Quality assurance panels for influenza virus detection and typing: Experiences from the UK and Europe

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Presentation Overview

Influenza Virus: Regular seasonal occurrence and constant pandemic threat

Annual HPA proficiency panel for molecular detection of (H5) influenza virus

Influenza proficiency testing in European reference labs
- Virus culture and strain characterisation (CNRL)
- PCR detection (WHO EQAP)

Future objectives
- Antiviral resistance detection
- Zoonotic viruses
Influenza Virus: Regular seasonal occurrence and constant pandemic threat

Influenza A and B
Influenza A: 16 haemagglutinin (HA) subtypes and 9 neuraminidase (NA) subtypes
H1, H2 & H3 have circulated widely in humans
Seasonal epidemics
Sporadic pandemics – 1918, 1957, 1968, 2009….

Avian reservoir for all HA subtypes
H5, H7 & H9 have sporadically infected humans
Cumulative cases of human infection with H5N1 worldwide 2003-10

Areas with confirmed human cases of H5N1 avian influenza since 2003

- Turkey: Cases: 12, Deaths: 4
- Azerbaijan: Cases: 9, Deaths: 5
- Egypt: Cases: 112, Deaths: 36
- Iraq: Cases: 3, Deaths: 2
- Pakistan: Cases: 3, Deaths: 1
- Bangladesh: Cases: 1, Deaths: 0
- Myanmar: Cases: 1, Deaths: 0
- Thailand: Cases: 25, Deaths: 17
- Indonesia: Cases: 188, Deaths: 139
- China: Cases: 39, Deaths: 28
- Lao People’s Democratic Republic: Cases: 2, Deaths: 2
- Viet Nam: Cases: 119, Deaths: 59
- Cambodia: Cases: 10, Deaths: 8

* All dates refer to onset of illness

The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement. © WHO 2010. All rights reserved.

Data Source: WHO
Map Production: Public Health Information and Geographic Information System (GIS) World Health Organization
Annual HPA proficiency panel for molecular detection of influenza virus

Background

Establish competency for H5 diagnosis in UK as part of National Pandemic Preparedness

- Need for rapid detection and confirmation of H5 infection
- Rapid availability of results for public health purposes

First panel distributed in 2005 following the establishment of the UK national laboratory network for influenza H5 diagnosis

Annual proficiency panel exercises have tested regional capability to detect diverse influenza strains including H5

- Testing diagnostic service from sample receipt to final report
- Real time reporting
UK national laboratory network for influenza H5 diagnosis

Validated assays → National SOPs → Training programme

Website → National Laboratory H5 Network

Support and development → Proficiency tests 05 -09 → Resources
Annual HPA proficiency panel for molecular detection of influenza virus

Aims of 2005 proficiency panel

1. Implementation of H5 molecular assays
2. Investigate ability to provide confirmed H5 diagnosis
3. Indicate problems with specificity/sensitivity of assay systems
4. Required rapid decision-making and reporting within a defined time frame
5. Test operational capability for H5 diagnostics and drive improvements in quality
Annual HPA proficiency panel for molecular detection of influenza virus

**Participation**

Panel distributed to 20 labs who were initial members of H5 network

Hospital labs in UK and Republic of Ireland

Courier delivery within a specified 1 hr time slot to all labs on the same day

Preliminary diagnostic results within 6 hrs

Confirmed results within 24 hrs

**In 2010 (panel 5):**

10 labs who recently joined the network during the 2009 pandemic response participated for the first time in 2010
Annual HPA proficiency panel for molecular detection of influenza virus

Composition

Pandemic preparedness (H5N1 from different clades, avian subtypes, zoonotic viruses)
Seasonal influenza viruses (H1N1, H3N2, influenza B)
Negative controls

In 2010 (panel 5):

• H1N1 (2009) virus
• Antiviral drug resistant and sensitive viruses
Annual HPA proficiency panel for molecular detection of influenza virus

Preparation

High titre virus stock prepared in tissue culture cells or eggs
Dilution series tested by diagnostic and subtyping RT-PCR assays
Appropriate virus concentration(s) selected to test assay sensitivity
Panel members prepared as simulated clinical respiratory samples containing inactivated virus
Annual HPA proficiency panel for molecular detection of influenza virus

Results analysis

Results returned to HPA Respiratory Virus Unit for analysis

Result for each sample analysed to measure performance of:

- Influenza A diagnosis
- H5 diagnosis
- Seasonal influenza subtyping

In 2010 (panel 5):

- H1N1 (2009) virus diagnosis
- Antiviral susceptibility screening
Time to Submission of Preliminary Report 2005-2010

*Proficiency panel not performed due to pandemic

<table>
<thead>
<tr>
<th>Year</th>
<th>Mean (hrs)</th>
<th>Median (hrs)</th>
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<tbody>
<tr>
<td>2005</td>
<td>14.5</td>
<td>15.5</td>
</tr>
<tr>
<td>2006</td>
<td>6.3</td>
<td>6.1</td>
</tr>
<tr>
<td>2007</td>
<td>6.0</td>
<td>5.5</td>
</tr>
<tr>
<td>2008</td>
<td>5.9</td>
<td>5.4</td>
</tr>
<tr>
<td>2010</td>
<td>5.8</td>
<td>5.8</td>
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Types of errors
- False negatives most frequent error
- Few false positive errors
Total Number of Errors in H5 Detection from 2005-2010

Total Number of H5 Errors

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of errors</th>
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<tbody>
<tr>
<td>2005</td>
<td>5</td>
</tr>
<tr>
<td>2006</td>
<td>4</td>
</tr>
<tr>
<td>2007</td>
<td>1</td>
</tr>
<tr>
<td>2008</td>
<td>1</td>
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<tr>
<td>2010</td>
<td>0</td>
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</table>
Number of Laboratories Performing Seasonal Influenza A and Flu B Subtyping, 2005-2010

Number of laboratories performing seasonal Influenza A and Flu B subtyping

Year | Number of laboratories (total of 17)
--- | ---
2005 | 9
2006 | 3
2007 | 15
2008 | 15
2010 | 15

Legend:
- Flu A Subtyping
- Flu B Subtyping
## Comparison of WHO EQAP and CNRL EQA programme

<table>
<thead>
<tr>
<th>WHO EQAP</th>
<th>CNRL EQA</th>
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<tbody>
<tr>
<td>Influenza A</td>
<td>Influenza A and B</td>
</tr>
<tr>
<td>H1, H1(2009), H3 &amp; H5</td>
<td>H1, H1(2009) &amp; H3</td>
</tr>
<tr>
<td>Detection &amp; subtyping</td>
<td>Detection &amp; subtyping&lt;br&gt;Virus culture &amp; strain characterisation&lt;br&gt;Antiviral resistance detection</td>
</tr>
<tr>
<td>Viral RNA</td>
<td>Simulated respiratory specimens (live virus)</td>
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<tr>
<td>Worldwide distribution to WHO National Influenza Centres (NICs)</td>
<td>Distribution within Europe</td>
</tr>
<tr>
<td>Report results within 5 weeks of receipt</td>
<td>Report detection results within 7 days; virus culture results within 14 days</td>
</tr>
<tr>
<td>2 panels / year</td>
<td>Panels from HPA in 2008 &amp; 2010</td>
</tr>
</tbody>
</table>
CNRL EQA panel (Nov 08) and WHO EQAP (Jun 09) – Improvement in performance between panels

Frequent errors – false negatives (H5 samples)
Guidance documents & Questionnaires

Tele-conferences

↔

EQA Results

Follow up

Proficiency testing

↓

Training Courses
Conclusions

Proficiency testing is of key importance in establishing and maintaining high quality laboratory networks for influenza virus.

Five years of annual proficiency testing demonstrates that the UK has a robust diagnostic service for influenza, including H5.

Comprehensive follow up of EQA results with participating laboratories supports network development.

An ambitious CNRL EQA programme aims to improve quality of influenza virus culture and strain characterisation within Europe – a key factor in vaccine virus strain selection.

WHO EQAP distributed to laboratories with diverse capability – aims to drive quality in global influenza virus detection.
Future Objectives

HPA proficiency testing

Antiviral resistance detection

Zoonotic virus detection e.g. European swine viruses

Detection of generic influenza A and viruses of pandemic concern including diverse clades of H5N1 influenza virus

CNRL EQA programme

CNRL EQA programme 2010

• Culture of contemporary influenza viruses including H1N1 (2009)
• Antiviral resistance detection
• Measure quality of genotypic and phenotypic methods of detection of virus resistance
Acknowledgements

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