Guidance for Laboratory Diagnosis of Influenza A H1N1 in HA Hospitals

1 **Definition of confirmed case of influenza A (H1N1)**

Confirmed case of influenza A(H1N1) virus infection is defined as an individual with laboratory confirmed influenza A(H1N1) virus infection by one or more of the following tests:

i. Real-time RT-PCR
ii. Viral culture
iii. Four-fold rise in influenza A (H1N1) virus specific neutralizing antibodies

2 **Preferred specimens**

i. Nasopharyngeal aspirate or nasopharyngeal or throat and nasal swabs in viral transport medium

ii. Clotted blood for serology tests

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Test / specimen collection</th>
<th>Timing of specimen collection</th>
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</thead>
<tbody>
<tr>
<td>Respiratory aspirates (nasopharyngeal, tracheal)</td>
<td>Direct detection and culture: Aspirate respiratory secretions into viral transport media (T/M).</td>
<td>As soon as possible after onset of illness.</td>
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<tr>
<td>Respiratory swabs (nasopharyngeal, throat and nasal)</td>
<td>Culture: Vigorously swab mucous membrane, especially inflamed areas, with a cotton-tipped swab. Place into viral transport media (T/M) and break off shaft.</td>
<td>As soon as possible after onset of illness.</td>
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<tr>
<td>Paired sera</td>
<td>Viral titre: Collect 5 ml of blood sample in a plain container without anticoagulants.</td>
<td>Acute and convalescent samples 10-14 days apart.</td>
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</table>
Specimen labeling

Specimen container must be labeled with 2 unique patient identifiers matching the information on the request form.

Specimen packaging

Triple packaging system should be used for transport:

- Primary container containing the specimen must be watertight, leak-proof, and properly and securely capped or screwed.
- Secondary leak-proof container should be used to protect the primary container. Request form must be placed outside the secondary container, using a separate plastic bag.
- Tertiary container (transport box) should have adequate strength for its capacity and intended use which can be cleansed and disinfected. It should bear the biohazard warning label.

Specimen transport

Specimens should be sent to laboratory as soon as possible. If delay is unavoidable, keep at 4°C for up to 72 hours. They should be kept at 4°C and upright during transport to minimize the possibility of spillage.


3 **Diagnostic Tests for Novel Influenza A H1** (refer to point 6 for operational arrangement)

3.1 RT-PCR

i. To use reverse transcription (RT) real-time-PCR assays to detect the M gene, current human virus H1 and H3 genes and novel virus H1 gene

ii. If M gene negative, or if M gene positive AND novel virus H1 PCR negative AND either current human virus H1 or H3 PCR positive, novel influenza A H1 can be ruled out.

iii. A positive M gene and novel influenza A H1 gene result indicates infection with the novel virus

iv. Turnaround Time

- Daily testing on specimens received before 12 noon
- Result within same day (both positive and negative)

3.2 Viral culture

It would require virus isolation (in a BSL-3 facility). The turnaround time may be up to 1 week.
3.3 Serology for antibodies
Test on paired serum samples, taken within 7 days of onset and at least 2 weeks from onset.

4 Role of Rapid Influenza antigen Test
i. On NPA, Sensitivity (95%); Specificity (91%)
ii. Detect a range of animal influenza A subtypes, including H5N1 and H9N2
iii. Data, however, is based on human influenza strains
iv. Should be able to detect this Influenza a H1N1 virus but may give false negative results.

5 Guide to Request for Testing for Novel Influenza Virus to CHP-PHLSB
5.3 Contact Information - Lab testing enquiry (Tel: 2319 8252-4)
5.4 Indications for Testing - Fulfilled "Criteria for Reporting"
5.5 Laboratory Request Forms - DH 2542
5.6 Transport of Specimens
i. Upright, avoid leakage at 4°C, reach lab within 24 h of collection
ii. Outside normal working hours, hospital has to arrange for prompt delivery of specimens to PHLSB.

6 Operational arrangement
6.1 M gene realtime PCR (with effect from 30 April 2009):
   i. PHLC will serve KE and HKE clusters
   ii. All other clusters will be served by themselves

6.2 All hospitals are requested to send any specimen INTENDED for M gene realtime PCR to PHLC to perform additional parallel testing for the novel H1 virus.

6.3 Special arrangement for the holiday on 3 May 2009:
   - PHLC will receive specimens for testing from KE and HKE clusters as in 6.1 above (all such specimens should either have been notified via eFlu or NDORS. Please inform MCO or Dr Jance Lo (telephone number: 9124-8341) beforehand;
   - PHLC will receive specimens from all other hospitals for testing as in 6.2 above
- PHLC laboratory will be open for 3 hours (from 9 a.m. to 12 p.m.) everyday to receive specimens. Special arrangement is required outside this period (please call Dr Janice Lo)
- Specimens should be delivered to Room 841 (please call 2319-8261 so that laboratory staff can come to open the door for the messenger)

References
2. Ruest A, Michaud S, Deslandes S, Frost EH. Comparison of the Directigen flu A+B test, the QuickVue influenza test, and clinical case definition to viral culture and reverse transcription-PCR for rapid diagnosis of influenza virus infection. J Clin Microbiol 2003;41:3487-93
3. Guidance to Influenza Laboratories. WHO Diagnosing Swine Influenza A/H1N1 Infections of current concern 25 April 2009