Laboratory diagnosis of influenza

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Influenza Viruses

- Orthomyxoviruses can be identified as influenza A, B and C viruses
- Influenza A infects a variety of different host species
  - There are 15 subtypes of H and 9N. H1N1, H2N2 and H3N2 have been recognized to cause human infection for decades
    - H1N1 - 1918 - 1957
    - 1977 -
    - H2N2 - 1957 - 1968
    - H3N2 - 1968 –
- H1N2 – 2002 --
- Single stranded RNA virus with a core of 8 segments. Each segment corresponds to a gene
**Influenza Viruses**

- The envelop haemagglutinin (HA) attaches to host-cell receptors and stimulates the production of neutralizing antibody.
- The surface spike neuraminidase (NA) permits transport of virus through mucin on cells of respiratory tract.
- The nucleoprotein (NP) contains type specific antigens that distinguish between influenza type A, B and C.
- PB1 and PB2 are polymerases involved in mRNA synthesis and PA in virion RNA synthesis.
- M gene codes for membrane or matrix protein.
- NS genes code for two proteins in infected cells involve in protein and RNA synthesis.
Source of specimens

- Clinical specimens from hospitals for virology studies.
- Swabs from sentinel surveillance sites
- Swabs from investigation of institutional outbreaks
- Confirmatory service for private laboratories with positive screening tests.
Specimen collection

- Take specimen early during illness. Best result in the first 3 days of illness
- Throat / nose swabs
  - Nasopharyngeal aspirate
  - Tracheal aspirate
  - Bronchio-alveolar lavage
  - Sputum
- Transport to lab. as soon as possible. Place at 4°C if delay is inevitable (Isolation rate unchanged for up to 4 days at 4°C)
- Place at -70°C if not cultured within 4 days
Laboratory Methods

- Cell culture
- Immunofluorescence test
- Haemagglutination inhibition test
- Polymerase chain reaction
- Nucleotide sequencing
Rapid diagnosis of influenza A virus

- Directigen Flu A (Becton Dickinson)
- Now® Flu A (Binax)
- Immunofluorescence test
- Reverse transcription-PCR (In-house)
Directigen Flu A (Becton Dickinson)

- An enzyme immunoassay (EIA test) membrane test for influenza A viral antigen (nucleoprotein)
- Results are available in 15 minutes
Result of Directigen Flu A
<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPA</td>
<td>92%</td>
<td>88</td>
</tr>
<tr>
<td>PS</td>
<td>67%</td>
<td>92%</td>
</tr>
<tr>
<td>NPS</td>
<td>88%</td>
<td>97%</td>
</tr>
</tbody>
</table>
Now® Flu A (Binax)

- An immunochromatography (ICT) technology
- Requires only one step
- Results are available in 15 minutes
Result of Now® Flu A (Binax)
Correlation between culture and immunofluorescence

<table>
<thead>
<tr>
<th></th>
<th>IF screen +ve</th>
<th>IF screen −ve</th>
<th>IF screen +ve Both IF</th>
<th>Culture +ve</th>
<th>Culture +ve Both IF</th>
<th>Culture −ve</th>
<th>Culture −ve Both IF</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV</td>
<td>54</td>
<td>5</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influenza A</td>
<td>115</td>
<td>13</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influenza B</td>
<td>94</td>
<td>29</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parainfluenza 1</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parainfluenza 2</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parainfluenza 3</td>
<td>70</td>
<td>8</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenovirus</td>
<td>99</td>
<td>58</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>439</strong></td>
<td><strong>113</strong></td>
<td><strong>30</strong></td>
<td></td>
<td><strong>1566</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

# some specimens had more than a single virus detected. There the total number of viruses detected was greater than the number of specimens examined.
Reverse transcription-PCR

- For sub-typing and detection
- Principle
  - Sub-type (H1 or H3) specific primer
  - Target on viral hemagglutinin gene
  - Viral RNA is extracted and transcribed into cDNA
  - cDNA is then used as a target for polymerase chain reaction
  - Each reaction is analysed by agarose gel electrophoresis and ethidium bromide staining
RT-PCR result (H3)
Cell culture

Inoculate into MDCK cells to isolate influenza virus

Identify by IF and HI tests

Specimens are also inoculated into LLC-MK2, HEp-2c, RD to isolate other respiratory viruses
Serology

Based upon a significant rise (generally 4 fold) in antibody titre to a given viral antigen over the course of the patients’ illness.

- Complement fixation test (CF) type specific
- Haemagglutination inhibition test (HI) subtype specific
- Neutralization test measure protection most sensitive and specific
Typing of Viruses

- All the influenza virus isolates would be typed with antisera from WHO.
- Atypical isolates would be sent to WHO Collaborating Reference Laboratories (e.g. US, UK etc) for further typing.
WHO Influenza Surveillance

World Health Organization

United States  United Kingdom  Australia  Japan

WHO Influenza Collaborating Reference Laboratories

110 WHO National Influenza Centres
## Antigenic analyses of influenza A H3N2 viruses

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Isolation Date</th>
<th>Post infection ferret sera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A/Pan 2007/99</td>
</tr>
<tr>
<td>A/Panama/2007/99</td>
<td>2560</td>
<td>640</td>
</tr>
<tr>
<td>A/New York/55/01</td>
<td>2560</td>
<td>5120</td>
</tr>
<tr>
<td>A/Egypt/130/02</td>
<td>640</td>
<td>640</td>
</tr>
<tr>
<td>A/Fujian/411/02</td>
<td>80</td>
<td>&lt;</td>
</tr>
<tr>
<td>A/Finland/170/03</td>
<td>160</td>
<td>80</td>
</tr>
<tr>
<td>A/Scotland/4952/03</td>
<td>320</td>
<td>160</td>
</tr>
<tr>
<td>A/Wyoming/3/03</td>
<td>1280</td>
<td>320</td>
</tr>
<tr>
<td>A/UK/1861/03</td>
<td>320</td>
<td>80</td>
</tr>
<tr>
<td>A/Kumamoto/102/02</td>
<td>320</td>
<td>80</td>
</tr>
</tbody>
</table>

* *=<40
### Antigenic analyses of influenza B viruses

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Isolation date</th>
<th>B/Shan&lt;sup&gt;7&lt;/sup&gt;/7/97</th>
<th>B/Shan&lt;sup&gt;7&lt;/sup&gt;/7/97</th>
<th>B/HK&lt;sup&gt;335/01&lt;/sup&gt;</th>
<th>B/Te&lt;sup&gt;80/02&lt;/sup&gt;</th>
<th>B/Bris&lt;sup&gt;32/02&lt;/sup&gt;</th>
<th>B/Sich&lt;sup&gt;379/99&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>B/Shandong/7/97</td>
<td>2560</td>
<td>320</td>
<td>320</td>
<td>80</td>
<td>160</td>
<td>&lt;</td>
<td>&lt;</td>
</tr>
<tr>
<td>B/Hong Kong/335/01</td>
<td>1280</td>
<td>320</td>
<td>320</td>
<td>320</td>
<td>160</td>
<td>&lt;</td>
<td>&lt;</td>
</tr>
<tr>
<td>B/Tehran/80/02</td>
<td>1280</td>
<td>320</td>
<td>320</td>
<td>320</td>
<td>160</td>
<td>&lt;</td>
<td>&lt;</td>
</tr>
<tr>
<td>B/Brisbane/32/02</td>
<td>1280</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
</tr>
<tr>
<td>B/Sichuan/379/99</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
</tr>
<tr>
<td>B/Hong Kong/466/03</td>
<td>13.3.03</td>
<td>MDCK2 μl</td>
<td>40</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
</tr>
<tr>
<td>B/Hong Kong/553/03</td>
<td>31.3.03</td>
<td>MDCK2 μl</td>
<td>5120</td>
<td>320</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
</tbody>
</table>

<sup>1</sup> << 40

<sup>2</sup> hyperimmune sheep serum
## Specimens by Age Groups (1998-2002)

<table>
<thead>
<tr>
<th>Age Group positive</th>
<th>Specimens number</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 4</td>
<td>53944</td>
<td>19259 (35.7)</td>
</tr>
<tr>
<td>5 – 14</td>
<td>13108</td>
<td>5118 (39.0)</td>
</tr>
<tr>
<td>15 – 24</td>
<td>2516</td>
<td>820 (32.6)</td>
</tr>
<tr>
<td>25 – 29</td>
<td>7115</td>
<td>1908 (26.8)</td>
</tr>
<tr>
<td>≥60</td>
<td>4184</td>
<td>1092 (26.1)</td>
</tr>
</tbody>
</table>
Respiratory Viruses by Year (1998-2002)

- **Flu**
- **Adeno**
- **RSV**
- **Para**

Year:
- 1998
- 1999
- 2000
- 2001
- 2002
Recent Infections by Animal and Avian Influenza A Viruses

- 1997  Influenza A H5N1 virus (chick)
- 1999  Influenza A H9N2 virus (quail)
- 1999  Influenza A H3N2 virus (swine)
- 2001  Influenza A H1N1 virus (swine)
- 2003  Influenza A H5N1 virus (chick)
Confirmed Cases of H5N1 Infection in 1997

- 18 cases in total
- M : F = 8 : 10
- 11 children under 14, 7 adults
- 3 from Hong Kong Island
  - 6 from Kowloon
- 3 from New Territories East
- 6 from New Territories West
- History of possible exposure to poultry in 7
- 6 died 2 children <14, 4 adults
All eight DNA gene segments of each of these viruses are of avian origin.

The human and chick viruses showed a high degree of homology.

The avian influenza virus H5N1 crossed the avian-human species barrier without prior adaptation in another mammalian species.
Confirmed cases of H5N1 in 2003

- Father 33 and son 9
- Returned from Fujian after staying for 10-14 days
--Daughter 8 yrs. died of pneumonia in Fujian
- Father died on 17.2.2003
- Son recovered uneventfully
Confirmed cases of H5N1

**NPA**  H5N1 virus isolated in MDCK cells  
PCR   - H5 specific

- A/HK/212/03 similar to A/HK/213/03
- No human influenza gene segments present
- All the internal genes are different from A/HK/156/97
- Antigenically different from A/HK/156/97
Avian-to-Human Transmission of Influenza H9N2 Virus

- H9N2 viruses were isolated from two children with mild influenza-like symptoms.
- H9N2 viruses have been isolated from different species of birds and pigs.
- H9N2 viruses are widespread and have been found in Germany, Iran, Pakistan, Saudi Arabia.
Swine-to-Human Transmission of Influenza Virus

- H3N2 virus isolated from 10 month-old baby with mild influenza symptoms
- Antigenically distinct from circulating human virus
- Related to early human and swine H3N2 virus infection
- Closely related to swine H3N2 virus prevalent in the Europe since the 80’s
- Resistant to anti-influenza drugs amantadine and rimantadine
Swine-to-Human Transmission of Influenza Virus

- H1N1 virus isolated from 3 month-old baby with mild influenza symptoms
- Antigenically distinct from circulating human virus
- Related to early swine H1N1 virus infection
- Antigenically related to swine H1N1 virus A/New Jersey/8/76
- None of the other family members were positive for the virus
Conclusion

Laboratory diagnosis important for

• Treatment

• Infection control

• Investigation and management of outbreak

• Systematic collection and analysis of data provide coherent information on trends of infection

• Facilitate rapid isolation of new strains of influenza virus

• Detailed characterization of pathogens provide information for vaccine formulation