unit of MDL.

  For submitter calls, MDL requires the following:
  
  o Lab-confirmed H1N1 infection.
  
  o Source: with the exception of ETA and BAL, must be from a sterile site source.
  
  o Illness: pneumonia or severe illness requiring hospitalization.

  Testing is for surveillance purposes only; reports will not be issued at this time. For further information regarding isolate submission and testing, contact MDL at 510-412-3903.

Thank you for your ongoing commitment to the 2009 H1N1 influenza response.